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Toward Balancing the Pros and Cons of Spreading Olive Mill Wastewater in Irrigated Olive Orchards

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Citation: Kurtz, M.P.; Dag, A.; Zipori, I.; Laor, Y.; Buchmann, C.; Saadi, I.; Medina, S.; Raviv, M.; Zchori-Fein, E.; Schaumann, G.E.; et al. Toward Balancing the Pros and Cons of Spreading Olive Mill Wastewater in Irrigated Olive Orchards. *Processes* **2021**, *9*, 780. <https://doi.org/10.3390/pr9050780>

Academic Editors: Sebastián Sánchez Villascclaras and Juan Francisco García Martín

Received: 31 March 2021

Accepted: 25 April 2021

Published: 28 April 2021

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Abstract: The controlled application of olive mill wastewater (OMW) as a by-product of the olive oil extraction process is widespread in olive oil-producing countries. Therefore, a sustainable approach necessarily targets the positive effects of soil resilience between successive annual applications to exclude possible accumulations of negative consequences. To investigate this, we applied 50, 100, 100 with tillage and 150 m³ OMW ha⁻¹ y⁻¹ for five consecutive seasons to an olive orchard in a semi-arid region and monitored various soil physicochemical and biological properties. OMW increased soil water content with concentration of total phenols, cations, and anions as well as various biological and soil organic matter indices. Soil hydrophobicity, as measured by water drop penetration time (WDPT), was found to be predominantly in the uppermost layer (0–3 and 3–10 cm). OMW positively affected soil biology, increased the activity and abundance of soil arthropods, and served as a food source for bacteria and fungi. Subsequent shallow tillage reduced the extent of OMW-induced changes and could provide a simple means of OMW dilution and effect minimization. Despite potentially higher leaching risks, an OMW dose of 50–100 m³ ha⁻¹ applied every two years followed by tillage could be a cost-effective and feasible strategy for OMW recycling.

Keywords: olive mill wastewater; phenolic compounds; bait-lamina; Collembola; biodegradation; water re-use

1. Introduction

Olive mill wastewater (OMW) is the liquid by-product obtained from three-phase (solid, aqueous, oil) olive oil extraction systems. Up to 30 million m³ OMW is generated annually in the Mediterranean region [1]. OMW consists of vegetation water, tissues of olive fruits, and process water used in different stages of the extraction. Therefore, it has a high biological and chemical oxygen demand, high concentrations of soluble phenolic compounds (Total Phenols; TP), oil residues, as well as the residual solid content (Total Suspended Solids) and, therefore, it cannot be processed by conventional sewage treatment plants [2].

Numerous studies advised the controlled spreading of OMW on cultivated soils as a viable recycling approach. It was further suggested that OMW could be considered as a useful, low-cost soil amendment and fertilizer [2]. Specially, in regions having low

soil organic matter (SOM) content and consequently low soil fertility, OMW can serve as a readily available organic matter (OM) source. Annual allowed application rates vary in different countries with, for example, $50 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ for Italy and Israel [3,4] and $30 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ for Catalonia [5]. These recommendations were formulated to avoid issues associated with discharging OMW into the public sewage network, from sealed pipes to the collapse of pumping stations and sewage treatment plants. To overcome such negative effects, different approaches have been considered including engineered-oriented technologies (flotation, anaerobic digestion) or recycling (co-composting the OMW with other organic solid wastes) [6]. None has been applied on a large scale, as these approaches failed to overcome the existing challenges due to high costs for infrastructure, instruments, chemicals and transportation, low efficiency, low intake capacities, or the final treated wastewater did not fulfill the respective water regulations (e.g., [2])

OMW spreading in olive orchards has the potential to be a cost-effective recycling solution, since soil plays a key role for the transport and biodegradation of pollutants by filtering, storing, and transforming OMW constituents. From an economic and logistic point of view, on-site application of OMW near mills directly after its generation from October to January would be preferred. However, an optimal dose and application practice should be based on local variables related to climatic conditions, soil properties, and crop management [7,8]. Assuming a linear relationship between the magnitude of OMW-induced changes and OMW dose would be an oversimplification and has been disproved for processes such as phenolic compound degradation [5] or degree of weed germination inhibition [9,10]. Recently, the practice of shallow tillage following OMW application was considered by Levy et al. [11] and Zipori et al. [12] with the purpose of avoiding possible negative effects on soil physical and hydraulic properties. Evidently, reservations about tillage are based on its possible drawbacks such as a disturbed faunal community, disrupted soil aggregates, and reduced nutrients and soil organic carbon [13].

Phytotoxicity induced by the accumulation of phenolic substances [9], soil hydrophobicity [14], salinization, and acidification [15] are prominent adverse effects caused by OMW application. Still, only a few studies trace such effects with a particular focus on small-scale spatial resolutions. Recent findings from irrigated olive orchards in the semi-arid region of Israel suggest that such adverse effects may be overlooked unless the soil profile is analyzed at a high resolution. For example, Peikert et al. [14] found persistent hydrophobicity, higher electrical conductivity (EC), and sorption capacity toward agrochemicals, mainly in the very topsoil layer (0–3 cm). Steinmetz et al. [16] identified hydrophobicity and adverse effects toward soil biology exclusively in the upper soil layers (0–3 cm). Moreover, OMW toxicity toward soil biota is poorly understood. In ecotoxicological assessments (OMW from an evaporation pond, [17]) and short-term field studies ($150 \text{ m}^3 \text{ OMW ha}^{-1}$, [8]), OMW was pointed out to stress soil biota. Due to the degradation of OMW-derived phenolic compounds, it is likely that adverse effects disappear with time, as shown for recurring soil microbiological activity [18] or seed germination [9,19]. Therefore, the aims of this study were (i) to characterize soil alterations at increased resolution across soil profiles by assessing OMW-induced effects on soil surface hydrophobicity and other physicochemical properties, at annual doses in a range somewhat above what is considered to be practical; and (ii) to assess residual effects of OMW application on soil biota and microbiota as sensitive indicators to soil resilience between successive annual applications. For this, we hypothesized the following:

1. A relatively low OMW dose in the range of $50 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ balances the low degradation rates of OMW organic residues expected during the cold and wet winter season, and the soil can recover between consecutive winter applications;
2. Toxic effects of OMW toward soil biota will disappear between successive winter applications; and
3. Soil tillage following OMW application will enhance soil biodegradation rates of OMW constituents and therefore reduce the negative effects of OMW on soil physicochemistry and biology.

To test our hypotheses, we conducted a five-year field study at an olive orchard (cv. 'Leccino') on Loess soil in a semi-arid region and analyzed at increased resolution the spatio-temporal effects of OMW before and after successive annual winter applications. Specifically, we assessed soil surface hydrophobicity, analyzed physicochemical properties in aqueous extracts of soil samples from five depths, determined the amount and thermal stability of soil organic matter, and as biological parameters, bait lamina consumption and abundance of soil invertebrates using pitfalls were studied to detect OMW-derived toxic effects.

2. Materials and Methods

2.1. Study Area and Sampling Design

The field study was conducted between the years 2012 and 2016 at the Gilat Research Center of the Israeli Agricultural Research Organization, Northern Negev (31°20' N, 34°40' E). The orchard (cv. 'Leccino'; 7-year-old) is typical for intensive olive cultivation featuring a high mature tree density (450 trees ha⁻¹). The row spacing in this rectangular orchard is 7 m, while it is 3.5 m for the tree spacing in the row. No fertilizers were applied during the 5 years of the OMW application; irrigation was generally performed from March to October with a Kc of 0.55 relative to Penman ET₀, resulting in an average annual amount of 650 mm.

OMW was applied to the soil using a spreading tank at different annual doses of 0 (control), 50, 100, and 150 m³ ha⁻¹ y⁻¹. Additionally, a fifth treatment included an annual application of 100 m³ ha⁻¹ followed by a shallow tillage to a depth of 5 cm, using a hand rototiller, three weeks after OMW application after the upper layer was dried. OMW was applied shortly after the end of the olive milling season (January–February). All five treatments were designed in five replicates in a randomized block design (a total of 25 plots; more details in [11]).

