University of Koblenz and Landau
Institute for Environmental Sciences
Landau, Germany

Agricultural Research Organization
Gilat Research Center
Negev, Israel

Bachelor thesis

Persistence of chemical and biological effects of olive mill wastewater seasonally applied to loessial olive orchard soil

BY ZACHARIAS STEINMETZ

Student ID 210 200 186

Supervisors

Prof. Dr. Gabriele E. Schaumann

Dr. Arnon Dag

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Abstract

During olive oil production, large amounts of olive mill wastewater (OMW) are generated within a short period of time. OMW has a high nutrient content and could serve as fertilizer when applied on land. However, its fatty and phenolic constituents have adverse effects on soil properties. It is still unknown how seasonal fluctuations in temperature and precipitation influence the fate and effect of OMW components on soil properties in a long-term perspective. An appropriate application season could mitigate negative consequences of OMW while preserving its beneficial effects. In order to investigate this, 14 L OMW m^{-2} were applied to different plots of an olive plantation in winter, spring, and summer respectively. Hydrological soil properties (water drop penetration time, hydraulic conductivity, dynamic contact angle), physicochemical parameters (pH, EC, soluble ions, phenolic compounds, organic matter), and biological degradation (bait-lamina test) were measured to assess the soil state after OMW application. After one rainy season following OMW application, the soil quality of summer treatments significantly decreased compared to the control. This was particularly apparent in a three-times lower biodegradation performance, ten-fold higher soil water repellency, and a four-fold higher content of phenolic compounds. The soil properties of winter treatments were comparable to the control, which demonstrated the recovery potential of the soil ecosystem. Spring treatments resulted in an intermediate response compared to summer and winter treatments, but without any precipitation following OMW application. Significant accumulation or leaching effects to deeper soil were not observed. Therefore, the direct application of legally restricted OMW amounts to soil is considered acceptable during the moist seasons. Further research is needed to quantify the effect of spring treatments and to gain further insight into the composition and kinetics of organic OMW constituents in the soil.

Zusammenfassung

Bei der Olivenölproduktion fallen innerhalb kürzester Zeit große Mengen Olivenabwasser (OMW) an. OMW kann aufgrund seines hohen Nährstoffgehalts als landwirtschaftlicher Dünger eingesetzt werden. Doch seine öligen und phenolischen Bestandteile schaden dem Boden. Es ist nicht bekannt, inwiefern jahreszeitliche Temperatur- und Niederschlagsschwankungen den Verbleib und die Wirkung der Abwasserkomponenten im Boden längerfristig beeinflussen. Um dem nachzugehen, wurden jeweils 14 L OMW m^{-2} im Winter, Frühling und Sommer auf verschiedenen Parzellen einer Olivenplantage ausgebracht. Hydrologische Bodeneigenschaften (Wassertropfeneindringzeit, Wasserleitfähigkeit, Kontaktwinkel), physikalisch-chemische Parameter (pH, EC, lösliche Ionen, phenolische Verbindungen, organischer Kohlenstoff) sowie der biologische Abbau (Köderstreifen) wurden erfasst, um den Zustand des Bodens nach der Applikation zu beurteilen. Nach einer Regensaison war die Bodenqualität der im Sommer behandelten Flächen signifikant reduziert. Dies wurde insbesondere anhand einer dreimal niedrigeren biologischen Fraßaktivität, zehnmal höherer Hydrophobizität, sowie einem viermal höheren Gehalt an phenolischen Substanzen im Vergleich zu den Kontrollflächen deutlich. Die Ausbringung im Winter zeigte gegenteilige Effekte, welche das natürliche Regenerierungspotential des Bodens erkennen lassen. Der Einfluss der Frühlingsapplikation lag zwischen den zuvor genannten. Es wurden keinerlei Anzeichen auf Verlagerung von OMW-Bestandteilen in tiefere Bodenschichten beobachtet. Während der feuchten Jahreszeiten gilt die Ausbringung gesetzlich begrenzter Mengen Olivenabwasser somit als vertretbar. Weitere Forschung ist notwendig um den Einfluss von Frühlingsapplikationen zu quantifizieren und weitere Erkenntnisse über die Zusammensetzung und Mobilität organischer OMW-Bestandteile im Boden zu gewinnen.

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List of abbreviations

AAS	Atomic absorption spectroscopy
BCE	Before common era
CAE	Caffeic acid equivalents
C_{org}	Organic carbon
cv.	cultivar
DCA	Dynamic contact angle
EC	Electrical conductivity
$EC_{1:5}$	EC measurements in 1:5 aqueous soil extracts
ICP-OES	Inductively coupled plasma optical emission spectroscopy
K_s	Saturated hydraulic conductivity
LOD	Limit of detection
LOQ	Limit of quantification
OM	Organic matter
OMW	Olive mill wastewater
p	p-value
PCA	Principal component analysis
$pH_{1:5}$	pH measurements in 1:5 aqueous soil extracts
PVC	Polyvinyl chloride
SD	Standard deviation
SPC	Soluble phenolic compounds
SWR	Soil water repellency
tot	total content
UV	Ultra violet
WDPT	Water drop penetration time
Θ_{adv}	Advancing contact angle

1 Introduction

1.1 Characteristics and environmental impact of olive mill wastewater (OMW)

Olive oil production represents a traditional branch of Israeli agriculture, predominantly organized as family-owned business (Azbar et al., 2004). The first cultivation of olive trees can be dated to approximately 5 500 BCE (Galili et al., 1997). In 2011, the total annual olive oil production reached 12 300 t (FAOSTAT, 2013). Olive orchards are mainly located in rural areas. Due to the (semi-)arid climate in the Near and Middle East region, water shortage is a common problem. To secure agricultural yields during hot and dry summers, continuous irrigation is required and widely applied, particularly in intensive agriculture. Irrigation water is withdrawn from precipitation, rivers, aquifers (ground and fossil water), desalinated seawater as well as reclaimed wastewater from urban and agricultural sources (Frenken, 2009).

In Israel, the three-phase olive oil extraction process is dominant (Azbar et al., 2004). After the olive harvest, large amounts of olive mill wastewater (OMW) are produced within a short period of time. 1 kg of pressed olives generates 1 – 1.6 L OMW (Alburquerque et al., 2004), containing about 5 % solid, 1 % oily, and 94 % aqueous components (Aragón et al., 2001). OMW has a high nutrient and salt content. It could serve as fertilizer and as an additional water source for barren land (Mekki et al., 2006a, Sierra et al., 2007). But due to its large amount of organic compounds, such as fatty acids and (poly-)phenols, OMW is acidic (Lopéz et al., 1992) and has a high chemical and biological oxygen demand (Hamdi, 1993). The application of untreated OMW to soil can increase its long-term hydrophobicity (Mahmoud et al., 2010) as well as its acidity if not sufficiently buffered by organic matter (OM) or soil-situated carbonates (Laor et al., 2011, Sierra et al., 2001). If the soil surface becomes water repellent, irrigation efficiency may be reduced (Moore et al., 2010) and soil erosion may increase. Phenolic compounds and other organic substances such as carboxylic acids are phytotoxic and may harm microorganisms and edaphic mesofauna (Fiorentino et al., 2003, Obied et al., 2005, Paredes et al., 1987). Despite the beneficial effect of OMW contributing OM and plant nutrients, soil fertility is decreased when biodegradation of OMW compounds is inhibited due to the presence of persistent toxic agents (Mekki et al., 2008). Therefore, ecosystem structure and performance can be negatively affected.

Since sewage treatment plants do not accept OMW for purification, improper treatment and illegal disposal remain a serious environmental concern (Azbar et al., 2004, Roig et al., 2006).

Uncontrolled discharge into the environment is not recommended because soil quality may be reduced. Alternative decentralized wastewater management strategies are crucial. Apart from physical (e.g. filtration), chemical (oxidation etc.), and (micro-)biological pretreatments (Roig et al., 2006), the direct application of controlled doses of untreated OMW to natural soil under appropriate environmental conditions might be one of the potentially most practical and least expensive options for rural OMW management (Chartzoulakis et al., 2010).

1.2 Unresolved issues

It is generally understood that OMW significantly deteriorates chemical and biological soil properties in a short-term perspective up to three months after application (e.g. Di Bene et al., 2013, Pierantozzi et al., 2013, Piotrowska et al., 2011, Saadi et al., 2007). Contrary to this, medium- and long-term effects up to one year after repeated OMW application are still challenged regarding both its benefits and negative consequences for the environment: Even 15 years after repeated OMW application in winter, Mahmoud et al. (2010) observed a reduction of soil hydraulic conductivity. Mekki et al. (2006b, 2007) detected potentially toxic phenolic compounds along with a significant decrease of several soil biota communities six months after an OMW application in winter, whereas Di Bene et al. (2013) and Chartzoulakis et al. (2010) found phenolic compounds degrading less than six months after repeated spring and winter applications. Negative effects on other chemical and biological soil properties were not found. Chartzoulakis et al. (2010) even showed beneficial long-term effects on the nutrient content of treated soils.

Fate and effects of released OMW compounds depend on biotic factors like soil biota activity as well as on abiotic factors like soil type, pH, nutrient content, soil water and precipitation, and temperature (Saadi et al., 2013). Hence, winter application may lead to significant leaching of toxic agents due to precipitation. But due to low temperatures, biological degradation is not at its optimum. In spring, after the rainy season, moist and temperate soil conditions could better (re-)enable soil organisms to turn OMW components into less toxic substances (Abid and Sayadi, 2006) without significant leaching risk. In contrast, extreme hot and dry conditions during summer may disable edaphic metabolism or irreversibly harm dormant or less active edaphon and the surrounding vegetation (Saadi et al., 2007) so that OMW might become persistent in the soil. Except first approaches by Di Bene et al. (2013), none of the aforementioned studies took into account seasonal variation following OMW application. Therefore, it is still unknown how seasonally prevailing environmental conditions during and after the application influence the mobility and effectiveness of OMW components on chemical soil properties and on the ecological response in a long-term perspective. Due to the lack of standardized test conditions regarding OMW composition as well as the application season and doses, universally applicable concepts are still outstanding. Additional research, especially on soil water repellency (SWR) and biological effects with respect to the OMW application date, is necessary. An increase of knowledge could help to sustainably minimize negative consequences of OMW application while taking advantage of its beneficial effects on soil properties.

1.3 Objective and hypotheses

The objective of this thesis was to explain how a different seasonality of OMW application affects chemical and biological soil properties. The presented results are supposed to provide detailed insight into the complex interactions of applied OMW, soil quality, and degradation performance. This leads to the following hypotheses:

- High temperatures and low soil water content following OMW application continue to reduce biodegradation of OMW associated OM.
  - → OMW components have the potential to effect changes in soil quality (pH, nutrients, OM, phenolic compounds, and biological activity) persisting at least for one year.
  - → The persistence of these OMW components is stronger when applied in summer than when applied in spring or winter.
- Low temperatures in combination with high water content and substantial water transport towards groundwater favor the accumulation of less soluble organic substances and leaching of soluble cations.
  - → The water infiltration capacity (estimated by SWR and hydraulic conductivity) is higher on plots treated in winter compared to spring or summer treatments.
  - → Repeated winter applications lead to an accumulation of phenolic compounds at a certain soil depth. In contrast, soluble ions primarily leach to deeper soil layers or groundwater. Detectable traces of the leaching path remain apparent.

In order to verify/refute these hypotheses, various chemical and biological soil properties were measured to estimate the soil condition after OMW application. Interdependencies between individual soil parameters are shown and interpreted.

