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Article

Effects of Olive Mill Wastewater on Soil Microarthropods and Soil Chemistry in Two Different Cultivation Scenarios in Israel and Palestinian Territories

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Abstract: Although olive mill wastewater (OMW) is often applied onto soil and is known to be phytotoxic, its impact on soil fauna is still unknown. The objective of this study was to investigate how OMW spreading in olive orchards affects Oribatida and Collembola communities, physicochemical soil properties and their interdependency. For this, we treated plots in two study sites (Gilat, Bait Reema) with OMW. Among others, the sites differed in irrigation practice, soil type and climate. We observed that soil acidity and water repellency developed to a lower extent in Gilat than in Bait Reema. This may be explained by irrigation-induced dilution and leaching of OMW compounds in Gilat. In Bait Reema, OMW application suppressed emergence of Oribatida and induced a community shift, but the abundance of Collembola increased in OMW and water-treated plots. In Gilat, Oribatida abundance increased after OMW application. The effects of OMW application on soil biota result from an interaction between stimulation of biological activity and suppression of sensitive species by toxic compounds. Environmental and management conditions are relevant for the degree and persistence of the effects. Moreover, this study underlines the need for detailed research on the ecotoxicological effects of OMW at different application rates.

Keywords: olive mill waste water; hydrophobicity; Oribatida; Collembola; field study

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1. Introduction

Olive mill wastewater (OMW) is produced during the three-phase olive oil extraction process [1]. Typically, OMW is acidic (pH 4–5), has a high chemical and biological oxygen demand, high concentrations of cations and anions, nutrients, fats, oil and polyphenolic compounds [2]. As discharge of OMW into wastewater treatment plants is generally forbidden, it is often discharged in an uncontrolled manner into the environment [3]. Generally, OMW treatment could reduce negative biological effects as shown in the study of Mekki, *et al.* [4] and in avoidance tests using the collembolan species *Folsomia candida* [5]. However, treatment options are not feasible in countries where olive oil production is decentralized and/or family-owned, as for example in Greece [6], Israel or the Palestinian Authority [1]. On the other hand, the Israeli Ministry of Environmental Protection allows land application at rates up to 40 $m^3 \cdot ha^{-1} \cdot year^{-1}$, which is expected "to be on a safe side" [7].

OMW could serve as fertilizer [8–10], but it may also render soils water repellent or modify the sorptive capacity of soil for organic pollutants on a long-term scale [9,11–13], and leaching of OMW components to groundwater cannot be excluded [14,15]. Moreover, OMW is phytotoxic to, for instance, spinach [16] and cress [17]. Additionally, OMW amendment increased total soil respiration rate and number of heterotrophic microflora including fungi and coliforms but reduced the C_{tot}-normalized respiration rate, suggesting a reduced ability of the soil microflora to degrade the organic matter [4]. Overall, ecotoxicological data available mainly address impacts on plants and microbial parameters, but not ecotoxicity to other edaphic species including soil invertebrates [18]. To our best knowledge, this situation has not changed since 2012, although soil invertebrates are highly relevant for ecosystem services like decomposition of organic matter, nutrient cycling and detoxification of wastes [19]. Moreover, they play a central role for the resilience of ecosystems and they serve as indicators for soil quality due to their intermediate position in food chains [20]. Thus, it is highly relevant to understand how OMW application to soils affects soil invertebrate communities and soil properties. As biological effects of OMW amendment of soil have been observed during the early weeks after OMW-soil contact [17,21,22], this study also focuses on this period and investigates short-term effects. However, the impacts of OMW application on soil biology are still largely unknown and they are likely to depend on climate, soil type and management method. Therefore, in order to plan long-term studies, in a first step, the variety of conditions should be narrowed down to central specific aspects.

The objective of this study was to explore how edaphic Oribatida and Collembola communities are affected by OMW spreading in olive orchards during the first three weeks of OMW-soil contact and link these changes with soil physicochemistry. We hypothesized that the impact of OMW application on the Oribatida and Collembola communities is a result of: (i) the interaction between toxic effects of OMW constituents and water availability, the latter being controlled by soil moisture and soil water repellency, and (ii) the interaction between toxic and beneficial effects of different OMW constituents. We furthermore hypothesized that (iii) the OMW-derived phenolic compounds are mainly responsible for the toxic effects on soil microarthropods.