Soil samples were taken at a depth of 0–10 cm twice a year, 1–2 months before OMW application (autumn) and 1–2 months after application (spring). A more detailed field survey ("detailed survey") was conducted in January 2014 (shortly before the winter application of 2014) and included soil samples from 0–3, 3–10, 10–20, 20–40, and 40–60 cm depth.

Three unified soil samples were taken from each plot at a perpendicular distance of 1.5 m from the drip irrigation line (at an area that does not receive irrigation water), which is located in the center between two trees. The detailed survey of January 2014 was conducted at a distance of 80 cm from the drip line (also at an area that does not receive irrigation water). This distance was chosen based on soil hydrophobicity.

2.2. Soil and OMW Analyses

Soil water content was determined gravimetrically at 105 °C according to ISO 11456 [18]. Loss on ignition (LOI₅₅₀) was determined by igniting the sample to 550 °C (>4 h). Additionally, soil samples were air-dried, gently manually ground in a mortar to destroy larger aggregates, and sieved (<2 mm). A representative sample of the control plots (no OMW) was used to measure soil texture, bulk density, and effective cation exchange capacity [19]. Soil pH and EC from all treatments were measured in a 1:5 aqueous suspension of soil and water (shaken for 24 h and filtered through 0.45 µm filter) [19]. From these extracts, TP was determined after Box (1983) using the Folin–Ciocalteu reagent (Sigma-Aldrich, Germany) (cf. [20]) and caffeic acid as a reference (gallic acid for the detailed survey using a caffeic acid–gallic acid conversion factor of 1.2). Dissolved organic carbon (DOC) was measured using a multi N/C 2100 analyzer (Analytik Jena, Germany). Ultraviolet absorbance (UVA) at 254 nm was measured and used to calculate the specific ultraviolet absorbance, SUVA (=UVA/DOC × 100; L mg⁻¹ m⁻¹). Soluble ion contents, F⁻, Cl⁻, NO₂⁻, NO₃⁻, PO₄³⁻, and SO₄³⁻ were analyzed using an ion chromatograph (881 Compact IC pro, Metrohm, Switzerland). Total cation contents (Na⁺, K⁺, Mg²⁺, Ca²⁺, Mn²⁺, and Fe²⁺) were analyzed by inductively coupled plasma optical emission spectroscopy (Agilent 720 ICP-OES) in microwave-assisted reverse aqua regia (HCl + 3HNO₃) extraction at a pH < 2.

To characterize soil water repellency, water drop penetration time (WDPT) was determined directly in the field. One drop of tap water (100 μL) was placed onto the soil surface in 20 cm (4 cm for the detailed survey) distance intervals, and the time of complete penetration of each droplet was counted and classified from wettable to completely water repellent according to Bisdorn et al. [21].

Thermogravimetric analysis and differential scanning calorimetry of 10–20 mg soil samples (sieved to 1 mm) were conducted for the detailed survey on a TG DTA/DSC Apparatus STA 449F3 Jupiter (NETZSCH, Germany) coupled with the mass spectrometer MS 403 Aëolos II (NETZSCH, Germany). The sample was heated from 20 to 1000 $^{\circ}\text{C}$ at a heating rate of 10 $^{\circ}\text{C min}^{-1}$. An empty crucible was used as a reference. Smoothed raw data of the thermogram, a heat flux curve, and mass spectrometer signals (18 for H_2O and 44 for CO_2) were evaluated (more details in Peikert et al. [22] and Tamimi et al. [23]). According to Plante et al. [24], the thermogram was analyzed for two fractions: labile organic matter ($\text{LOI}_{\text{labile}}$, calorific value of the labile fraction, $\text{CV}_{\text{labile}}$), and recalcitrant organic matter ($\text{LOI}_{\text{recalcitrant}}$, $\text{CV}_{\text{recalcitrant}}$). The weight loss and the calorific value in the labile and recalcitrant fractions were summed up to determine the part caused by organic matter (LOI_{TGA} , CV_{TGA}). Then, the thermal stability of a sample was defined by the relative amount of organic matter that oxidizes with respect to the total weight loss (i.e., $\text{LOI}_{\text{recalcitrant}} / (\text{LOI}_{\text{recalcitrant}} + \text{LOI}_{\text{labile}})$).

OMW analyses were conducted on filtered samples (0.45 μm). Analyses included pH, EC, TP, DOC, ion contents, as well as thermogravimetry.

2.3. Biological Activity and Invertebrates

Invertebrates were sampled using pitfall traps before (2012 and twice 2014) and after (2012 and 2014) OMW application. Within each plot, two pitfall traps were set to level with the soil surface and placed in 40 cm distance (at an area that does receive irrigation water) from the irrigation line in the middle between two trees. Pitfall traps were filled to one-third with a 1:2 propylene-glycol and tap-water solution, which was emptied weekly for two weeks. Catches were transferred to 70% ethanol and determined on family level subsequently and summed up for both traps.

Biotic degradation processes were estimated before OMW application for the detailed survey using bait-lamina sticks (terra protecta GmbH) according to Kratz [25]. The 16 apertures of these perforated PVC strips were filled with a mixture of 70% cellulose, 27% wheat bran, and 3% activated carbon. Parallel to the irrigation line, 32 sticks were placed equidistantly distributed in 80 cm distance on both sides of the plot. Sticks were placed after soil sampling, removed after 14 days, and empty apertures were counted.

2.4. Data Analysis

Statistical analysis and plotting were done using R Statistics [26]. Each parameter was analyzed according to Lane [27] and Hothorn et al. [28], finding a suitable generalized linear model analysis of deviance [29]. Differences between treatments were identified (significance levels $p = 0.05, 0.01, \text{ and } 0.001$) applying Bonferroni-adjusted Tukey tests post-hoc. The invertebrate community structure was analyzed using nonmetric multidimensional scaling [30]. The community structure was related to treatment and environmental parameters from the first soil sampling layer as described by Oksanen et al. [31].

3. Results and Discussion

3.1. Olive Mill Wastewater (OMW) and Untreated Soil Characteristics

The OMW and soil used in this study was already characterized by Zipori et al. [11] as well as Levy et al. [3] and is typical for OMW characterized in the Mediterranean region [6,10]. OMW was acidic ($\text{pH } 4.4 \pm 0.3$) and loaded with conductive ions and inorganic material ($\text{EC } 12.1 \pm 1.2 \text{ dS m}^{-1}$, K^+ being most abundant with $5.3 \pm 0.9 \text{ g L}^{-1}$). Total phenols ($2.7 \pm 0.3 \text{ g L}^{-1}$) were prominent in its organic fraction (Biological oxygen demand of $35.8 \pm 10.0 \text{ g L}^{-1}$). Additionally, organic parameters were analyzed solely

in the detailed survey and can be seen in Table 1. The soil in Gilat is a sandy loam with 50% sand, 35% silt, and 15% clay, a pH of 8.2, and an effective cation exchange capacity of 33 mmolc kg⁻¹.

Table 1. Selected properties of the OMW used in 2012 and 2013 (preceded the detailed survey). Other properties of the OMW used during 2012–2016 are found in Zipori et al. [11]. LOI, loss on ignition; CV, calorific value.

Parameter		OMW	
		2012	2013
SUVA	L mg C ⁻¹ m ⁻¹	0.11 ± 0.01	0.13 ± 0.01
LOI	%	58 ± 0.9	64 ± 0.5
LOI _{labile}	%	48.9 ± 0.6	51.9 ± 0.7
LOI _{recalcitrant}	%	9.1 ± 0.4	12.2 ± 0.3
CV _{LOI}	kJ g ⁻¹	19.7 ± 1.6	19.0 ± 1.3
CV _{labile}	kJ g ⁻¹	4.4 ± 0.2	4.1 ± 0.2
CV _{recalcitrant}	kJ g ⁻¹	9.4 ± 0.5	9.1 ± 0.6

3.2. Soil Hydrophobicity

The first WDPT recording was done in 2013, after two successive years of winter OMW applications. During the detailed survey, the control plots exhibited a distance-dependent hydrophobic profile (Figure 1a). Between 0–48 cm and 128–200 cm distance from the irrigation line, the soil was classified as wettable to slightly water repellent according to the arbitrary classification of Bisdom et al. [21]. However, at a distance of 48–128 cm, the soil had a median WDPT of 50.5 ± 70 s and can be classified up to strongly water repellent. OMW application increased WDPT most substantially after an application of 100 m³ OMW ha⁻¹ with a median of 90 ± 111 s (*p* < 0.001). In all OMW treatments, single spots showed WDPTs above 600 s, which is considered as severely water-repellent soil.