2 Methods

2.1 Study area and experimental design

Field measurements and soil sampling were conducted at an intensively cultivated olive orchard located in the Northern Negev desert near Gilat, Israel (UTM 36 R 658723 E 3468186 N). A loessial arid brown soil (silt loam) dominates in the Northern Negev. The climate is semi-arid (Dan et al., 1972a). Summer temperatures average 26 °C. In the winter, mean temperatures drop to 12 °C. Total annual precipitation ranges from 150 to 250 mm (q.v. appendix, Fig. I, MOAG, 2012). The orchard measures approximately 14 000 m^2. The olive trees (*Olea europaea* L., cv. Barnea) are arranged in a rectangular grid. The distance between the tree lines measures 7 m. Within each line, the trees stand 3.5 m apart from each other (q.v. appendix, Fig. II). Along the lines, a drip irrigation system is installed delivering fresh water (electrical conductivity 0.4 – 0.7 mS cm^{-1}) and fertilizers (150 kg N, 250 kg K_2O, 60 – 80 kg P_2O_5 ha^{-1} a^{-1}). Irrigation is applied twice a week during the dry season, indicated by less than 25 mm precipitation per day. Altogether, 16 plots of interest were randomly selected along the tree lines, measuring at least 2 m × 2 m edge length. OMW was applied in four different treatments (Tab. 1) consisting of four replicated plots each.

Tab. 1: Date and amount of different OMW applications.

Treatment	2011	2012	2013
Winter†	7 L m^{-2}, in January	14 L m^{-2}, in January	
Spring		14 L m^{-2}, in March	14 L m^{-2}, in February
Summer		14 L m^{-2}, in July	
Control	no OMW application		

Every treatment was replicated four times. †additionally, preliminary water drop penetration time (WDPT) measurements were conducted on plots of an adjacent orchard where 15 L OMW m^{-2} were applied in January 2012 & 2013, respectively.

With respect to irrigation, a moist regime (0 – 40 cm from the irrigation line) and a dry regime (80 – 200 cm) were distinguished within each plot (q.v. appendix, Fig. II).

2.2 Soil sampling and field studies

In August 2013, a systematic sampling was carried out according to the prevailing water regime. Soil samples were taken using a soil auger in the moist regime at 30 cm and in the dry regime at 150 cm distance from the irrigation line. The sampled depths were set to 0 – 3 cm, 3 – 10 cm, 10 – 20 cm, 20 – 30 cm, 30 – 60 cm, and 60 – 90 cm. The soil samples were homogenized, air-dried for 300 h and sieved at 2 mm mesh width. Afterwards, the soil water content was determined in accordance with ISO 11465.

In-field water drop penetration time (WDPT) measurements provided information about SWR. Therefore, leaf litter was manually removed. Drops of 100 µL tap water were carefully placed every 4 cm from the irrigation line to the edge of each plot. WDPT results were averaged within 40 cm long sections. The analysis was complemented by in situ mini-disc infiltrometer measurements (Decagon Mini Disk Infiltrometer Model S). For this purpose, a defined volume of fresh water was infiltrated in the moist and in the dry regime of each plot. The effective saturated hydraulic conductivity K_s [cm s^{-1}] was estimated based on the cumulative infiltration I [cm] per time t [s] according to Eq. 1 and 2 (van Genuchten, 1980, Philip, 1957, Zhang, 1997).

$$I = C_1 \cdot t + C_2 \cdot \sqrt{t} \qquad (1)$$

$$K_s = \frac{C_1 \cdot (\alpha r)^{0.91}}{11.65 \cdot (n^{0.1} - 1) \cdot e^{7.5 \cdot (n - 1.9) \cdot \alpha h_0}} \qquad n < 1.9 \qquad (2)$$

The Philip parameter C_1 is a proxy for hydraulic conductivity [cm s^{-1}]. C_2 expresses soil sorptivity [cm s$^{-1/2}$]. h_0 [cm] is the suction rate and r [cm] the disc radius of the infiltrometer. α [cm^{-1}] and n [1] are van Genuchten parameters describing the soil type (silt loam, q.v. ch. 2.1, p. 4).

2.3 Bait-lamina test

Soil fauna activity and degradation performance were estimated using bait-lamina sticks (terra protecta GmbH). The bait-lamina sticks used in this experiment consisted of perforated PVC-strips with 16 apertures, filled with a standard substrate mixture of 70 % cellulose, 27 % wheat bran, and 3 % active charcoal (cf. Kratz, 1998). For each plot, one base group of bait-lamina sticks was systematically distributed parallel to the irrigation line in 30 cm (moist regime) and 150 cm distance (dry regime), respectively. One base group consisted of 16 bait-lamina sticks equidistantly arranged in a 1 m long row. Blank samples were taken to quantify the mechanical abrasion of bait substance due to hard and rough soil. The aim was for an average consumption rate of around 40 % of bait material during the study period. After four consecutive days of exposition, the sticks were removed from the soil. The sticks were carefully cleaned using a brush. Empty apertures were counted (cf. Larink, 1994).

2.4 Soil physical chemistry

To quantify basic soil properties, soluble cations were extracted from 5 g soil using 25 mL of distilled water (weight/volume ratio 1:5). The mixtures were shaken for 24 h, centrifuged, filtered (cf. Hurraß and Schaumann, 2006), and stored at 4 °C prior to use. The pH and electrical conductivity of the 1:5 soil extracts ($pH_{1:5}$ and $EC_{1:5}$) were measured using calibrated electrodes. Cation concentrations (K^+, Na^+, Mg^{2+}, Ca^{2+}) of the soil extracts were determined using atomic absorption spectroscopy (PerkinElmer AAnalyst 200). Chloride was titrimetrically measured in acidified extracts using an automatic chloridometer (Sherwood Scientific 926 Chloride Analyzer MK II). After the removal of inorganic carbon with hydrochloric acid, total organic carbon (C_{org}) was determined by dry combustion (elementar vario MICRO cube) in accordance with ISO 10694. Soluble phenolic compounds (SPC) were characterized colorimetrically by adding 200 µL Folin–Ciocalteu reagent and 1.2 mL saturated $NaHCO_3$ solution to 2.6 mL of a 0.45 µm filtrated 1:10 aqueous soil extract (cf. Box, 1983, Gutfinger, 1981). Caffeic acid was used as standard reference. After 1 h of incubation at laboratory temperature, the extinction of the mixtures was photometrically measured at a wavelength of 725 nm (Analytik Jena Specord 50). Furthermore, the dynamic contact angle (DCA) of the upper soil layer was examined using the Wilhelmy plate method (DataPhysics DCAT series 21) according to Diehl (2009). The advancing contact angle Θ_{adv} is defined in Eq. 3 where F_b [N] is the force of buoyancy, ΔF_g [N] is the change of the plate's weight while immersed in the water, γ [N m^{-1}] is the surface tension between the liquid (l) and the gas (g) phase, and l_w [m] is the wetted length of the plate (Bachmann et al., 2003).

$$\cos(\Theta_{adv}) = \frac{F_b - \Delta F_g}{\gamma_{lg} \cdot l_w} \qquad (3)$$

DCA measurements served as an estimate for SWR. The higher the Θ_{adv} the more water repellent is the soil (Bachmann et al., 2003).

2.5 OMW characterization

Three replicates of OMW applied in 2011 and 2013 were digested by microwave induced reverse aqua regia (HCl + 3 HNO_3) to quantify total cation concentrations (K_{tot}, Na_{tot}, Mg_{tot}, Ca_{tot}) using inductively coupled plasma optical emission spectroscopy (Agilent 700 series ICP-OES). Chloride concentrations were determined via ion chromatography (Metrohm Professional IC). Electrical conductivity (EC) and pH were measured electrochemically. C_{org}

was measured using an organic carbon analyzer (Analytik Jena multiNC 2100S). SPC were determined in a 1:500 diluted OMW sample using Folin–Ciocalteu reagent as mentioned above (q.v. ch. 2.4, p. 6).

2.6 Data analysis

The results are presented as mean ± standard deviation (SD). Mass concentrations refer to the soil dry weight. For relative measurement methods (SPC and soluble cations), the limit of quantification (LOQ) and the limit of detection (LOD) were calculated in accordance with DIN 32645. Due to the absence of normally distributed data proved by the Shapiro–Wilk test, the non-parametric Holm (1979) adjusted pairwise Wilcoxon rank sum test was applied to identify significant differences between treatments, the prevailing water regime, and soil depths. A highly inhomogeneous small-scale distribution necessitated to perform null hypothesis significance testing for WDPT data per treatment only. The significance level was set at $\alpha = 0.05$. Statistical analyses were performed using the R project (version 3.0.2) for statistical computing (R Core Team, 2012), including the ggplot2 package for graphical outputs (cf. Wickham, 2009). A standardized principal component analysis (PCA) was carried out using `prcomp` to summarize data, identify interdependencies between individual soil parameters, and link certain soil parameters to the different OMW treatments (cf. Jolliffe, 2002).

3 Results

3.1 OMW properties

OMW is a brown suspension of both liquid and solid components with a distinct smell of olives. The analyzed OMW (Tab. 2) was rather acidic and particularly rich in C_{org}, SPC, and total potassium (K_{tot}).

Tab. 2: Selected properties of OMW applied to the study area in 2011 and 2013.

Para-meters	**pH**	**EC**	**K_{tot}**	**Na_{tot}**	**Mg_{tot}**	**Ca_{tot}**	**Cl^-**	**C_{org}‡**	**SPC**
		[mS cm^{-1}]	[mg L^{-1}]					[g L^{-1}]	[mg CAE L^{-1}]
OMW	4.6	9.9 ± 0.4	3 700 ± 400	440 ± 30	210 ± 20	140 ± 20	1 200 ± 90	35 ± 1	2 900 ± 40

Results are given as mean ± SD of two replicates. Soluble phenolic compounds (SPC) are defined by caffeic acid equivalents (CAE). ‡measurement of OMW applied in 2011 only.

3.2 Soil pH and soluble ions

Across all treatments, the acidity (Fig. 1) of the 1:5 soil extracts ranged from $pH_{1:5}$ 7.5 to 8. Almost no significant differences were found between the dry and the moist regime. With respect to soil depth, a distinct distribution pattern was observed. Whereas lowest values ($pH_{1:5}$ 7.5) were determined in the top soil (0 – 3 cm), $pH_{1:5}$ significantly increased until a maximum of $pH_{1:5}$ 8 at a depth of approximately 10 cm, to decrease again entering deeper soil layers (10 – 90 cm). This trend was particularly significant for plots treated in summer. The most acidic soil ($pH_{1:5}$ 7.5) was found in the upper soil layer (0 – 3 cm) of OMW-treated plots.