In order to evaluate these hypotheses, we conducted field studies in two different cultivation scenarios: an olive orchard with extensive and rain-fed olive cultivation in a Mediterranean climate (Bait Reema, Palestinian Authority) and an intensively cultivated and irrigated olive orchard in a semi-arid climate (Gilat, Israel). We assessed the effects of a single OMW spreading event on soil arthropod communities and their relation to changes in soil chemical properties during three weeks of OMW-soil contact. Moreover, we compared two cultivation scenarios, expecting that the change in water availability regulates OMW impacts in Bait Reema, while toxic effects of OMW affect the development of the microarthropod community in Gilat.

2. Material and Methods

2.1. Study Design

2.1.1. Site Description

Field studies were conducted in Bait Reema (West Bank, Palestinian Authority) and in Gilat (South District, Israel); (Figure 1). The fields are representative of two typical olive cultivation practices applied in the Palestinian Authority and Israel, respectively [23,24]. Bait Reema is located within the central mountains in the West Bank with ridges more than 800 m above sea level. The olive orchard in Bait Reema (32°1′ N, 35°5′ E) represents extensive agricultural practice in the West Bank. Hot summer Mediterranean climate is predominant with an average annual precipitation of 615 mm (526.1 mm in 2011, data from Ramallah 20 km SSE of Bait Reema, Palestinian central bureau of statistics 2011 [25]). The total area of the olive orchard is 2 ha and the trees are arranged in an irregular manner and low density (50 trees ha^{-1}). Boles, branches and crown differ from tree to tree and no irrigation is applied. The soil type is a brown rendzina [26].

The Gilat Agricultural Research Center (31°20′ N, 34°40′ E) of the Israeli Agricultural Research Organization is located in the lowlands of the western Negev desert. Semi-arid climate dominates with an average annual precipitation of 213 mm (230.8 mm in 2011, data from meteorological station in Gilat). The size of the whole orchard is 0.75 ha. Olive trees in Gilat are arranged in a regular grid with a distance of 3.5 m between adjacent trees in a row and 7 m between rows (450 trees ha^{-1}). Along the rows, a drip irrigation system (drippers spaced 0.5 m along the covered drip line) is installed delivering fresh water (electrical conductivity 0.4–0.7 $mS\cdot cm^{-1}$) and fertilizers (150 kg N, 250 kg K_2O, 60–80 kg P_2O_5 $ha^{-1}\cdot a^{-1}$) two times a week during summer. The soil type is a light brown sandy loam [26].

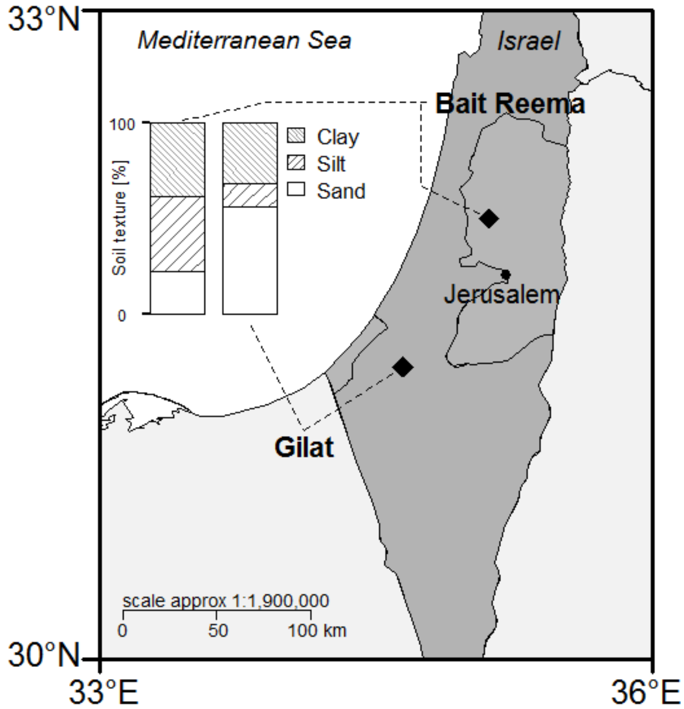

Figure 1. Map of Israel and the Palestinian Authority, asterisks indicate the two sampling sites Bait Reema and Gilat. Bars show the soil texture of both sites. Distance between the sites is approximately 100 km.