The repellency classes (subplots in Figure 1) show that the strongest hydrophobizing effects were found on 100 m³ OMW ha⁻¹ y⁻¹ treated plots, which was followed by 50 and 150 m³ OMW ha⁻¹ y⁻¹ treated plots, whereas the tilled plots with an application of 100 m³ OMW ha⁻¹ were even less hydrophobized than the control plots. This hydrophobicity effect was measured during summer in the following three successive years of winter OMW applications. The hydrophobicity effect was not intensified over the years but rather diminished substantially. In 2014 (Figure 1b), water repellency was higher near the irrigation line (100 and 150 m³ ha⁻¹ y⁻¹), and also at 48 cm distance but only at the 150 m³ ha⁻¹ y⁻¹. Plots receiving an OMW application rate of 150 m³ OMW ha⁻¹ y⁻¹ showed high WDPT at 48 cm with a total of 50% of all spots of WDPT ≥ 5 s. The water repellency and its spatial distribution diminished in the following year (Figure 1c). However, only OMW-treated plots, also tilled ones, showed WDPT above 5 s in the last assessment (Figure 1d).

The noticeable distance-dependent spatial distribution of water repellency is mainly due to the field microtopography. Starting at the drip line, which is arranged at the tree trunks, there is a slight downward slope in the 50 cm parallel to the drip line. The area between the tree lines is flat. In these sinks, OMW, as well as natural organic matter such as olive leaves or fruits, accumulate and cause repellency. Harman et al. [32] found that the microtopography strongly influences soil hydraulic conductivity and soil organic matter contents in semi-arid shrubland. Steinmetz et al. [15] observed a similar spatial distribution with higher WDPT in control as well as OMW-treated plots in the first 60 cm (“irrigation zone”) compared to the region between 80 and 200 cm (“dry zone”) in the same study region where our study was conducted but in a different field experiment. However, 18 months after the application of 140 m³ OMW ha⁻¹ y⁻¹, they found no increased water repellency compared to control plots. This suggests that the additional time of six months might have been sufficient to degrade OMW-derived organic compounds. Similar to our study, Mahmoud et al. [33] found persistent water repellency after repeated OMW applications in winter.

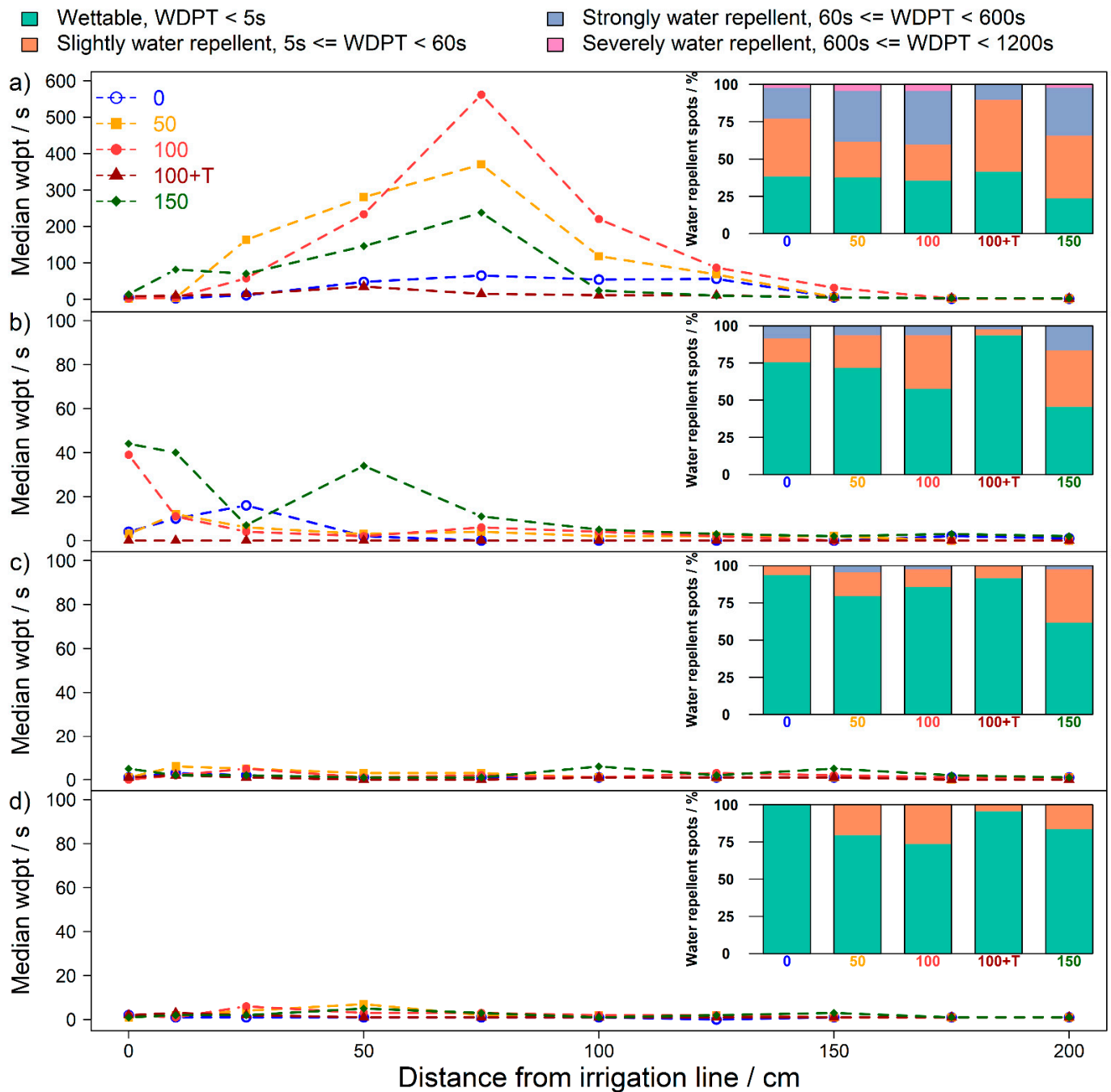


Figure 1. Median values of water drop penetration time (WDPT) measured in distance steps of 20 cm from the drip irrigation line. (a) 2013; (b) 2014; (c) 2015; (d) 2016. Subplot (stacked bars) shows the frequency distributions of four hydrophobicity categories ranging from wettable to strongly water repellent. OMW application rates are 0 (control), 50, 100, and 150 m³ ha⁻¹ y⁻¹ and 100 m³ ha⁻¹ y⁻¹ followed by shallow tillage (100 + T).

Tillage reduced the occurrence of water repellency and its spatial distribution as the natural and OMW-derived organic compounds were incorporated into deeper soil layers and crusts destroyed. However, the simple addition of natural and OMW-derived organic compounds does not solely lead to the expression of water repellency in soils. It can be caused by the natural organic compounds in the soil if they form a specific molecular arrangement triggered by the water content [34]. The critical water content of a soil is suggested to define a transition zone between a water repellent and a wettable state [35] and is a function of the soil organic carbon [36]. Due to their significantly lower soil organic carbon, only the control plots had critical water content below the measured water

content in the upper layer, which could additionally lead to increased water repellency. Soil hydrophobicity is especially problematic in rainfed olive orchards, as it will increase the water run-off and reduce the amount of rain that will be absorbed by the soil. Based on the observations shown in Figure 1a, it was decided to focus the detailed survey at a distance of 80 cm.