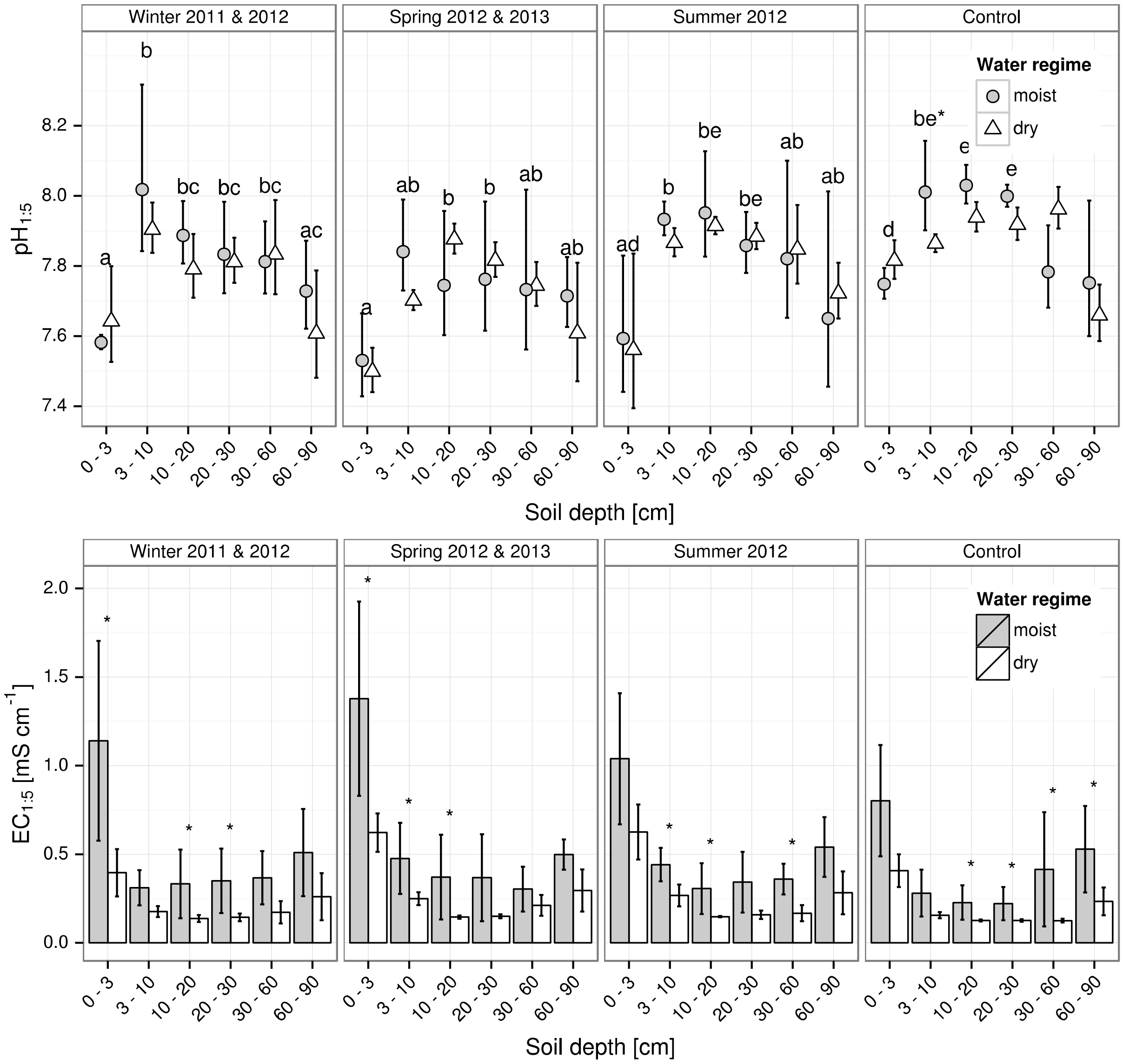

Fig. 1: pH and EC (mean ± SD of four replicates) of 1:5 aqueous soil extracts obtained from different depths. Different letters on top of the bars indicate significant relations (Wilcoxon, $p < 0.05$) between treatments and depths. Asterisks depict significant differences between the moist and the dry regime. No letter or asterisk means no significance to other groups.

With respect to untreated soil (control), OMW application significantly ($p = 0.016$) increased soil acidity in the upper soil layers of spring and winter treatments. Although the difference between summer treatment and control was just below the threshold of significance ($p = 0.062$), a rough trend for acidification was observed.

EC measurements in 1:5 soil extracts served as an estimate for total soluble ion load. As depicted in Fig. 1, $EC_{1:5}$ ranged from 0.13 mS cm^{-1} to 1.38 mS cm^{-1}. $EC_{1:5}$ distribution showed highest values in the upper soil layer (0 – 3 cm depth). $EC_{1:5}$ minima were observed between 10 and 20 cm depth, increasing again with depth afterwards. Across all treatments, $EC_{1:5}$ was lower in the dry regime compared to the moist regime. This distribution was partly significant. Significant differences between the treatments were not found.

Details of soluble ion distribution are depicted in Fig. 2. Across all treatments, soluble potassium (K^+) contents were highest in the upper soil layer, sharply decreasing with depth. From 0 to 3 cm soil depth, OMW-treated plots showed significantly higher ($p < 0.044$) K^+ loads compared to untreated plots (control). Maximum K^+ contents of (600 ± 200) mg kg^{-1} were observed in the top soil layer (0 – 3 cm) of plots treated in spring. The K^+ content at summer treatments was slightly lower. The upper soil layer of plots applied with OMW in winter showed approximately half the K^+ load compared to spring treatments. The K^+ content in deeper soil (10 – 90 cm) averaged (20 ± 30) mg kg^{-1}. Contrary to this, OMW application did not significantly affect magnesium (Mg^{2+}) and calcium (Ca^{2+}) contents throughout all treatments. Nevertheless, a trend was visible showing slightly lower Mg^{2+} and Ca^{2+} means in the top soil layer of untreated soil compared to its OMW-treated counterparts. Alkaline earth metal load significantly ($p < 0.019$, except Mg^{2+} at winter treatments) decreased from the upper soil layer to its subsequent one (3 – 10 cm). Across all treatments, almost no significant differences of K^+, Mg^{2+}, or Ca^{2+} contents were observed between the moist and the dry regime. In contrast, both soluble sodium (Na^+) and chloride (Cl^-) contents were mostly higher in the moist regime compared to the dry regime. Whereas Na^+ contents averaged (200 ± 200) mg kg^{-1} in the moist regime, in the dry regime mean ion contents of (20 ± 40) mg kg^{-1} were found. Cl^- contents were (300 ± 300) mg kg^{-1} in the moist regime and (60 ± 90) mg kg^{-1} in the dry regime. Across all treatments and depths, the mean molar ratio of Na^+ to Cl^- was 1:0.98. Significant differences between treatments and soil depths have not been identified. Nevertheless, Na^+ and Cl^- depth distribution revealed a rough trend similar to EC distribution showing minimum Na^+ and Cl^- loads at approximately 10 to 20 cm depth, increasing both towards upper and deeper soil layers. Mean Na^+ and Cl^- contents were slightly

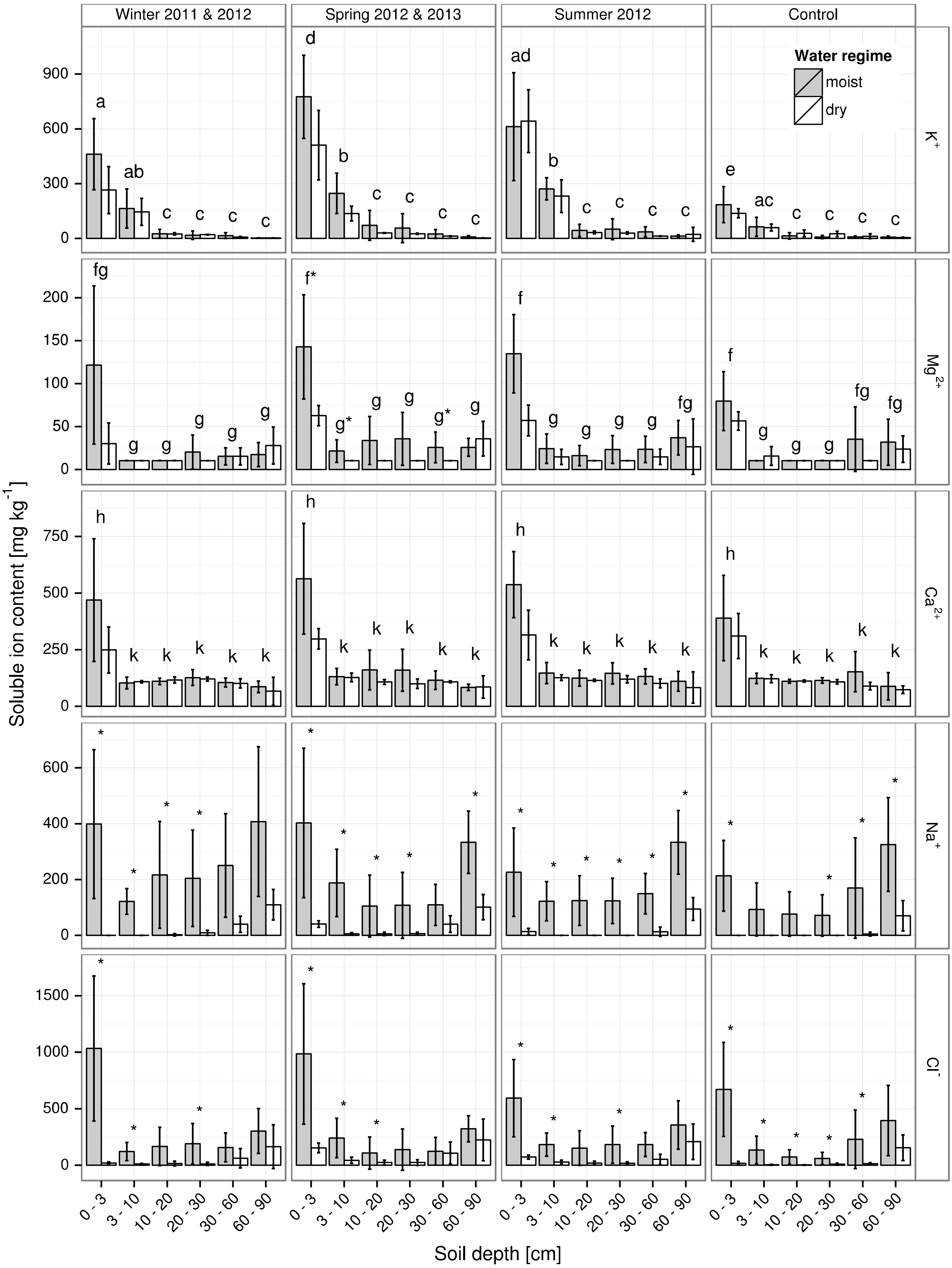

Fig. 2: Soluble ion contents (K^+, Mg^{2+}, Ca^{2+}, Na^+, Cl^-) with respect to the soil depth and the water regime, given as mean ± SD of four replicates. Different letters on top of the bars indicate significant relations (Wilcoxon, $p < 0.05$) between treatments and depths. Asterisks depict significant differences between the moist and the dry regime. No letter or asterisk means no significance to other groups.

higher in plots where OMW was applied twice (spring and winter) compared to summer and control treatments.

3.3 Organic compounds

C_{org} served as an estimate for soil OM. As shown in Fig. 3, significantly ($p < 0.028$) highest mean C_{org} contents of (20 ± 10) g kg^{-1} were obtained in the upper soil layer (0 – 3 cm) of summer and spring treatments, being approximately twice as high when compared to winter treatments with (7 ± 3) g kg^{-1}. Lowest C_{org} loads were determined in the control plots with (6 ± 3) g kg^{-1}. Winter treatments did not differ significantly from the control. C_{org} was mostly observed to be slightly higher in the moist regime than in the dry regime although no statistically significant difference was established. In treated plots, C_{org} decreased significantly with depth, whereas it stayed at a rather constant level of approximately 4 g kg^{-1} across all depths of the control plots.

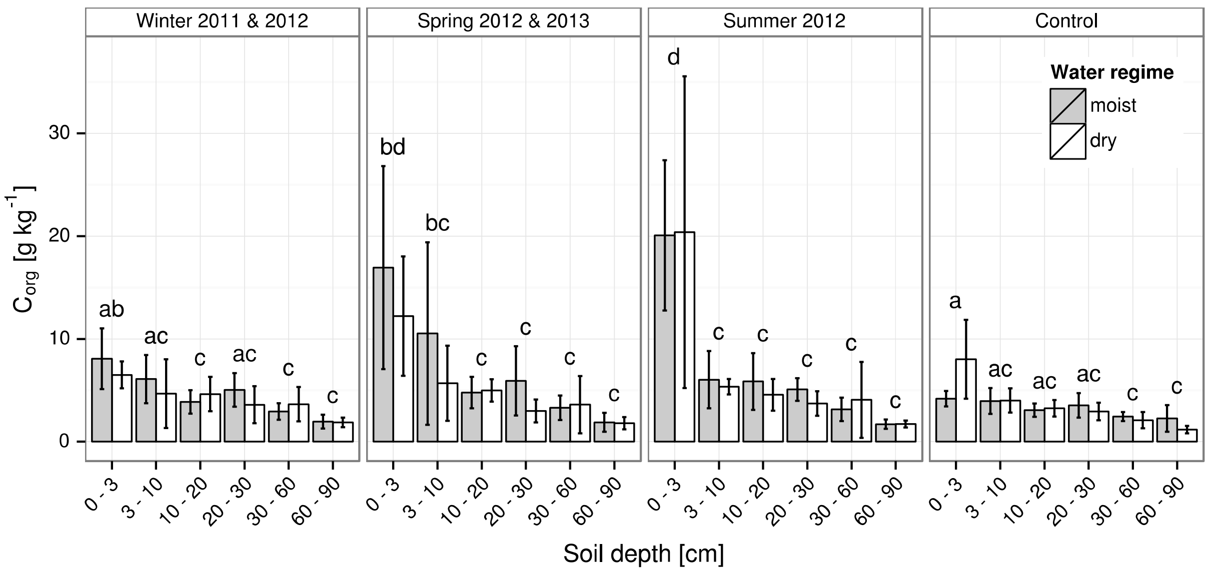

Fig. 3: Total organic carbon (C_{org}) at different depths given as mean and SD of four replicates. Different letters on top of the bars show significant relations (Wilcoxon, $p < 0.05$) between treatments and depths, regardless of the water regime.