2.1.2. Experimental Design

In each study site, OMW was applied in four randomly selected shaded plots in August 2011. Four additional plots received tap water and served as control. Plots were located in the shady areas under the trees because a preliminary screening had shown sufficiently high biological activity only in those areas. In Gilat, the dry and sun-exposed areas between the tree lines are additionally compacted by tractors, and no extraction of microarthropods was possible in the upper soil layer (up to 10 cm). In Bait Reema, almost no microarthropod abundance was found in the sun-exposed areas up to the same depth. Due to site-specific reasons, plot size in Bait Reema was 2.5 × 1.5 m and in Gilat 2 × 2 m with a buffer zone of at least one meter between the plots. The OMW applied on both fields originated from an olive mill in Bait Reema extracted during the harvest season of 2010/11. The composition of the OMW is given in Table 1. Important characteristics are the high phenolic content (3.5 $g \cdot L^{-1}$), its acidity (pH of 4.6) and high potassium content (5290 $mg \cdot L^{-1}$). Application amounts were 147 $m^3 \cdot ha^{-1}$ in order to consider a worst-case scenario and to realistically simulate the effect of extreme amounts. This amount is three times higher than recommended by the Israeli government [7]. OMW was applied manually using water gardening cans in order to avoid soil disturbance and to allow equal distribution. The top soil (0–10 cm) was sampled weekly, starting three weeks before the application in order to identify natural variation in soil properties and microarthropod assemblage and to obtain a no water application control. Sampling was continued during the three weeks following OMW application in order to monitor the OMW application impacts. A pooled sample from six randomly chosen sampling points within each plot was taken and used for chemical analysis and soil microarthropod extraction. The minimum distance of the sampling points to the plot border was 20 cm.

Table 1. Characteristics of OMW from Bait Reema used for application in both fields [13]. Errors are within the last digit of each value.

Parameter	OMW
pH	4.6
EC ($mS \cdot cm^{-1}$)	10.8
Dry mass ($mg \cdot g^{-1}$)	53
DOC ($g \cdot L^{-1}$)	26
Phenols ($g \cdot L^{-1}$)	3.5
$SUVA_{254\ nm}$ ($L \cdot mgC^{-1} \cdot m^{-1}$)	1.3
K^+ ($mg \cdot L^{-1}$)	5290
Ca^{2+} ($mg \cdot L^{-1}$)	252
Mg^{2+} ($mg \cdot L^{-1}$)	171
Na^+ ($mg \cdot L^{-1}$)	105
Cl^- ($mg \cdot L^{-1}$)	1278
PO_4^{3-} ($mg \cdot L^{-1}$)	765
SO_4^{2-} ($mg \cdot L^{-1}$)	158

2.2. Soil Analysis

Soil water content was determined gravimetrically before air drying and measured on site according to ISO 11465:1993-12. A pooled sample of about 100 g from the six sampling points of each plot was taken and air dried before shipping. The highly aggregated samples were gently grinded manually, sieved to 2 mm and air dried for chemical analysis. Elemental analysis (carbon, hydrogen and nitrogen, DIN ISO 10694:1996-08, Vario micro cube, Elementar Analysensysteme GmbH, Germany), as well as loss on ignition (LOI) and carbonate content (as percentage of mineral mass) by thermogravimetric analysis (TGA) using a Simultaneous Thermal Analysis device (STA 449 F3 Jupiter, Netzsch, Germany) were determined on these soil samples. Additionally drops of 100 μL tap water were carefully placed on the soil to measure water drop penetration time (WDPT, [27]).

The pH was determined according to DIN ISO 11265:1997-06. Aqueous extracts of the soil samples were produced by shaking the soil-water mixtures for 24 h and centrifugation at a relative centrifugal acceleration of 3720 g for 10 min. Soil to solution ratio was 1:5 for determination of anions (fluoride, chloride, nitrate, phosphate and sulfate), content of soluble phenolic compounds (SPC), water extractable dissolved organic carbon (DOC) and specific ultra violet absorbance at 254 nm ($SUVA_{254\ nm}$). For determination of soluble cations, a soil to solution ratio of 1:10 was used. Cations and anions were determined on an ion chromatograph (881 Compact IC pro, Metrohm, Herisau, Switzerland). SPC was determined using the Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) resulting in a blue color complex [28] with tannic acid as a calibration standard. DOC was determined from the difference between total carbon and total inorganic carbon in the extracts using a multi N/C analyzer (2100S, Analytik Jena, Germany). Effective cation exchange capacity (CEC_{eff}), exchangeable cations and base saturation of single soil samples of each treatment and time point were determined according to DIN ISO 11260:1994-08.