3.3. Effects of OMW Application on Soil Properties

The net effect (delta) of the OMW treatments relative to the control plots is presented in Figure 2. Following OMW application, a short-term increase in soil water content was observed at the top 0–10 cm layer (Figure 2a). This increase was highest in tilled plots with a surplus of up to 3%, 2% after an application of 100 and 150 m³ OMW ha⁻¹ y⁻¹ and 1% after 50 m³ OMW ha⁻¹ y⁻¹. Interestingly, higher soil water content by 1.8% was still observed at the 150 m³ ha⁻¹ y⁻¹ treatment ($p < 0.05$) in the detailed survey (Figure 3a) just before the winter application of 2014. Neither irrigation water (starting in March) nor the cumulative amount of rain (226.5 mm between the previous application of 2013 and the detailed survey), which are both the same for the entire experimental plot, could be the source for differences in water contents among treatments. On the other hand, crust formation after OMW application due to fast drying could be responsible for this effect by covering the exchange surface and reducing drying kinetics [37]. Such an effect of crusts formed following OMW application has been seldom referred to in the literature, although biological soil crusts are known to positively affect the water balance in semi-arid ecosystems by modifying hydrological processes [38]. Moreover, the contribution of OMW to soil organic matter can increase the soil water-holding capacity [39]. This effect must be investigated further during spring and summer months, as any water surplus could be beneficial in this semi-arid ecosystem. Finally, although not measured in this study, improved soil aggregation, which might be a result of OMW application [40], may lead to higher water infiltration rates, which in turn increases the water content.

TP ($p < 0.001$) and DOC ($p < 0.05$) generally increased at the top 0–10 cm soil layer in OMW-treated plots (Figure 2b,c, respectively). This increase was OMW dosage-dependent in the detailed survey (Jan 2014) also in a depth of 10–20 cm (TP, $R^2 = 0.23$, $p < 0.001$, DOC, $R^2 = 0.07$, $p < 0.05$; not shown). The significant difference and dose-dependent correlation were even stronger at a depth of 0–10 cm. In the tilled plots, TP was also higher compared to the non-tilled control treatment down to a depth of 20–40 cm ($p < 0.05$). DOC as well as TP accumulated over the years, whereby this effect was strongest after an OMW application rate of 150 m³ OMW ha⁻¹ y⁻¹ (1.9 mg TP L⁻¹ y⁻¹); only soil samples before an OMW application were evaluated and linearly correlated to the OMW application rate. In general, TP was high in the upper soil layer (e.g., control plot in the detailed survey 28 ± 9 mg kg⁻¹) and then ranged between 14 ± 2 mg kg⁻¹ and 17 ± 3 mg kg⁻¹ in the beneath layers (Figure 3b). The same trend was observed for dissolved organic carbon (DOC, correlation coefficient with TP was 0.74, $p < 0.001$, data not shown).

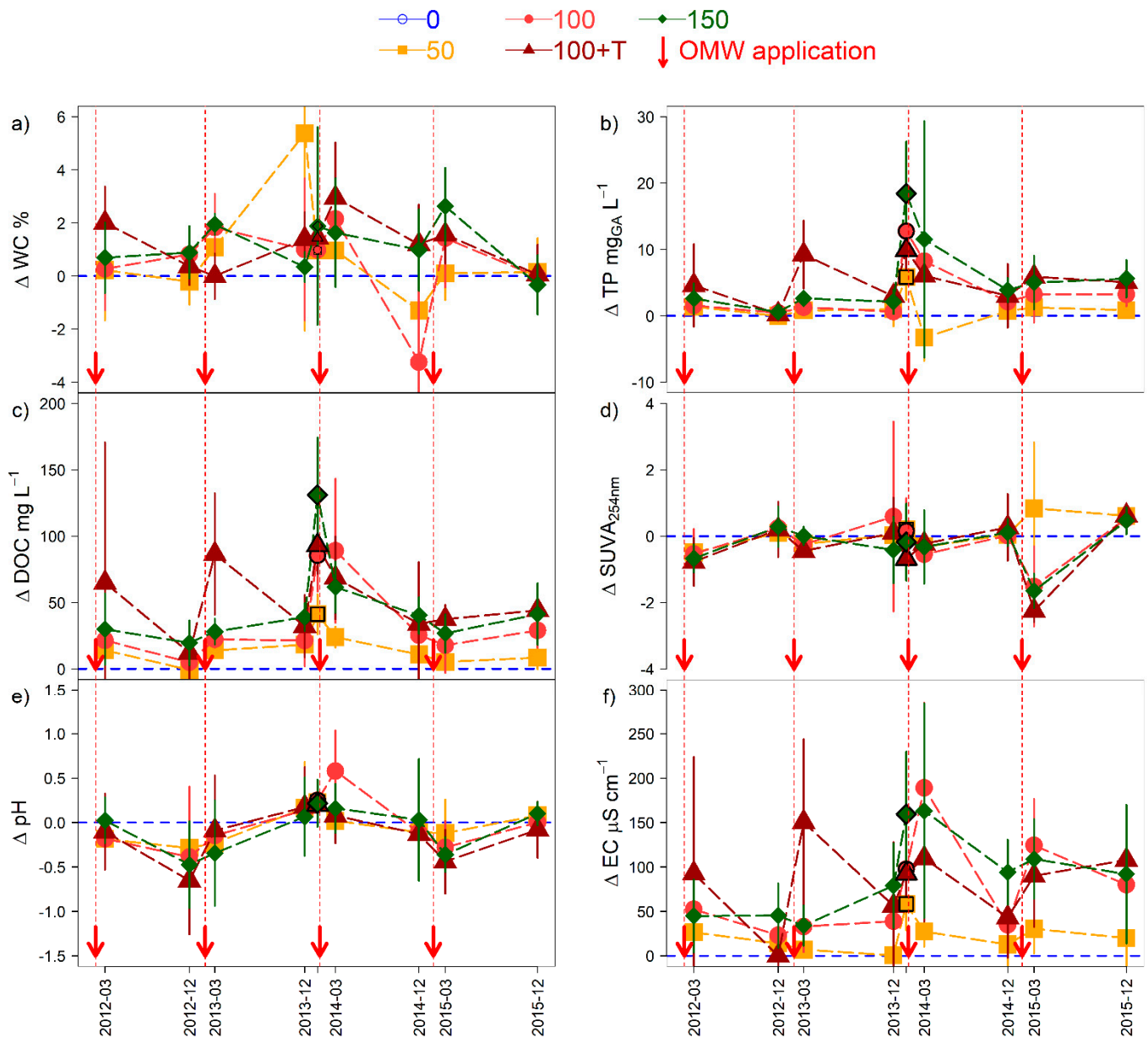


Figure 2. Evolution of physicochemical soil properties at a depth of 0–10 cm, expressed as the difference between OMW-treated and control plots. (a) Gravimetric water content; (b) total phenolic compounds (TP); (c) specific UV absorbance (SUVA); (d) pH; (e) electrical conductivity (EC); and (f) dissolved organic carbon (DOC). Bold red arrows with dashed lines mark OMW application dates. OMW application rates are 0 (control), 50, 100, and 150 m³ ha⁻¹ y⁻¹ and 100 m³ ha⁻¹ y⁻¹ followed by shallow tillage (100 + T).