OMW treatments significantly ($p < 0.002$) affected the content of SPC in the top soil layer (0 – 3 cm) when applied in spring and summer (Fig. 4). There, maximum SPC contents of approximately 90 mg CAE kg^{-1} were observed. In the upper soil layer, SPC contents of spring and summer treatments were approximately four times as high, compared to both control and winter treatments. Regarding spring and summer applications, the mean content of SPC in the

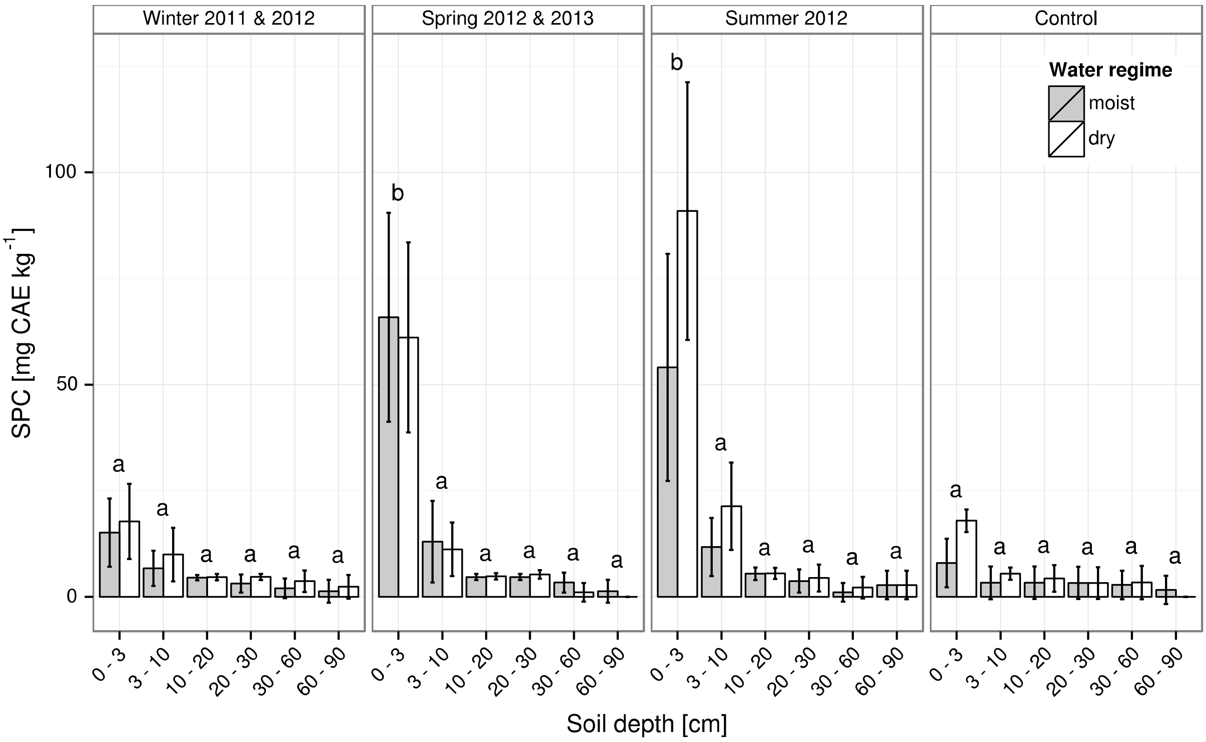

Fig. 4: Soluble phenolic compounds (SPC as mean ± SD of four replicates) at different depths given in caffeic acid equivalents (CAE). Values below LOQ were set to LOD, values below LOD were omitted. Different letters on top of the bars indicate significant relations (Wilcoxon, $p < 0.05$) between treatments and depths, regardless of the water regime.

upper soil layer was, as well, approximately four times higher compared to the subsequent soil layer (3 – 10 cm). No significant difference was identified between winter treatments and untreated soil (control). No significant relation was shown regarding the water regime either. Especially in deeper soil layers (10 – 90 cm) of the winter and control plots, SPC were often observed to be below LOQ or LOD.

3.4 Soil water repellency (SWR) and hydraulic conductivity

Across all treatments, WDPT raw data showed a highly inhomogeneous distribution pattern. The highest WDPTs with more than 2 400 s were observed on plots treated in summer. Mean WDPTs per every 40 cm are given in Fig. 5. The average WDPT on plots treated in winter was as low as on the control plots, both ranging from 0 to 28 s. Contrary to this, OMW applied in spring and summer significantly affected WDPT approximately 10 to 20 times more ($p < 2 \cdot 10^{-16}$). The mean categorized WDPT of spring treatments ranged from 96 to 222 s. On plots treated in summer, categorized WDPTs between 51 and 496 s were observed. No significant differences were identified between spring and summer treatments.

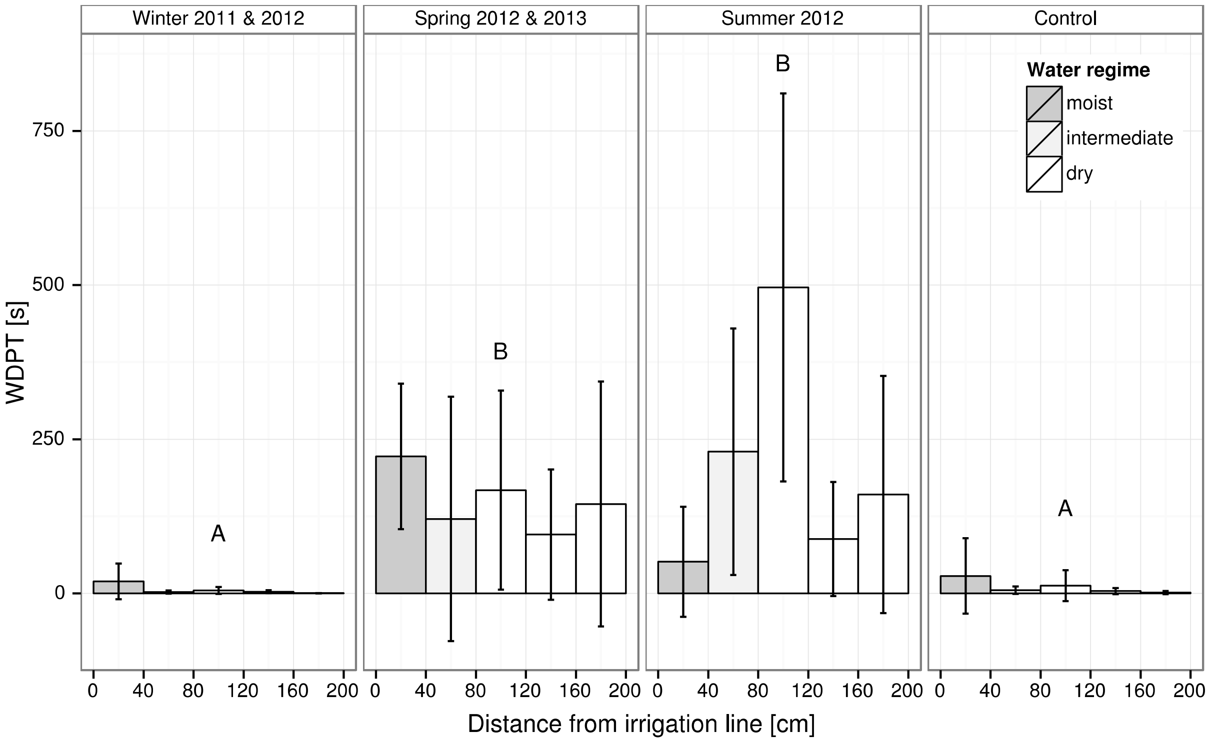

Fig. 5: Mean categorized in-field water drop penetration time (WDPT) of four replicates with respect to the distance from the irrigation line. Different capital letters on top of the bars express significant relations (Wilcoxon, p < 0.05) between treatments, regardless of the distance from the irrigation line or the prevailing water regime.

Preliminary results (q.v. appendix, Fig. III) showing mean categorized WDPT of four replicated plots where OMW was applied in winter 2012 & 2013 under comparable conditions (15 L OMW m^{-2} a^{-1}) ranged from 7 s to 277 s in the dry regime and averaged (150 ± 40) s in the moist regime. Accordingly, winter 2012 & 2013 treatments caused significantly ($p < 2 \cdot 10^{-16}$) higher WDPTs compared to OMW applications of winter 2011 & 2012 and control plots. OMW applied in winter 2012 & 2013 affected WDPT significantly ($p < 0.024$) less than spring treatments in the same years.

DCA results are given in Tab. 3 to complement WDPT data. Regardless of the water regime, the advancing contact angle Θ_{adv} of the summer and spring treatments was significantly higher ($p = 0.001$) compared to untreated soil (control). Plots treated in winter did not differ significantly from all other treatments. The prevailing water regime did not influence DCA.

Tab. 3: Advancing contact angle Θ_{adv} of the upper soil layer (0 – 3 cm) obtained by Wilhelmy plate method.

Treatment	**Winter** 2011 & 2012^{AB}		**Spring** 2012 & 2013^{B}		**Summer** 2012^{B}		**Control** ^{A}	
Water regime	moist	dry	moist	dry	moist	dry	moist	dry
Θ_{adv} [°]	60 ± 20	60 ± 20	70 ± 10	57 ± 4	60 ± 10	70 ± 10	43 ± 4	49 ± 2

Results are given as mean ± SD of four replicates. Different superscript letters mean significant relations (Wilcoxon, $p < 0.05$) between treatments, regardless of the water regime.

The saturated hydraulic conductivity K_s (Fig. 6) of the spring treatments was approximately 12 times lower ($p = 0.003$) compared to all other treatments. In the spring plots, K_s averaged (0.1 ± 0.2) cm h^{-1}. No significant relation was established among the winter, summer, and control plots. There, K_s means ranged from 0.9 to 2.4 cm h^{-1}. The prevailing water regime did not significantly affect K_s.

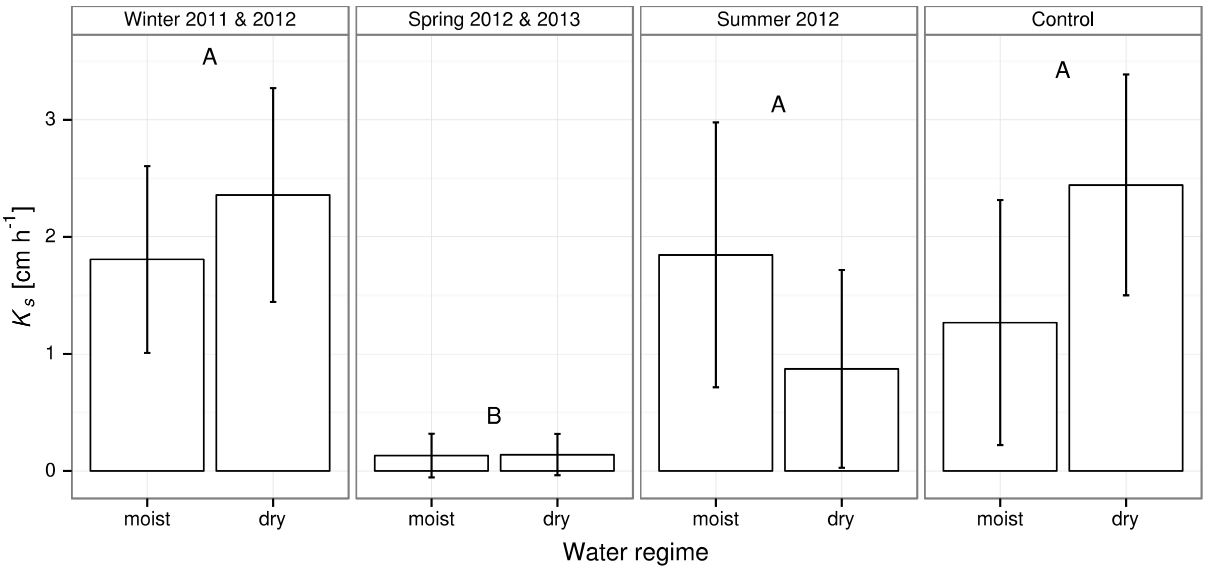

Fig. 6: Saturated hydraulic conductivity K_s estimated from manual infiltration measurements given as mean and SD of eight replicates with respect to the water regime. Different capital letters on top of the bars mean significant relations (Wilcoxon, $p < 0.05$) between treatments, regardless of the water regime.