2.3. Soil Arthropod Sampling and Community Analysis

Soil arthropod sampling was conducted in the early morning from 6 a.m. to 8 a.m. to avoid effects of the circadian rhythm of soil arthropods. Storage time in plastic containers was as short as possible (at most two hours) and processing of the samples followed subsequently. Soil arthropods were extracted using a modified Berlese-Tullgren extractor [29]. The 25 W filament bulbs were on top of the funnel at a distance of 20 cm to the soil surface. Specimens were collected in 70 % ethanol during 7 days of extraction. In a preliminary study, we identified 7 days as the optimal extraction time allowing a compromise between sufficient microarthropod extraction and decreasing water content and heat stress during extraction.

Individuals of Oribatid mites and Collembola were mounted in lactic acid on individual cavity slides which allow removal of pigmentation and fat, so that inner structures are visible [30]. Temporary mounts were generally used to identify small microarthropods [31]. Collembola were determined to family level according to Bellinger, *et al.* [32]. For the determination of Oribatida, the keys of Weigmann [31] were used. An Oribatid mite was classified as juvenile when typical adult structures like a thick cuticula, a complete cover through notogaster shields, lamellae or genital plates were missing or underdeveloped. Juvenile individuals were not differentiated further. Additionally, selected Oribatid mites (*Gymnodamaeus* sp., *Zygoribatula* sp. from both areas, *Zygoribatula cf. excavata*, juvenile Oribatida from both areas, *Oribatulidae*) were sent to Mark Maraun (University of Göttingen, Germany) for identification and confirmation. Other soil arthropods were determined to order level.

2.4. Data Analysis

Data were analyzed using the statistic software R, version 3.1 [33] with the packages vegan [34], MASS [35] and multcomp [36]. Shapiro-Wilk [37] and Levene's [38] test were used to analyze the data for normality and homogeneity of variance. Generalized linear models [39] were used with treatment, area and time as fixed factors. *Post hoc* tests for significant effects were Bonferroni-adjusted Tukey tests. For all tests, a significance level of $\alpha = 0.05$ was taken as default value. At the community level, Shannon-Wiener diversity index H′ [40] and Pielou's evenness were calculated using the number of specimens observed at the family level.

Treatment effects on community composition were identified by Bray-Curtis-derived [41] non-metric multidimensional scaling based on abundance at each sampling date. For this, the community matrix was Wisconsin double standardized [42]. Here, the family abundances are first divided by their maxima and then by site totals. Treatment effects on the soil arthropod community and their potential links to soil chemical properties were investigated by permutational multivariate analysis of variance, which is a robust way to describe how variation in community data is attributed to different treatments [43].

3. Results and Discussion

3.1. Comparison of Fields before Olive Mill Wastewater (OMW) Application

Soil properties of the upper 10 cm of the fields during the study period in Bait Reema and Gilat are shown in Tables 2 and 3. The soil in Bait Reema is a clayey loam with a pH of 8.2, a CECeff of 117 mmol·kg^{-1} containing 7.6 % water. In Gilat, the sandy clay loam had a pH of 8.8, a CECeff of 33 mmol·kg^{-1} and a water content of 9.1 %. With WDPT between 0 and 2 s, all soil samples in Bait Reema and Gilat were classified as wettable according to Bisdom, et al. [44]. Along with differences in management, soil texture and climate (Chapter 2.1, Figure 1), the sites in Bait Reema and Gilat also differ significantly in most investigated soil parameters (Table 2). Most striking are the higher LOI, inorganic carbon content, SPC, DOC, carbonate and water soluble ion content in Bait Reema than in Gilat. Moreover, the composition of the water soluble ions differs. Soluble fluoride, sulfate as well as total hydrogen and nitrogen content differed between both cultivation scenarios, but were unaffected by OMW and water application.

Among 6630 extracted soil microarthropods, Oribatida (60.5 %) and Collembola (16.4 %) were the dominant subclasses in both fields with the order Oribatida dominating among Acari. The remaining 25.1 % were attributed to other orders of Acari (8.9 %), Formicidae (4.8 %), Pseudoscorpionida (3.4 %), Coleoptera (2.5 %), Oniscidea (1.5 %), Araneae (1.0 %), Diptera (0.4 %), Oligochaeta (0.3 %), Myriapoda (0.2 %), Dermaptera (0.1 %) and Pauropoda (0.1 %).