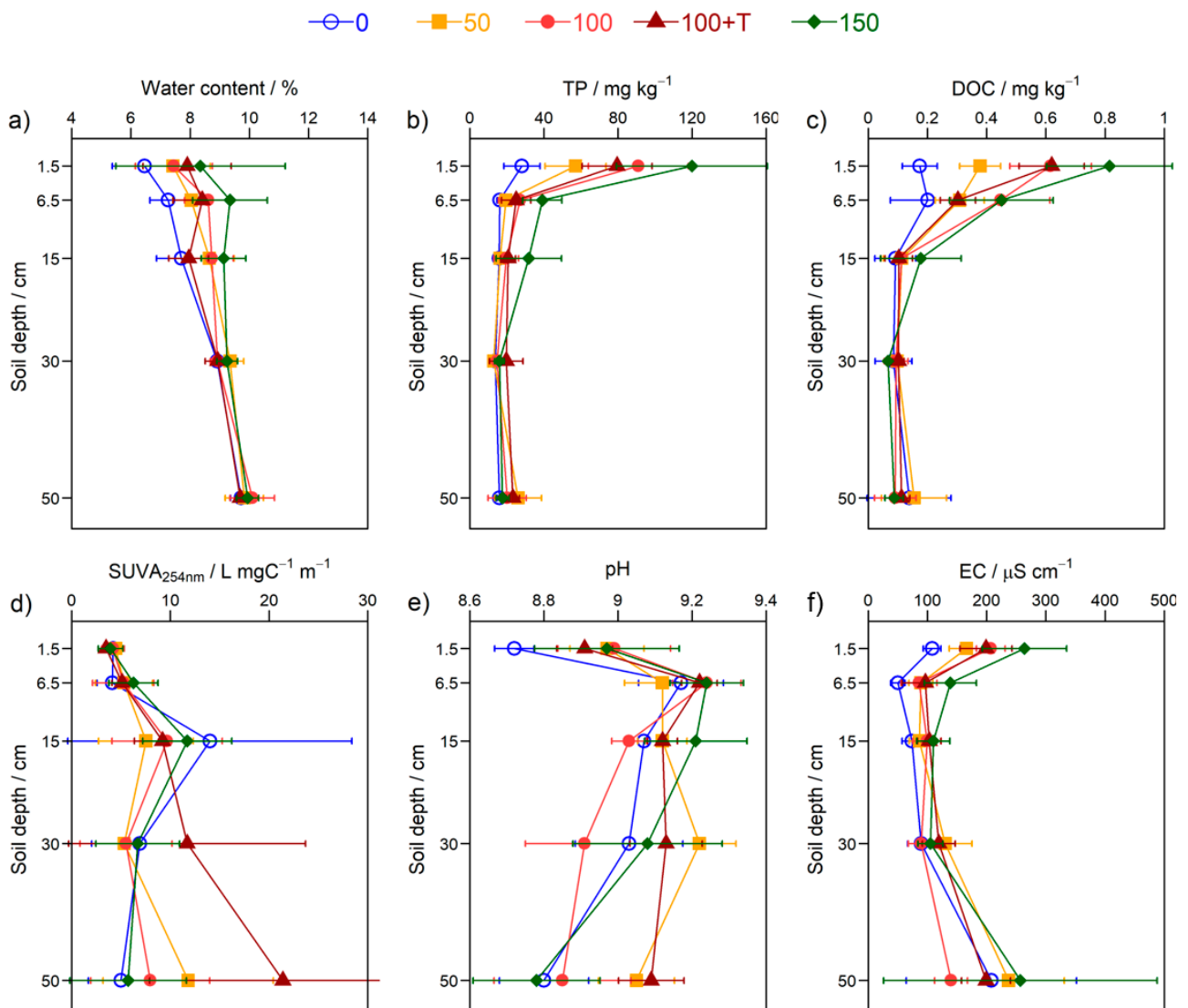


Figure 3. Depth soil profiles of (a) water content; (b) Total Phenols (TP); (c) dissolved organic carbon (DOC) (d) specific UV absorbance (SUVA); (e) pH; (f) electrical conductivity (EC) of the detailed survey before the third application of OMW. OMW application rates are 0 (control), 50, 100, and 150 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ and 100 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ followed by shallow tillage (100 + T).

OMW application affected the quality of soluble organic compounds expressed as SUVA₂₅₄ toward higher aromaticity solely following tillage in a depth of 40–60 cm ($p < 0.001$, Figure 3c). At a depth of 15 cm, OMW application favored a lower aromaticity compared to the control. During the whole study period, no differences in the top layer could be detected. At the end of the study period, no effect on the balance of soil H^+ could be detected (Figure 3d). However, in the detailed survey, soil pH in the control soil ranged from 8.7 to 9.2 with its minimum at 0–3 cm and maxima at 3–10 cm (Figure 3e). Twelve months after the two first annual OMW applications, soil pH increased to 9.0 ± 0.2 in all treated plots solely in the upper soil layer (0–3 cm; $p < 0.01$). Previous reports show the opposite or no effect after OMW application (e.g., Lanza et al. [41]; or the review by Mekki et al. [17]). However, the mineralization of OMW-derived carbon releases free OH^- ions, which in turn could lead to a ligand exchange with ions brought by OMW. Such effects are also reported in other studies on OMW land application [42–44].

A strong effect of OMW application was found for soil EC (Figure 3f). Only the lowest application rate of 50 $\text{m}^3 \text{OMW ha}^{-1} \text{y}^{-1}$ showed no increase compared to water-treated plots at the end of the study. In the detailed survey, the depth dependence of EC showed a

characteristic c-shape in all treatments. In a depth of 0–3 ($110 \pm 14 \mu\text{S cm}^{-1}$) and 40–60 cm ($210 \pm 100 \mu\text{S cm}^{-1}$), we found the highest EC in the control, while at a depth of 3–10 cm ($48 \pm 9 \mu\text{S cm}^{-1}$), it was lowest. OMW application did not affect this shape but increased the values especially in the upper soil layers ($p < 0.001$) and application rate dependent at 0–3 cm (adj. $R^2 = 0.65$, $p < 0.001$). This relation became weaker with increasing depth (3–10 cm, adj. $R^2 = 0.48$, $p < 0.001$; 10–20 cm, adj. $R^2 = 0.23$, $p < 0.001$). Ca^{2+} was the most abundant cation in each layer of the control plots between 130 and 180 mg kg^{-1} (Figure 4), which was followed by K^+ (43 to 130 mg kg^{-1} , Figure 4a). While PO_4^{3-} and NO_2^- were distributed equally within the soil layer ($11.5 \pm 1.2 \text{ mg kg}^{-1}$ and $5.0 \pm 0.3 \text{ mg kg}^{-1}$), NO_3^- was high in the upper soil layer ($11 \pm 16 \text{ mg kg}^{-1}$) and lower in the layers beneath ($5.3 \pm 0.7 \text{ mg kg}^{-1}$). Conversely, SO_4^{3-} increased with increasing depth. These relations changed after OMW application. The absolute and relative effect of annual application of $150 \text{ m}^3 \text{ OMW ha}^{-1} \text{ y}^{-1}$ was by far highest for K^+ ($590 \pm 100 \text{ mg kg}^{-1}$). Even in a depth of 10–20 cm, plots receiving this application rate of OMW contained K^+ contents of $160 \pm 110 \text{ mg kg}^{-1}$ while control plots contained only $54 \pm 21 \text{ mg kg}^{-1}$. Up to this depth, OMW application increased K^+ contents linearly with the applied application rate. Similar differences and application rate dependencies were found for Mn^{2+} , Na^+ , Cl^- , and PO_4^{2-} (Figure 4). For sulfate, this was only found between 0 and 3 cm, while calcium, magnesium, and iron showed this relation only in depth of 3–10 cm. Linear relations were always stronger and more significant when the tillage treatment was not incorporated into the model. K^+ , Mg^{2+} , Ca^{2+} , Fe^{2+} , and Mn^{2+} as well as SUVA were elevated in tilled plots at a depth of 40–60 cm compared to the control and all other OMW treatments, but these trends were not significant.

OMW spreading led to an application rate-dependent increase of cations as well as anions in the soil. Ions were contributed by OMW itself but also mobilized from the solid soil phase as shown especially for Fe^{2+} and Mn^{2+} . Aharonov-Nadborny [45], who investigated the leaching of soil cations after OMW application, suggested that cation mobilization is mainly due to the formation of cation–organic complexes with the OMW-derived dissolved organic matter and cation exchange. In loess soil, they found a high leaching potential for Mn, which can be confirmed in our study. Especially tillage increases this risk, as we found higher cation contents in the lowest investigated soil layer.

Even the lowest application rate of $50 \text{ m}^3 \text{ OMW ha}^{-1}$ lead to significant physicochemical changes 12 months after two annual OMW applications. TP, DOC, and EC as well as most anions (Cl^- , PO_4^{3-} , SO_4^{3-}) and cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+}) accumulated linearly with the OMW application rate. This was attenuated but still significant for the application of $100 \text{ m}^3 \text{ OMW ha}^{-1}$ plus tillage. In the same study area, 18 months (6 months more compared to our study) after two annual OMW applications of 70 and $140 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$, pH, EC, all cations (except K^+), C_{org} , TP, WDPT, as well as bait consumption were unaffected [15]. It is assumed that increasing temperatures and increasing soil humidity stimulate soil resilience. Moreover, OMW-derived changes disappeared already within three weeks after OMW application of $140 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ in the summer in this study region [8]. Such seasonal effects were also shown for sandy clay loam treated with OMW $40 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ and $80 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ [11] and $150 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ for clay loam [3,4]. These works highlight the unfavorable environmental conditions resulting in low biological activity and a risk for leaching when spreading OMW during winter, as done in our study.