3.5 Biological degradation performance

In the dry regimes of all plots practically no decomposition of bait material was observed (Fig. 7), except where *Prosopis farcta* (Banks & Sol.) J.F.Macbr. grew in close proximity to bait-lamina base groups. In the moist regimes, bait consumption ranged from zero to all (16) apertures. Median zero consumption was found in the moist regime of plots treated in

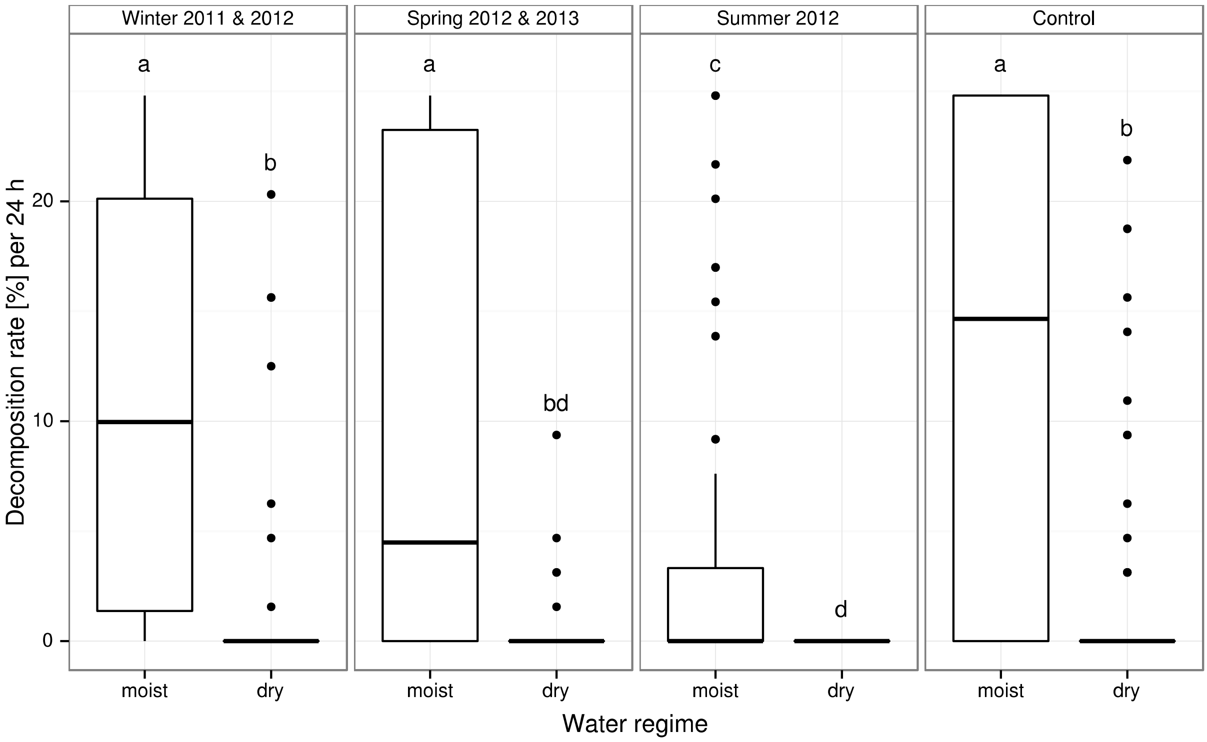

Fig. 7: Bait-lamina decomposition rates in the moist and dry regime of each plot given in percent of bait material consumed per 24 h. Each box shows the median and ranges from the 25 to the 75 percentile, the whisker length was set to 1.5 times the inter quartile range. Dots represent outliers. Letters indicate significant differences (Wilcoxon, $p < 0.05$) between the treatments and the water regime.

summer. The median decomposition rate in the moist regime of all other plots was approximately (10 ± 10) % per 24 h. A slight but insignificant trend was observed showing highest median bait consumption on untreated plots, following winter and spring treatments. Regardless of the water regime, OMW treatment in summer significantly ($p < 0.049$) affected degradation, being approximately three times lower compared to all other treatments.

3.6 Parameter interactions

The explanatory power of the first two principal components is 74.1 % of the total variation of the PCA depicted in Fig. 8. It is observed that winter and control treatments mostly cluster together in the first and second quadrant. In contrast, spring and summer treatments cluster together in the third and fourth quadrant. Biodegradation rates and all soluble ions except K^+ are organized in one group, where Na^+ and Cl^- mostly load together, indicating a significant positive correlation. The divalent cations Mg^{2+} and Ca^{2+} correlate as well. K^+ contents load together with C_{org}, SPC, WDPT, and Θ_{adv}. These parameters are negatively correlated with pH and K_s. K^+, C_{org}, SPC, WDPT, Θ_{adv}, pH, and K_s show a correlation close to zero compared to

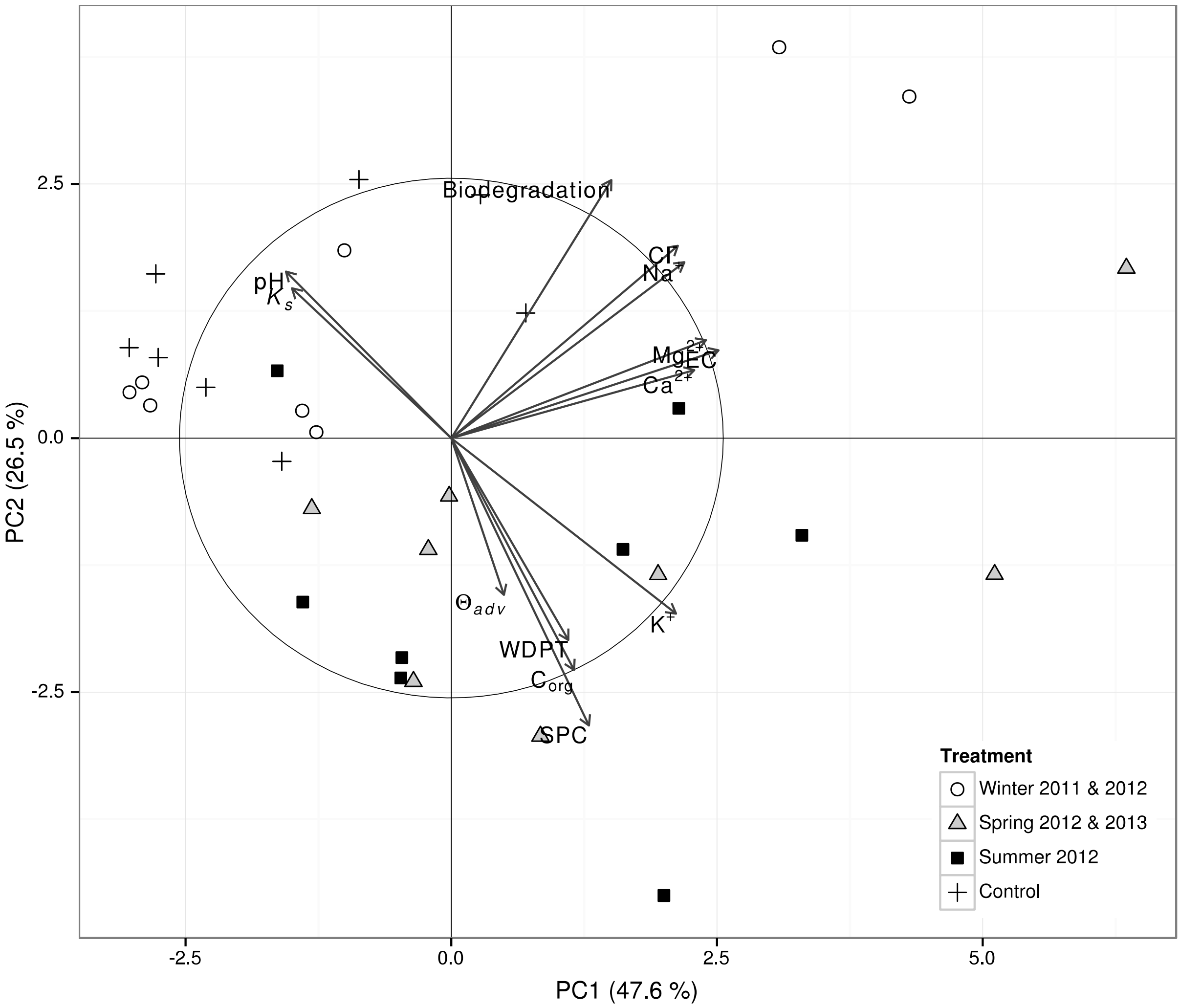

Fig. 8: Principal component analysis (PCA) based on chemical and biological parameters obtained from the upper soil layer (0 – 3 cm). Individual observations are displayed per treatment including both the moist and dry water regime. PC1 and PC2 are the first and the second principal components, respectively. The percentage of the total variance explained by principal components is given in parentheses. Parameter vectors going beyond the radius of the circle of equilibrium contribution can be interpreted with confidence (Borcard, 2011).

biodegradation, EC, Na^+, Cl^-, and divalent ions. The contribution of SPC to the soil parameters is found to be highest, followed by biodegradation and K^+. Contrary to this, Θ_{adv} has a comparably low influence. High values in WDPT, C_{org}, SPC, and K^+ are orientated towards spring and summer treatments. In contrast, winter and control plots are mostly described by high biodegradation, pH, and K_s. Observing soil parameters for summer treatments only (q.v. appendix, Fig. IV), SPC and biodegradation correlate negatively. For all other treatments, no significant correlation between SPC and biodegradation was established.

4 Discussion

4.1 Chemical soil properties

Applied OMW led to a slightly but significantly enhanced (ca. –0.4 pH units) soil acidity in the upper soil layer still observable after one rainy season following application. The presented distribution can be explained by bidirectional decationization. In the top soil, protons from OMW (pH 4.6) and natural weathering replaced soil cations. Due to irrigation and winter rain, these cations partly leached to subsequent soil layers (3 – 30 cm). In summer, soluble minerals from deeper soil layers (30 – 90 cm) were transported upwards due to evapo-transpiration. Natural weathering and acidic root exudates consequently caused a slightly enhanced soil acidity (Scheffer et al., 2010). Due to the proximity to the irrigation line, this process was more distinctive in the moist than in the dry regime. Under natural conditions, Dan et al. (1972b, cited from Singer, 2007) observed similar pH fluctuations. Seasonally different OMW applications did not significantly influence soil acidity. In the research area, a loessial alkaline silt loam dominates (Dan et al., 1972a) which is capable of buffering pH changes to a certain extent (Sierra et al., 2001). Severe soil acidification caused by repeated OMW application is therefore understood not to become a particular concern for soil ecology until carbonate buffers are exhausted. Piotrowska et al. (2011) and Magdich et al. (2013) observed an increasing acidity from pH 8.1 to 7.4 in alkaline sandy loam directly after application, whereas Pierantozzi et al. (2013) did not find any significant impact on the same soil type. Long-term effects of OMW on soil acidity of sandy to silty clay loam have not yet been demonstrated in related studies e.g. by Chartzoulakis et al. (2010) or Di Bene et al. (2013). Problems may arise in a long-term perspective. Acidification can become an issue even earlier when OMW is applied on humus-rich cambisols with a natural pH between 4 and 5.9 (Scheffer et al., 2010). In such cases, soil liming may be considered (e.g. Aktas et al., 2001) to avoid adverse effects on the edaphon as well as mobilization of heavy metals or aluminum.

The EC is proportional to the concentration of dissolved ions, particularly to sodium chloride. The interaction of various ions, their different solubility and molar conductivity in aqueous solutions interfere with $EC_{1:5}$ measurements (Liu et al., 2006). Due to these interferences, inconsistent data was obtained from $EC_{1:5}$ measurements. Nevertheless, high ion loads in the upper soil layers confirmed the significance of mineral input from OMW and irrigation water. In related studies, particularly high EC values in the top soil only persisted for two to four months if more than 16 L m^{-2} OMW were applied (Di Serio et al., 2008).

With an average K_{tot} content of (3 700 ± 400) mg L^{-1}, OMW is one of the main contributors to soil minerals. Due to its function as one of the plant's primary macronutrients, potassium is supposed to gradually decrease over time, serving as a mineral fertilizer (Arienzo et al., 2009). Therefore, soil K^+ significantly depends on the time elapsed since the last OMW application. But even 18 months, including one rainy season, as well as particularly high K requirements of *O. europaea* (Fernández-Escobar et al., 1999) were not able to bring upper soil K^+ loads back to normal conditions (viz. control plots). The K^+ surplus in plots repeatedly treated with OMW in winter was approximately 200 mg kg^{-1}. One single summer treatment resulted in a K^+ surplus of approximately 450 mg kg^{-1} after one rainy winter. Six months after application without any significant precipitation following, subsequent spring applications had almost the same impact (480 mg kg^{-1} K^+ surplus). The applied OMW consequently had the potential to alter the soil's potassium balance at least for one year, respectively one rainy season, after an application during the hot and dry season. Repeated spring and winter applications resulted in significant K^+ accumulations in the soil as well. This is in line with Chartzoulakis et al. (2010) who observed a surplus of approximately 600 mg kg^{-1} exchangeable potassium after two years of subsequent winter treatments (42 L m^{-2} a^{-1}). Six months after the application of 8 L OMW m^{-2} in spring, Di Bene et al. (2013) reported a surplus of approximately 80 mg kg^{-1} of exchangeable K. The K^+ content seems to be proportional to the applied OMW quantity. Surplus K^+ is susceptible to sorb to micaceous clay minerals (Arienzo et al., 2009). Nevertheless, subsequent OMW applications cause a higher K^+ input than output which improves long-term soil fertility on the one hand (Cucci et al., 2008). On the other hand, high quantities of exchangeable monovalent cations (Auerswald et al., 1996, Levy and Torrento, 1995) could decrease the aggregate stability and consequently enhance the soil erosion potential when replacing Ca^{2+} (Mekki et al., 2006b) in humus–clay bonds. Chartzoulakis et al. (2010) suggested a reduction in the application of potassium based fertilizers to olive orchards amended with OMW. A slight but insignificant K^+ leaching to the subsequent layer (3 – 10 cm) was observed. Regarding its low toxicity and high sorptivity to soil particles, K^+ leaching to deeper soil layers (> 30 cm) or groundwater is considered a negligible risk (Arienzo et al., 2009).

Mg and Ca serve as secondary macronutrients. Total Mg and Ca (< 210 mg L^{-1}) in the OMW applied were comparatively low (cf. Paredes et al., 1999). Slightly higher Mg^{2+} and Ca^{2+} contents in the soil of different seasonal OMW treatments indicated a small impact of OMW application on soil properties (cf. Mekki et al., 2006a). Pérez et al. (1986, cited from Paredes et al., 1999) showed that Mg from OMW can be less available to plants compared to natural soil-bound Mg. With respect to deeper soil layers, high Mg^{2+} and Ca^{2+} in the top soil may also be linked to the input of organic matter from leaf litter. Due to the identical distribution of Mg^{2+} and Ca^{2+} contents between treated and untreated soil, the presence of Mg and Ca in the deeper soil layers is assumed to be geogenic. Leaching of divalent cations is a natural process in forest and agricultural soils (Brady and Weil, 2008).

The high Na^{+} and Cl^{-} contents of the upper soil layers most likely originated from continuous irrigation. With a mean Na_{tot} content of approximately 440 mg L^{-1} and a Cl^{-} content of 1 200 mg L^{-1} OMW, the Na and Cl contribution of a maximum of two subsequent OMW applications had a rather small impact on soil mineral distribution. In comparison to that, the withdrawn irrigation water had an EC varying between 0.4 and 0.7 mS cm^{-1} which is equivalent to approximately 400 mg L^{-1} (cf. Hanson et al., 2006) of total dissolved salts. During the dry season, all plots were irrigated twice a week. Evapotranspiration consequently led to an accumulation of ions from irrigation water in the soil (Hanson et al., 2006). This is why high Na^{+} and Cl^{-} contents were only observed in the moist regimes of all plots, viz. near the irrigation line. No significant differences in Na^{+} and Cl^{-} distribution were found (cf. Chartzoulakis et al., 2010) between the different seasonal OMW treatments. Due to precipitation in winter, leaching of soluble minerals may be possible (Melgar et al., 2009). Particularly high Na^{+} and Cl^{-} contents in the deeper soil layers (30 – 90 cm) are assumed not to be caused by OMW constituents because control plots were comparably affected. Yet, no such accumulative effect has been observed for repeated OMW applications (Moraetis et al., 2011) but due to continuous irrigation in arid regions. Groundwater salinization may arise in a long-term perspective (Rebhun, 2004). Mekki et al. (2006b) preventively suggested not to exceed an application amount of 5 L OMW m^{-2} a^{-1} to avoid soil salinization.

After one rainy season following the last application, summer treatments showed significantly higher C_{org} contents compared to repeated winter applications or control plots. Without any significant rain following the last application, the C_{org} of spring treatments had an intermediate magnitude compared to winter and summer treatments. The organic fraction of OMW (ca. 35 g L^{-1}) is often hydrophobic (Mahmoud et al., 2010) so that it likely remains in the top soil. Under suitable environmental conditions, easily degradable carbon compounds are mineralized by microorganisms directly after application. This mineralization increases soil fertility and may positively affect soil stability (Magdich et al., 2013, Mekki et al., 2006a). Due to significantly reduced biological activity, the microbial mineralization process may have been inhibited in plots treated in summer. There, C_{org} is likely to consist of more stable and hydrophobic OM compounds which enhanced SWR as estimated by WDPT. With respect to the control plots, the depth profiles of plots treated in spring indicated that oily OMW constituents infiltrated to the subsequent (3 – 10 cm) soil layer. In plots treated in winter, potentially leached OM was likely to have been already degraded. Although highest C_{org} quantities were observed in the top soil of summer treatments, no such leaching effect was found. This may be the reason why K_s was less affected by OMW components compared to WDPT. Slightly but insignificantly higher C_{org} contents in the moist regime than in the dry regime may be attributable to the input of OM from trees and microbial biomass near the irrigation line. This is in line with the results obtained from WDPT and biological degradation measurements. In comparison to that, Chartzoulakis et al. (2010) and Di Bene et al. (2013) did not find any significant increase or accumulation of soil OM six months after subsequent OMW applications in spring and winter (8 – 42 L m^{-2} a^{-1}).

The impact of OMW on soil SPC was comparable to the seasonally different C_{org} distribution previously discussed. SPC in plots treated in summer remained elevated for at least one rainy season following application. Winter rain was not able to significantly diminish SPC. In contrast, after one rainy season following two repeated winter (2011 & 2012) applications, SPC contents decreased almost to those levels found in the control plots. Accordingly, Di Bene et al. (2013), Chartzoulakis et al. (2010), and Saadi et al. (2007) observed phenolic compounds degrading less than six months after applications of 8 – 42 L OMW m^{-2} in spring or winter. It can be interpreted that SPC were able to become persistent due to the prevailing environmental conditions. Soil temperature and soil moisture, in particular, exert considerable influence on OMW degradation (Sierra et al., 2007). Saadi et al. (2013) even assumed that the decomposition of phenolic compounds was regulated to a greater extent by soil temperature than by soil moisture as long as the moisture content does not fall below 20 % of the field water capacity. Despite periodical irrigation, soil moisture was possibly not sufficient to maintain biological degradation following OMW application in summer although soil temperatures were adequate. This is in line with the performed PCA which revealed that the biodegradation performance was largely independent of the SPC content when OMW was applied in spring or winter. Only for summer treatments was a negative correlation established. Regarding biological degradation, SPC were therefore assumed to be more easily degraded after an OMW application under moist and temperate soil conditions in spring or winter, compared to under hot and dry soil conditions in summer. Furthermore, SPC were found confined to the upper soil (0 – 10 cm), not significantly leaching or infiltrating into deeper layers (10 – 90 cm) where SPC were mostly found below LOQ. Although many phenolic derivatives show moderate to good solubility in water (Góral et al., 2011), these compounds were less mobile than assumed. Similar effects of phenol fate and transport in OMW-treated soils were shown by Chartzoulakis et al. (2010). Phenolic compounds tend to bind to other organic particles from OMW or be built into humins. In soil, these agglomerates tend to remain on the topsoil where they can form a visible crust. These phenolic humins can develop comparable chemical properties like humic acids and enhance SWR (Cox et al., 1997, Mahmoud et al., 2010). Contrary to this, Mekki et al. (2007) detected a new phenolic fraction at 1.2 m soil depth four months after OMW application to a sandy soil using high performance liquid chromatography. This indicates that SPC are not necessarily totally degraded or bound, but biochemically converted to other substances which may no longer be detectable using Folin–Ciocalteu reagent. According to the aforementioned authors, this new fraction was less phytotoxic compared to the phenolic compounds originally found in OMW. No information was provided concerning the molecular structure or other chemical properties. Such new substances are potentially less biodegradable (Ramos-Cormenzana et al., 1996). C_{org} measurements emphasized that OM residues are still found one rainy season after OMW treatment. It remains unknown whether a certain C_{org} proportion consists of transformed phenolic compounds. Due to the prevailing soil texture (silt loam) and the absence of detectable leaching paths of SPC between 10 – 90 cm, it is rather unlikely, but possible (Sierra et al., 2001), that SPC itself leached into deeper soil layers. However, the depth distribution of C_{org} may provide a certain indication that potential decomposition products have leached to the subsequent soil layers. In that case, a potential groundwater contamination (Spandre and Dellomonaco, 1996) may represent a serious environmental threat.

4.2 Soil hydrology

Due to a naturally uneven soil surface considerably influenced by agricultural vehicles, applied OMW ran off small soil heaps and accumulated in hollows. Consequently, OMW did not evenly settle after application which resulted in small-scale differences in the spatial distribution of OMW containing hydrophobic C_{org} (Mahmoud et al., 2010). When categorized into groups of 40 cm distance, a distinct medium-scale distribution pattern was observed. In the shady and moist regime, the degradation and metabolism of hydrophobic OMW constituents were mainly determined by the activity of soil fauna (cf. Abid and Sayadi, 2006).

Due to the absence of significant biological degradation processes in the dry regime together with hot and sunny conditions, repellent compounds like carboxylic acids may have been more susceptible to physically decompose (Hachicha et al., 2009), e.g. by heat or UV irradiation. With regard to WDPT, the biological degradation of hydrophobic compounds seems to be less effective compared to physical decomposition. In the intermediate region between the defined water regimes, tractor tracks compacted the soil inducing applied OMW to accumulate and enhance WDPT. Regardless of the distance from the irrigation line, SWR obtained from DCA and in-field WDPT measurements had the same magnitude at spring and summer treatments where C_{org} contents were still observed to be elevated. 215 mm of total precipitation (MOAG, 2012) during the following rainy season could not significantly diminish the hydrophobic C_{org} causing high SWR on plots treated in summer. Particularly lower C_{org} and WDPTs at winter 2011 & 2012 treatments emphasized that OMW applied in two subsequent winters did not persist for more than 18 months, respectively for one rainy season following the last application. One single application in summer was consequently more persistent than two subsequent winter treatments. This was most likely caused by inhibited biodegradation. Furthermore, preliminary results (q.v. appendix, Fig. III) indicated that subsequent winter applications (2012 & 2013) were slightly less persistent compared to subsequent spring (2012 & 2013) applications. It was not possible to resolve whether this different impact was attributed to seasonality or simply to the time elapsed since the last application. Mahmoud et al. (2010) found mean WDPTs of around 25 s after five years of continuous winter application, directly correlating with soil OM loads. Although feared by González-Vila et al. (1995), a severe long-term impact of OMW on SWR is assumed to be rather unlikely if summer applications are avoided.