Notably, the high resolution of soil profiles described in the present study revealed more OMW-derived effects; e.g., when analyzing the differences between treatments based on the weighted average of the three layers together (0–3, 3–10, and 10–20 cm), significant differences for all parameters became insignificant or were weaker (e.g., no difference between 0 and $50 \text{ m}^3 \text{ OMW ha}^{-1} \text{ y}^{-1}$ could be detected for TP or the significant application rate effect on K^+ disappeared). Some significances already disappeared when using the weighted average of only the depths 0–3 and 3–10 cm (e.g., Mn^{2+} , Fe^{2+} , SO_4^{3-} , SUVA, thermogravimetric-derived parameter).

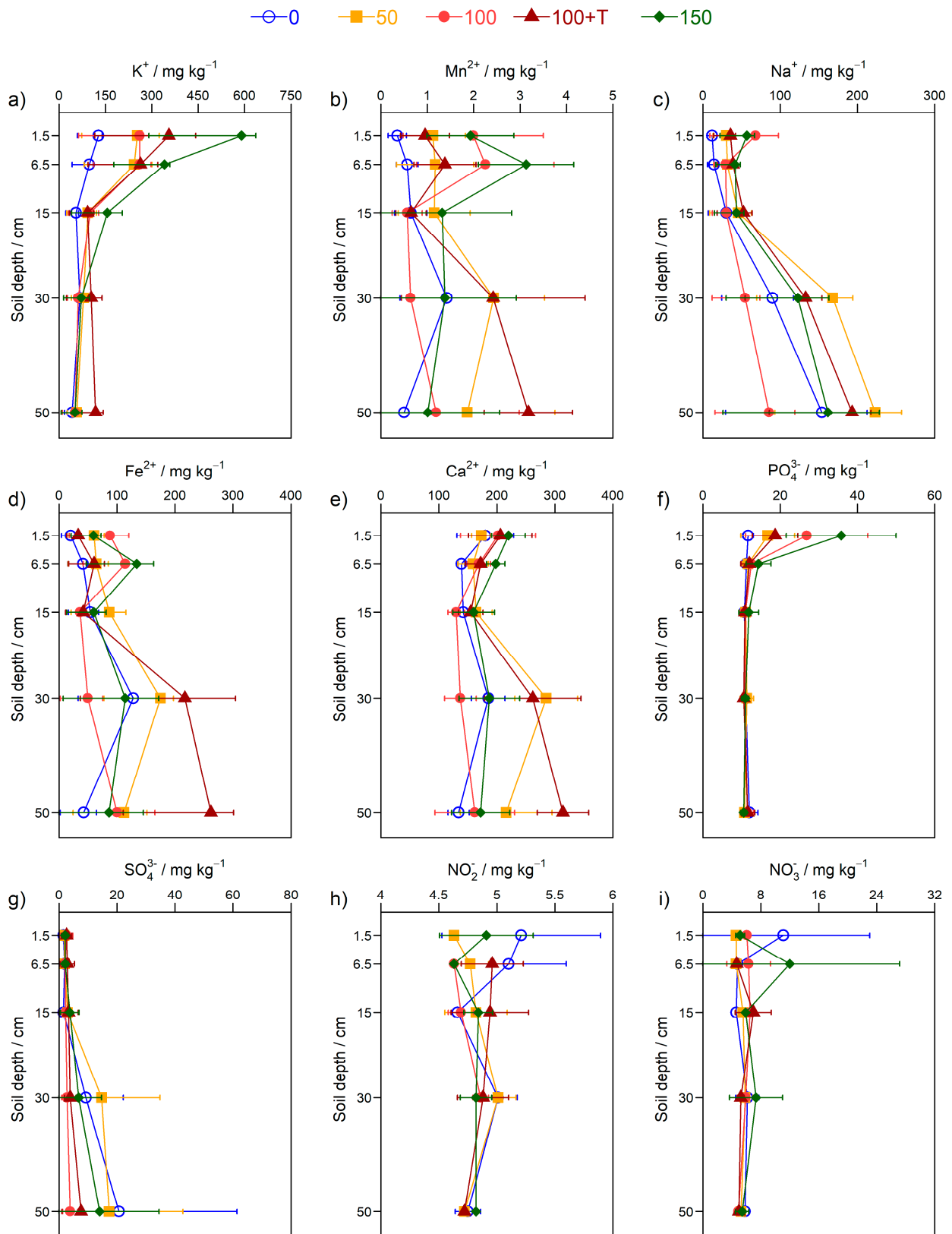


Figure 4. Depth soil profiles of K^+ (a), Mn^{2+} (b), Na^+ (c), Fe^{2+} (d), Ca^{2+} (e), PO_4^{3-} (f), SO_4^{3-} (g), NO_2^- (h), and NO_3^- (i) of the detailed survey before the third OMW application. OMW application rates are 0 (control), 50, 100, and 150 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ and 100 $\text{m}^3 \text{ha}^{-1} \text{y}^{-1}$ followed by shallow tillage (100 + T).

3.4. Changes in Thermal SOM Properties after OMW Application

During the thermoanalysis of soil samples from the detailed survey, temperatures of CO₂ evolution below 550 °C ranged from 286 ± 1 °C to 508 ± 6 °C. LOI_{labile} was identified by the thermogram and ranged from 286 ± 1 °C to 398 ± 3 °C (Figure 5a). LOI_{recalcitrant} ranged from 398 ± 3 °C to 508 ± 6 °C (Figure 5b). Only the application of 100 m³ OMW ha⁻¹ y⁻¹ with tillage significantly increased the LOI_{labile} compared to the control in both upper soil depths of 0–3 and 3–10 cm ($p < 0.01$). LOI_{recalcitrant} decreased after an application of 50 m³ OMW ha⁻¹ y⁻¹ in a depth of 0–3 cm and in all OMW treatments except 100 m³ OMW ha⁻¹ y⁻¹ tillage compared to the control in a depth of 3–10 cm ($p < 0.001$), while this was true only for the application of 50 m³ OMW ha⁻¹ y⁻¹ for the above soil layer. The application of OMW reduced the thermal stability in all OMW treatments compared with the control ($p < 0.001$, Figure 5c) in both soil layers except for the lowest application rate of 50 m³ OMW ha⁻¹ y⁻¹ (3–10 cm). The calorific value (CV_{labile}, Figure 5d) of the labile fraction decreased after an application of 100 m³ OMW ha⁻¹ y⁻¹ ($p < 0.01$).

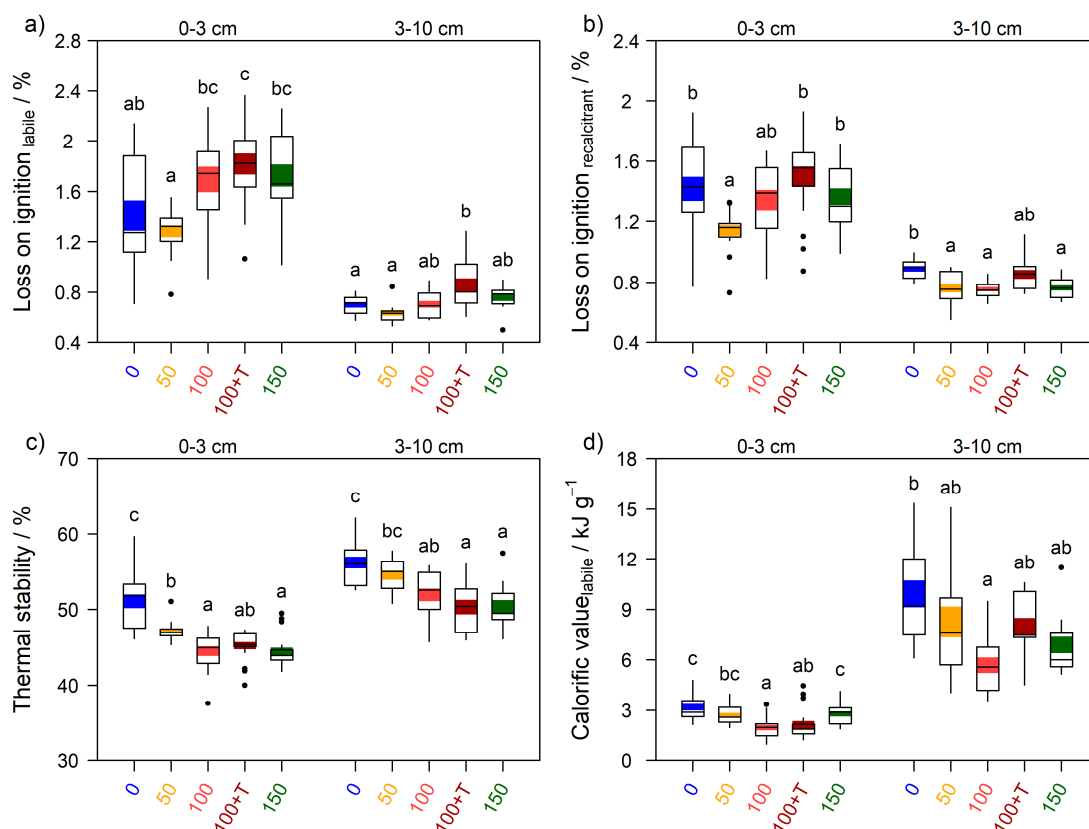


Figure 5. Thermogravimetric analysis of soil organic matter in soil depths of 0–3 and 3–10 cm: (a) mass loss on ignition of the labile temperature range; (b) mass loss on ignition of the recalcitrant temperature range; (c) thermal stability index; (d) calorific value of the labile fraction. Boxplots show the median and ranges from the 25th to the 75th percentile; the whisker length was set to 1.5 times the interquartile range. OMW application rates are 0 (control), 50, 100, and 150 m³ ha⁻¹ y⁻¹ and 100 m³ ha⁻¹ y⁻¹ followed by shallow tillage (100 + T). Colored overlays indicate the standard deviation of the mean. Mean values within one soil depth followed by the same letter were not statistically different ($p \leq 0.05$).

The reduced LOI_{recalcitrant} could be due to a priming effect, which was also identified as responsible by Tamimi et al. [23]. By adding easily degradable exogenous substrate (OMW) to the soil as an energy source for microorganisms, co-metabolized enzymes are synthesized by K-strategists capable of degrading recalcitrant OM [46]. This and the OMW-OM could also be one source for increased LOI_{labile} in OMW-treated plots, as the degraded recalcitrant OM is converted to labile OM. Another explanation for the increased

labile organic carbon in tilled treatments could be due to residual plant materials and roots brought into the soil matrix through this management practice [47].

3.5. Effects of OMW on Soil Biological Activity and Invertebrates

In the detailed survey conducted on January 2014 after two OMW applications and just before the third winter application, the median biological activity in terms of consumed bait material showed a distinct depth dependency (Figure 6). Moreover, OMW application positively affected soil biological activity in the upper soil layer (0–0.5 cm). Overall, a significant effect on soil biological activity could be found for OMW application rates of $100 \text{ m}^3 \text{ OMW ha}^{-1} \text{ y}^{-1}$ with and without tillage.

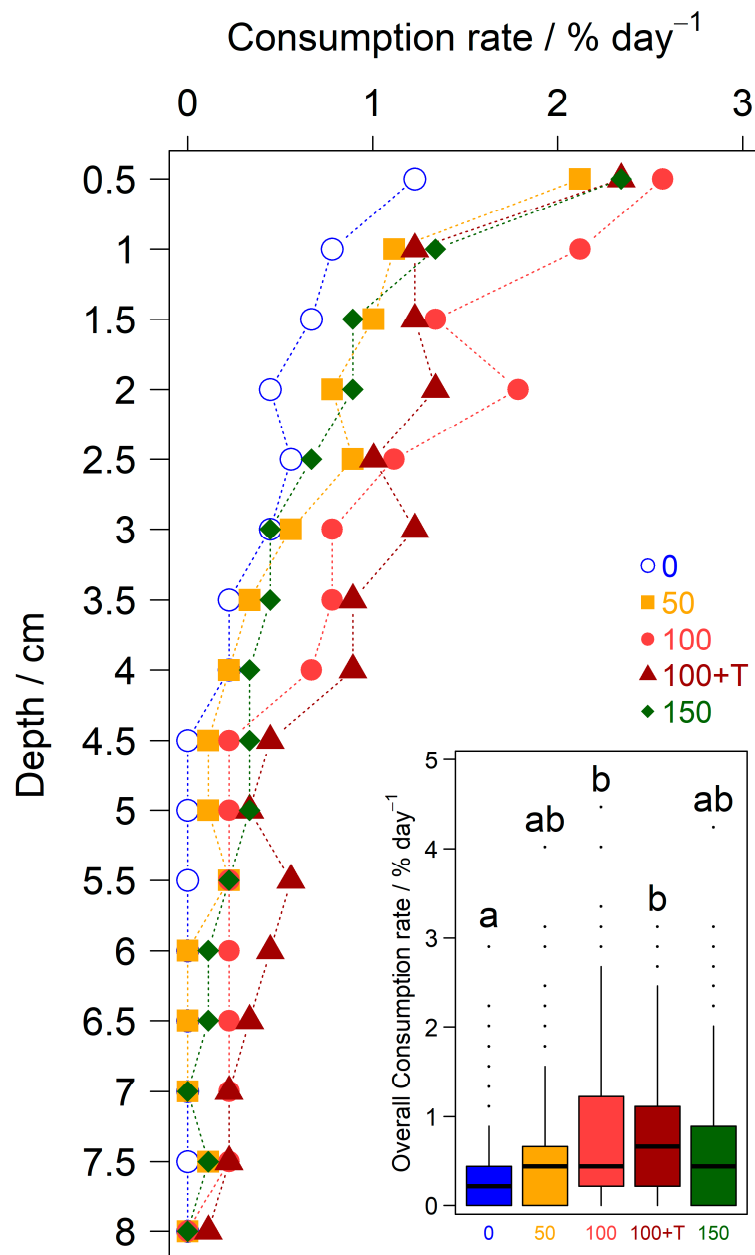


Figure 6. Soil biological activity as consumed bait material per day. The subplot shows the median and ranges from the 25th to the 75th percentile, the whisker length was set to 1.5 times the interquartile range. OMW application rates are 0 (control), 50, 100, and $150 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ and $100 \text{ m}^3 \text{ ha}^{-1} \text{ y}^{-1}$ followed by shallow tillage (100 + T). Dots represent outliers. Different small letters indicate significant differences between OMW applications ($p < 0.05$).

During the detailed survey, the abundance of Collembola (Figure 7) was not affected by OMW. In addition to grubs, Coleoptera, Arachnida, Dermaptera, and Diplopoda were not affected and different compared with the control. During the whole study period, no clear pattern was found.