Subsequent spring treatments (2012 & 2013) resulted in a significantly lower saturated hydraulic conductivity K_s six months after OMW application, compared to control plots. OMW applications executed more than 12 months previously (summer and winter) did not significantly affect K_s. One rainy season following OMW application may therefore already be sufficient to recover K_s. In contrast to chemical soil properties and WDPT, this indicates that OMW does not persistently deteriorate the water infiltration capability of subsurface soil when OMW is applied in winter or summer. High C_{org} and K contents potentially inducing soil aggregate alteration were shown to be confined to the upper soil layer where they tended to affected WDPT rather than K_s (Mahmoud et al., 2010). Abu-Zreig and Al-Widyan (2002) as well as Pagliai et al. (2001) showed a slight increase of K_s directly and three months after a

16 L m^{-2} OMW application. By contrast, Mahmoud et al., (2010) reported a significant K_s reduction of approximately 18 % five years after subsequent applications of varying (sic) OMW amounts. On plots treated in winter, easy infiltration may have favored leaching of soluble compounds towards groundwater. However, the depth profile depicting selected soluble ions, C_{org}, and SPC did not significantly indicate any OMW induced leaching paths and/or accumulation processes.

4.3 Edaphic ecology

There is definite evidence showing a strong, persistently negative impact of a single OMW application during the hot and dry season (summer 2012) on the biological degradation processes in the soil. Even the latest and repeated spring application (2012 & 2013) did not lead to such an alarming decrease in edaphic biodegradation. This deviates from the physical and chemical soil properties discussed before (q.v. ch. 4.1, p. 17). Although soluble ions, SPC, C_{org}, WDPT, and K_s showed almost equal magnitudes in plots treated in summer and spring, degradation performance was significantly reduced for summer treatments only. This resulted in a negative correlation of SPC and WDPT with biological degradation for plots treated in summer. For winter and spring treatments biological degradation was largely independent of the SPC content and WDPT as shown by PCA.

Under comparable climatic conditions, OM decomposition mainly depends on the abundance and activity of soil microorganisms, viz. fungi, bacteria, and microflora. The particularly high content of toxic agents like monomeric phenolic compounds and carboxylic acids (Fiorentino et al., 2003), together with seasonally hot soil conditions may have built up a lethal combination of environmental stressors which led to a collapse of soil biota communities. Karpouzas et al. (2010) also hypothesized that altered soil OM and reductive soil conditions following OMW application negatively affected soil biota. All this may have outweighed a potentially beneficial effect of easily degradable OM on soil microorganisms directly after OMW application that was visible in the decrease of C_{org} in plots treated in spring and winter. Therefore, the impact of OMW on the edaphon is assumed to be strongly dependent on the season in which OMW is applied. Beyond that, it is unclear whether only dry soil conditions inhibit OM degradation by soil biota due to insufficient irrigation (Saadi et al., 2013), or whether artificial irrigation during summer even has a stress enhancing influence on soil biota due to elevated salt loads (cf. Mekki et al., 2006b). After 12 months, respectively one rainy season, no significant sign of soil biota recovery was apparent in plots treated in summer. Consequently, further degradation of OMW components was lastingly inhibited. In contrast, the edaphon apparently completely recovered from repeated OMW applications in spring or winter. This indicates that the natural attenuation mechanisms of the soil ecosystem remain intact when OMW is applied in appropriate doses and under suitable environmental conditions.

Bibliographic data regarding the state of edaphic ecology after OMW application is contradictory. The ecological response to OMW application is highly dependent on the observed taxa. Many authors reported increases in microorganism abundances such as acidophilic and spore-forming bacteria (Karpouzas et al., 2010, Paredes et al., 1987), yeasts and actinobacteria (El Hassani et al., 2010), as well as soil microflora (Mekki et al., 2009) during a short period (< 3 months) after OMW application. Saadi et al. (2007), Di Serio et al. (2008), and Di Bene et al. (2013) obtained comparable results from total microbial counts and microbial biomasses. While Mekki et al. (2009) observed a constantly low soil respiration following a 10 L m^{-2} OMW treatment, Di Serio et al. (2008) showed an increase of soil respiration two months after application of 8 and 16 L OMW m^{-2}. Long-term studies are rare. According to Mechri et al. (2008), OMW application generally affected bacteria abundances more than fungi abundances. This is probably why Di Bene et al. (2013) did not show any severe impact on fungal root colonization six months after a single spring and autumn OMW applications (8 L m^{-2} respectively). Soil respiration was not negatively affected in a long-term perspective. In contrast, Mekki et al. (2006b) showed a negative impact of OMW (10 L m^{-2}) on the colony formation of microflora, yeasts and moulds, actinobacteria, and nitrifiers persisting at least for six months. Negative long-term effects on soil organisms have only been observed when OMW was applied in high doses of more than 8 L m^{-2} (El Hassani et al., 2010).

4.4 Limitations of the study

Due to a total quantity of 192 soil samples as well as cost containments and time limitations, process analytical measurements were not replicated. It was assumed that the variance among the four-fold field replications of different OMW treatments was higher compared to process replications. It was consequently not completely feasible to evaluate possible errors in measurement and the accuracy of the methods used. Furthermore, irregularities occurred with regard to the most recent application period in 2013: OMW was repeatedly applied on spring plots instead of on winter plots. Due to the different times elapsed and the different number of rainy seasons following the last application, spring treatments had to be carefully interpreted when comparing their impact to summer and winter treatments.

5 Conclusion

The seasonally prevailing environmental conditions during OMW application significantly influenced the effects of OMW components on soil hydrological and physicochemical properties such as WDPT, soil acidity, soluble K, OM, and phenolic compounds. With regard to the control, these parameters were persistently elevated by two to ten orders of magnitude on plots where OMW was applied in summer. The bait-lamina test revealed a three-fold decrease in the biodegradation performance following summer application. Contrary to that, adverse effects on soil physical chemistry and ecology were demonstrated to be significantly reduced after one rainy season following winter application. This demonstrated the natural recovery potential of the soil when OMW is applied under temperate environmental conditions. It was not totally resolved for how many rainy seasons subsequent summer and spring applications would maximally persist. According to current information, summer applications are not recommended. The results obtained give rise to the assumption that the environmental impact of spring applications is comparable with that of winter treatments. Further research is needed here to draw distinct conclusions. So far, farmers have mostly applied OMW in autumn or winter in any case, in order to avoid costly storage until the coming application period. Significant leaching or accumulation of problematic substances such as phenolic compounds was not observed. However, further research on the fate and effect of phenolic compounds and their decomposition products in the soil is recommended due to their toxicity and potential water solubility. Furthermore, the effects of OMW containing carboxylic acids on soil properties are still unknown and possibly underestimated. Thermogravimetric analysis could help to thoroughly assess the impact of OMW on the composition of soil OM.

With regard to the results obtained in this study, direct OMW application to soil is considered acceptable under controlled conditions. This implies that OMW is not applied during hot and dry months (summer) and that certain threshold amounts are respected. In accordance with other authors (e.g. Mekki et al., 2006b) as well as the Italian law no. 574 (1996), maximum application amounts of 5 – 8 L OMW m^{-2} a^{-1} are recommended. These quantities ensure sufficient potassium and organic matter input for fertilizing purposes while lowering the risk of phenol contamination and soil water repellency. Dependent on the available infrastructure, cost-effective OMW pretreatments from simple sealed evaporation ponds and liming (Aktas et al., 2001) to more elaborated oxidation processes using ferric oxide (Rivas et al., 2001) can be taken into account.

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Appendix

The supporting material can be found on the enclosed CD-ROM or at the following link http://tinyurl.com/BSc-SOM-ZS. It contains in detail:

- Bachelor thesis (Portable Document Format)
- Laboratory protocol for Folin–Ciocalteu total phenol determination (OpenDocument text)
- Raw data and summarized results
  - Complete sample list with all measured values and experiments (OpenDocument spreadsheets)
  - Climate data (OpenDocument spreadsheet)
- Main statistical analysis (R Code)
- Full bibliographic data (BibTeX file)

Download links of the software used:

- The R project (version 3.0.2) for statistical computing (CRAN): http://www.r-project.org/
- LibreOffice Suite (version 4.1.3): http://www.libreoffice.org/

On the following pages VIII to XI, selected figures of the study area and preliminary results are depicted.

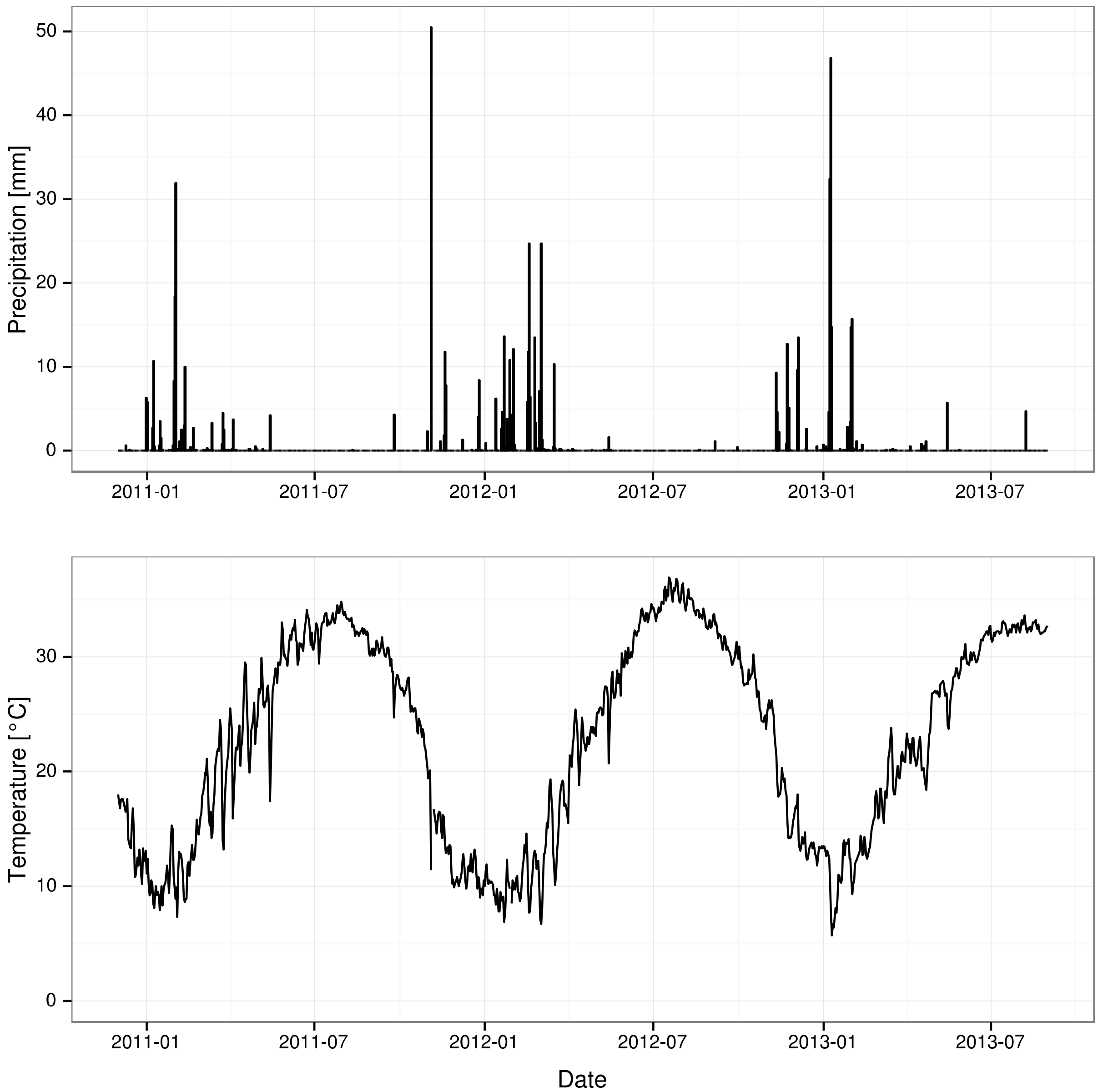

Fig. I: Daily precipitation and temperature 5 cm above ground level at Gilat research station, recorded from December 2010 to August 2013 (MOAG, 2012).