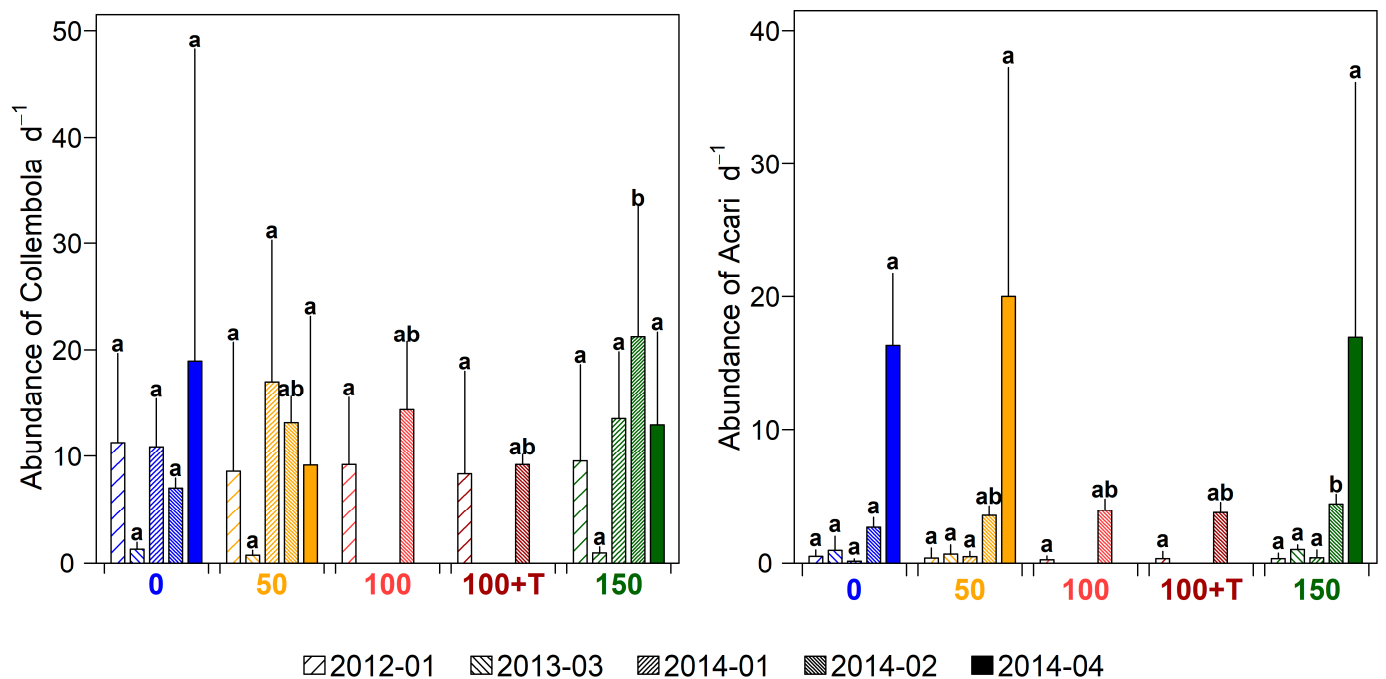


Figure 7. Abundance of Collembola and Acari per sampling day in five scoring events. OMW application rates are 0 (control), 50, 100, and 150 m³ ha⁻¹ y⁻¹ and 100 m³ ha⁻¹ y⁻¹ followed by shallow tillage (100 + T). Different small letters indicate significant differences within one OMW application rate ($p < 0.01$).

OMW application, independent of the application rate, created temporarily biologically favorable conditions resulting in higher biological activity and higher abundances of Collembola and Acari. Steinmetz et al. [15] did not find any effect on bait consumption after winter application but rather an adverse effect when applying OMW in summer due to high TP contents. To our best knowledge, the only field study available dealing with effects of OMW toward soil invertebrates was performed in a neighboring field, and the authors monitored the effects three weeks after the application of OMW in summer [8]. In their study, neither negative nor positive effects toward Collembola were found. The latter was surprising, as a high application rate of 150 m³ OMW ha⁻¹ y⁻¹ was applied four days before the first invertebrate sampling. Even more, positive effects on Acari abundance were discovered three weeks after the OMW application. It is to be noted that the abundance was much lower in this study compared to ours. During six sampling events, Kurtz et al. [8] found 56 individuals of Collembola and 716 individuals of Acari, whereas in this study, 4598 and 1268 individuals were found for Collembola and Acari during the detailed survey, respectively. This difference is mostly attributed to the sampling season (hot and dry summer vs. wet and mild winter) and sampling method (soil extraction with Berlese–Tullgren funnels vs. pitfalls). Conditions of ideal humidity, temperature, and food supply could be the reason for such high abundances due to induced swarming [48]. This is highlighted by the increased consumption of bait material, which indicates a stronger activity of soil mesofauna [49] in all depths down to 8 cm. OMW is known to increase the number of bacteria and fungi in soil [41,50], which in turn are preferred feeding sources for Collembola [51]. Moreover, Hentati et al. [16] showed that the standard soil arthropod *Folsomia candida* favors some soils from OMW ponds (with an EC > 1600 $\mu\text{S cm}^{-1}$) compared to a farmland ‘reference’ soil without OMW treatment. These results strongly suggest that the soil fauna is insensitive or even prefers OMW-derived edaphic changes

(i.e., phenols, carbon input, high salt contents, or hydrophobic films) after a time period of one year. The toxic effects of OMW-derived phenolic compounds disappeared through the degradation or polymerization of these OMW constituents. Soil fauna is known to stimulate microbial activity and modify the recalcitrance persistence of soil carbon pools [52]. Wang et al. [53] found that high numbers of Collembola transfer soil C from recalcitrant to more labile sources. Additionally to the priming effect, this can explain the decreased recalcitrant carbon in the lower soil layer as OMW application significantly increases soil biological activity.

3.6. Practical Implications

Considering national and international recommendations of 30–80 m³ OMW ha⁻¹ y⁻¹, the area available for spreading in olive orchards is sufficient to absorb the entire amount of OMW produced from olive oil production. In non-irrigated orchards, where olive production is in the range of 2–3 tons ha⁻¹, and the resulting OMW is ca. 2.5–3.5 m³ ha⁻¹ accordingly, the area available for spreading (e.g., 50% of the land) means a low dose of only 5–7 m³ ha⁻¹ y⁻¹, or 10–14 m³ ha⁻¹ every second year. Even in intensive irrigated orchards, where olive production is much higher, in the range of 10 tons ha⁻¹, and the resulting OMW is ca. 10–12 m³ ha⁻¹ accordingly, the area available for spreading (e.g., 50% of the land) means a dose of 20–22 m³ ha⁻¹ y⁻¹, or 40–44 m³ ha⁻¹ every second year. These volumes are much lower than the above-mentioned recommendations, indicating that spreading OMW in olive orchards is a sustainable practice. Potential annual contribution of OMW to soil nutritional status is significant and in line with the idea of turning waste into a resource. The application of 50 m³ OMW ha⁻¹ from our study would provide 318 kg K₂O ha⁻¹ y⁻¹ (annual recommendation in e.g., Israel is 300 kg K₂O ha⁻¹ y⁻¹) and save around 1500 € ha⁻¹ y⁻¹ [11].

4. Conclusions

OMW application in semi-arid areas with sandy loam soils has positive effects (accumulation of K⁺, PO₄³⁻, organic carbon, and increased biological activity and diversity) but also negative consequences (accumulation of phenolic substances, increased soil hydrophobicity, and salinity). Most of these effects are OMW dose-dependent and most substantial in the topsoil. The positive effects underline the strategy to apply low application rates of OMW in alternating locations. Especially tillage after OMW application needs to be further investigated as we found indicators (SUVA, K⁺, Mn²⁺) for higher leaching risks after this management practice. Since tillage ‘dilutes’ the OMW within the soil, the displacement of OMW constituents favors biological degradation as the effect of UV degradation is negligible in deeper soil layers. Although the strongly reduced soil hydrophobicity after tillage did not affect soil biology and even improved soil physicochemical properties, further investigations on higher leaching risks after this management practice are mandatory. A simple field kit or annual sampling to determine soil EC, sum of phenolic substances, and water drop penetration time would be sufficient for a rough estimation if another OMW application could be performed.

The overall results of this study indicate that applying the recommended doses of 30–80 m³ OMW ha⁻¹ y⁻¹ in olive orchards at a semi-arid region pose no clear negative effects on soil chemical and physical properties. The only measured negative effect is the temporary increase in soil surface hydrophobicity. To overcome this potential effect, shallow tillage may be applied subsequently after OMW spreading.

Author Contributions: Conceptualization: M.P.K., G.E.S., D.D. and A.D.; methodology, M.P.K., G.E.S., I.Z., Y.L., D.D., S.M., E.Z.-F. and A.D.; software, M.P.K.; validation, M.P.K., A.D. and Y.L. formal analysis, M.P.K., G.E.S., A.D. and D.D.; investigation, M.P.K., I.S., S.M., M.R., E.Z.-F., I.S.; resources, G.E.S., A.D., I.S.; data curation, M.P.K., C.B., A.D., Y.L., I.Z. and D.D.; writing—original draft preparation, M.P.K., C.B.; and D.D.; writing—review and editing, M.P.K., C.B., A.D., Y.L. and D.D.; visualization, M.P.K.; supervision, D.D. and G.E.S.; project administration, G.E.S. and A.D. funding acquisition, G.E.S. and A.D. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Deutsche Forschungsgemeinschaft (TRILAT-OLIVEOIL, SCHA 849/13 and also part of the MERC foundation (Research Project M31-019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available at the time of publication due to an ongoing data transfer to the corresponding author. An openly accessible link to the raw data will be provided in a timely manner.

Acknowledgments: We want to thank the researchers in Gilat and Landau for fruitful discussions and help in the laboratory. Finally, we thank Yulia Subbotin and Silvia Zypman for help in Gilat and Avi for hospitality in (Hapoel!) Be'er Sheva.

Conflicts of Interest: The authors declare no conflict of interest.

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