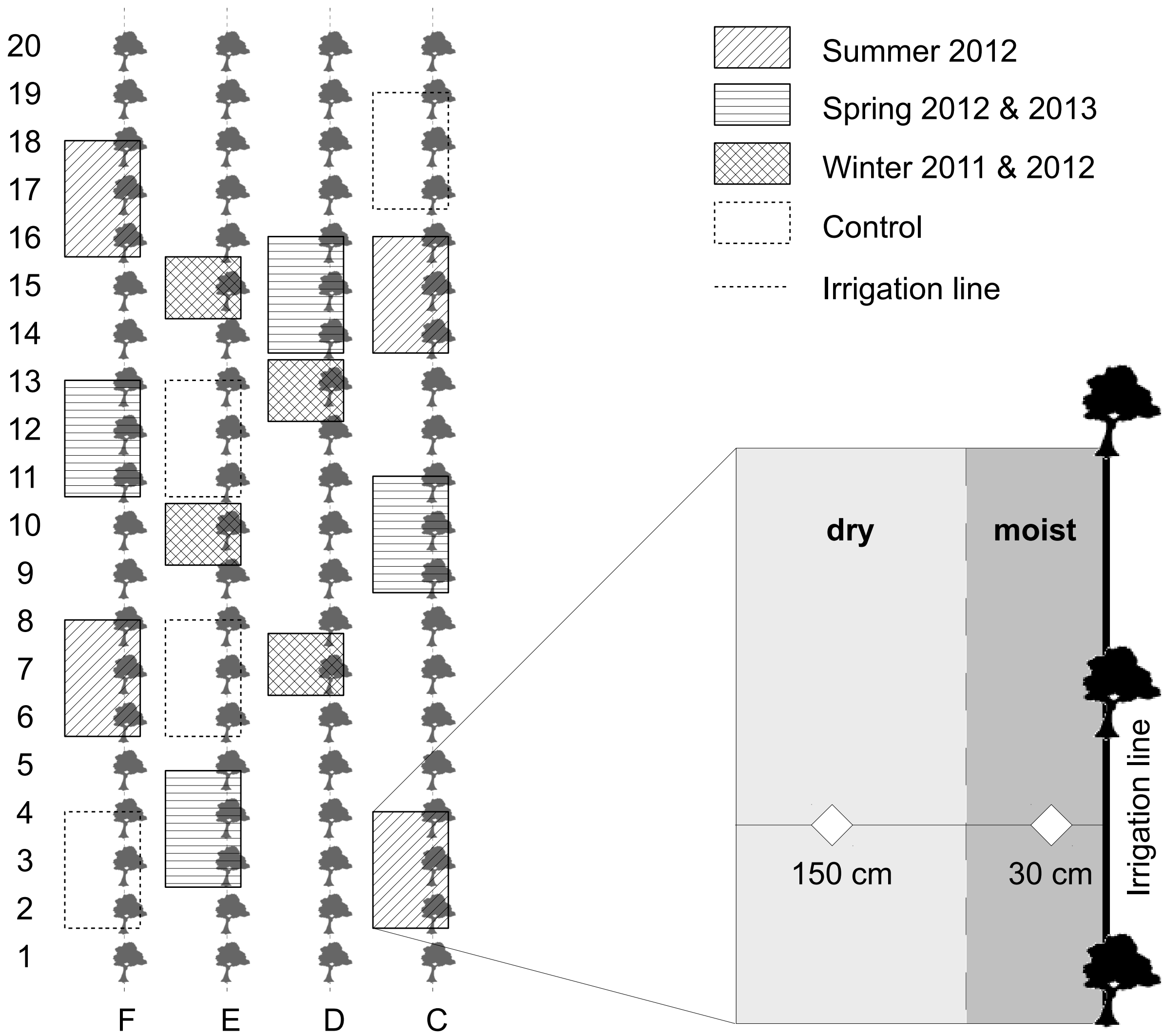

Fig. II: Study area and experimental design. The orchard measures approximately 14 000 m^2. The distance between the tree lines (capital letters, border tree lines A, B, G, and H not depicted) measures 7 m. Within each line, the trees stand 3.5 m apart from each other. These rows are consecutively numbered. Winter plots measure 2 m × 2 m edge length. Summer, spring, and control plots measure 2 m × 4 m edge length. ◇ = sampling positions in each plot, with respect to the distance from the irrigation line.

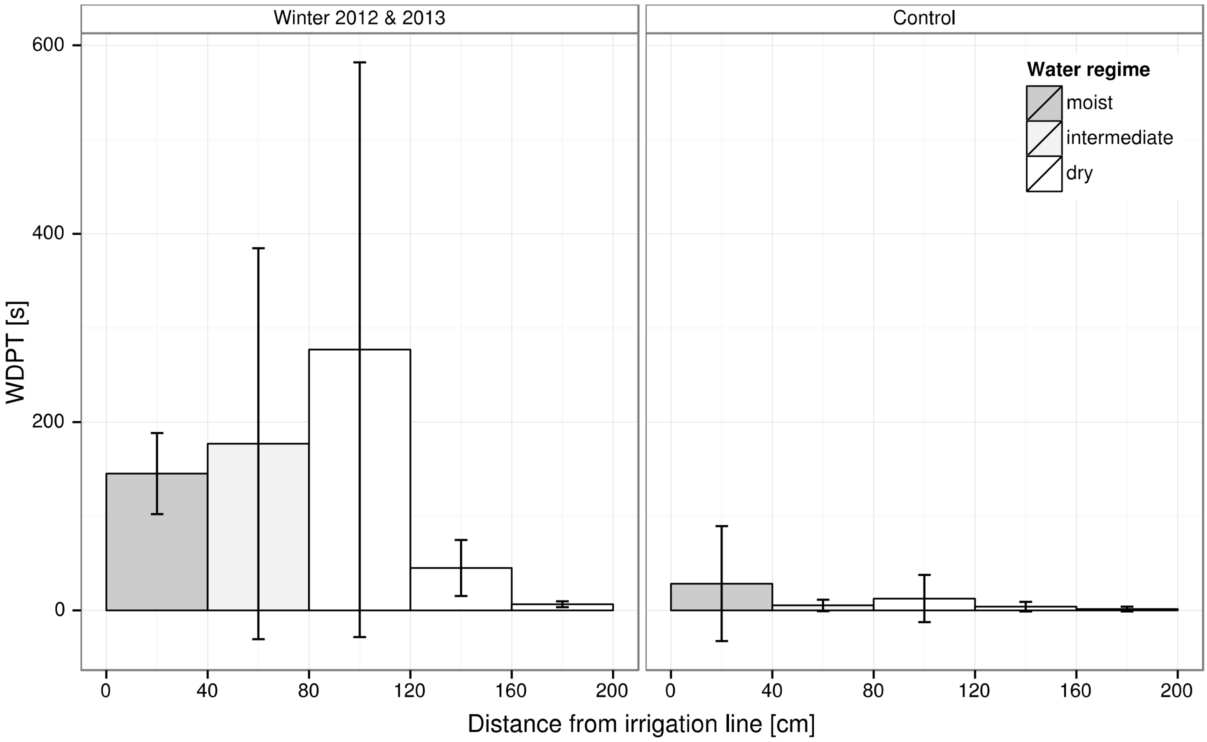

Fig. III: Mean in-field WDPT of four replicates with respect to the distance from the irrigation line, categorized into distances of 40 cm. Preliminary results from plots of an adjacent orchard treated with 15 L m^{-2} OMW in winter 2012 & 2013, respectively.

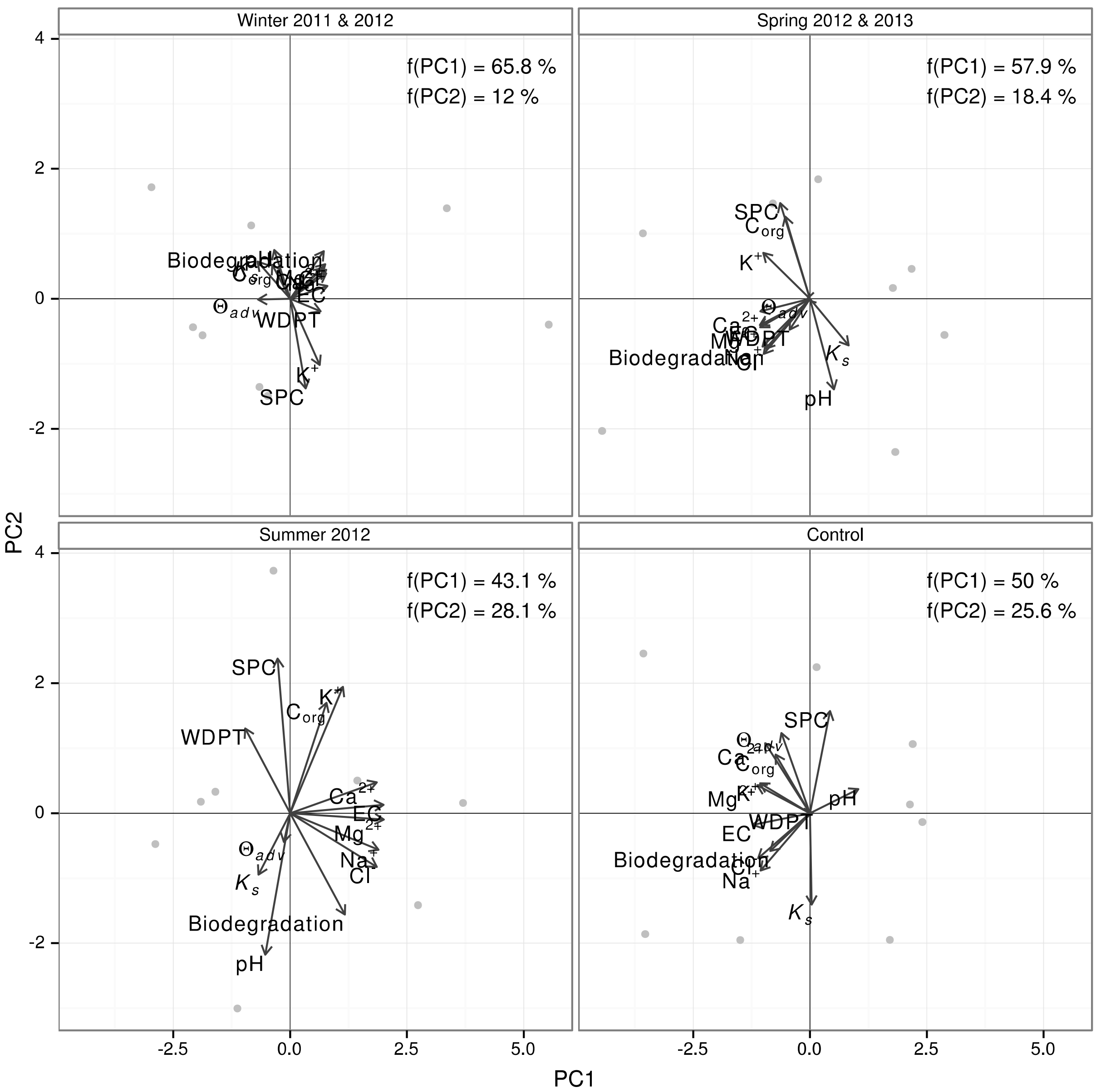

Fig. IV: Principal component analysis (PCA) per treatment based on hydrological, physico-chemical, and biological parameters from the upper soil layer (0 – 3 cm). Observations include both the moist and dry water regime, except biodegradation where only data from the moist regime was used. PC1 and PC2 are the first and the second principal components, respectively. F is the percentage of the total variance explained by each principal component.

Affidavit

Hereby, I affirm that this bachelor thesis, entitled “Persistence of chemical and biological effects of olive mill wastewater seasonally applied to loessial olive orchard soil“, has been independently written without additional means. I declare that no information and data were used which are not stated in this thesis. Furthermore, I confirm that this thesis has not been submitted to any other examination board.

Landau, November 29, 2013