Diversity and abundance for Oribatida and Collembola were higher in Bait Reema than in Gilat (Table 4, Figure 2). In Bait Reema, 52 % of Oribatida were juvenile while in Gilat this portion was 18 %. There were 14 families of Oribatida and twelve families of Collembola found in Bait Reema, whereas we found nine families of Oribatida in Gilat. Zygoribatula cf. excavata was by far the most abundant species in Gilat followed by *Zygoribatula* sp.

Table 2. Major characteristics of studied fields and chemical properties of soils before amendment with water and OMW. Errors are within the last digit of each value.

Parameter	Bait Reema	Gilat	Parameter	Bait Reema	Gilat
Annual Rainfall (mm)	19.2	20.4	**DOC (mg kg^{-1})**	590	132
Average Temperature (°C)	615	213	**SUVA$_{254\,nm}$ (L mgC^{-1}·m^{-1})**	1.8	1.9
Sand (%)	22	56	**SPC (mg TA·kg^{-1})**	35	9
Silt (%)	40	13	**EC (mS·cm^{-1})**	0.3	0.2
Clay (%)	38	31	**K^+ (mg·kg^{-1})**	357	71
Water content (%)	7.6	9.1	**Ca^{2+} (mg·kg^{-1})**	853	81
C_{tot} (g·kg^{-1})	71.3	21.5	**Mg^{2+} (mg·kg^{-1})**	80	20
LOI (g·kg^{-1})	101.5	23.4	**Na^+ (mg·kg^{-1})**	30	93
Carbonate (g·(kg mineral mass)$^{-1}$)	213	82.6	**Cl^- (mg·kg^{-1})**	67	163
δ13C (‰)	−9.2	−8.7	**NO_3^- (mg·kg^{-1})**	144	116
N (g·kg^{-1})	2.2	<0.1	**PO_4^{3-} (mg·kg^{-1})**	143	6
C/N	32.4	>215	**SO_4^{2-} (mg·kg^{-1})**	81	252
pH	8.2	8.8	**WDPT (s)**	2	1

Table 3. Effective cation exchange capacity (CECeff), exchangeable cations, base saturation (BS) and carbonates affected by application of OMW and water in Bait Reema and Gilat pre application (P) and after application of OMW (O) or water (W) during six weeks of sampling. Mean values for the whole study period followed by the same letter are not statistically different in Bait Reema or Gilat. Concerning CEC, only selected samples from each treatment were analyzed. Errors are within the last digit of each value.

	Treatment	CEC_{eff} mmol·kg^{-1}	Exchangeable Cations Na^+ mmol·kg^{-1}	K^+ mmol·kg^{-1}	Mg^{2+} mmol·kg^{-1}	Ca^{2+} mmol·kg^{-1}	BS %	Carbonate g·(kg mineral mass)$^{-1}$
Bait Reema	P	117 ^{a}	0.3 ^{a}	1.2 ^{a}	4.4 ^{a}	32.3 ^{a}	64 ^{a}	213 ^{a}
	W	117 ^{a}	0.2 ^{a}	1.3 ^{a}	5.9 ^{b}	35.0 ^{b}	71 ^{a}	218 ^{a}
	O	116 ^{a}	0.2 ^{a}	3.1 ^{b}	5.1 ^{c}	32.8 ^{a}	68 ^{a}	202 ^{a}
Gilat	P	33 ^{a}	0.5 ^{a}	0.5 ^{a}	2.1 ^{a}	12.2 ^{a}	90 ^{a}	82.7 ^{a}
	W	32 ^{a}	0.4 ^{a}	0.5 ^{a}	2.2 ^{a}	11.1 ^{ab}	87 ^{a}	87.8 ^{a}
	O	35 ^{b}	0.4 ^{a}	1.4 ^{b}	2.5 ^{a}	10.9 ^{b}	83 ^{b}	86.7 ^{a}

Table 4. Shannon-Wiener diversity index (H′) and Pielou's evenness (J) found in Bait Reema and Gilat pre application (P) and after application of OMW (O) or water (W) at the 1st, 2nd and 3rd sampling interval.

	Treatment	Bait Reema 1	Bait Reema 2	Bait Reema 3	Gilat 1	Gilat 2	Gilat 3
H′	P	1.74	1.93	1.90	0.82	0.83	0.64
	W	2.08	1.72	1.53	0.70	0.56	0.91
	O	2.22	1.81	2.27	0.55	1.12	1.23
J	P	0.83	0.85	0.85	0.81	0.67	0.73
	W	0.75	0.67	0.60	0.63	0.51	0.91
	O	0.84	0.76	0.88	0.88	0.87	0.83

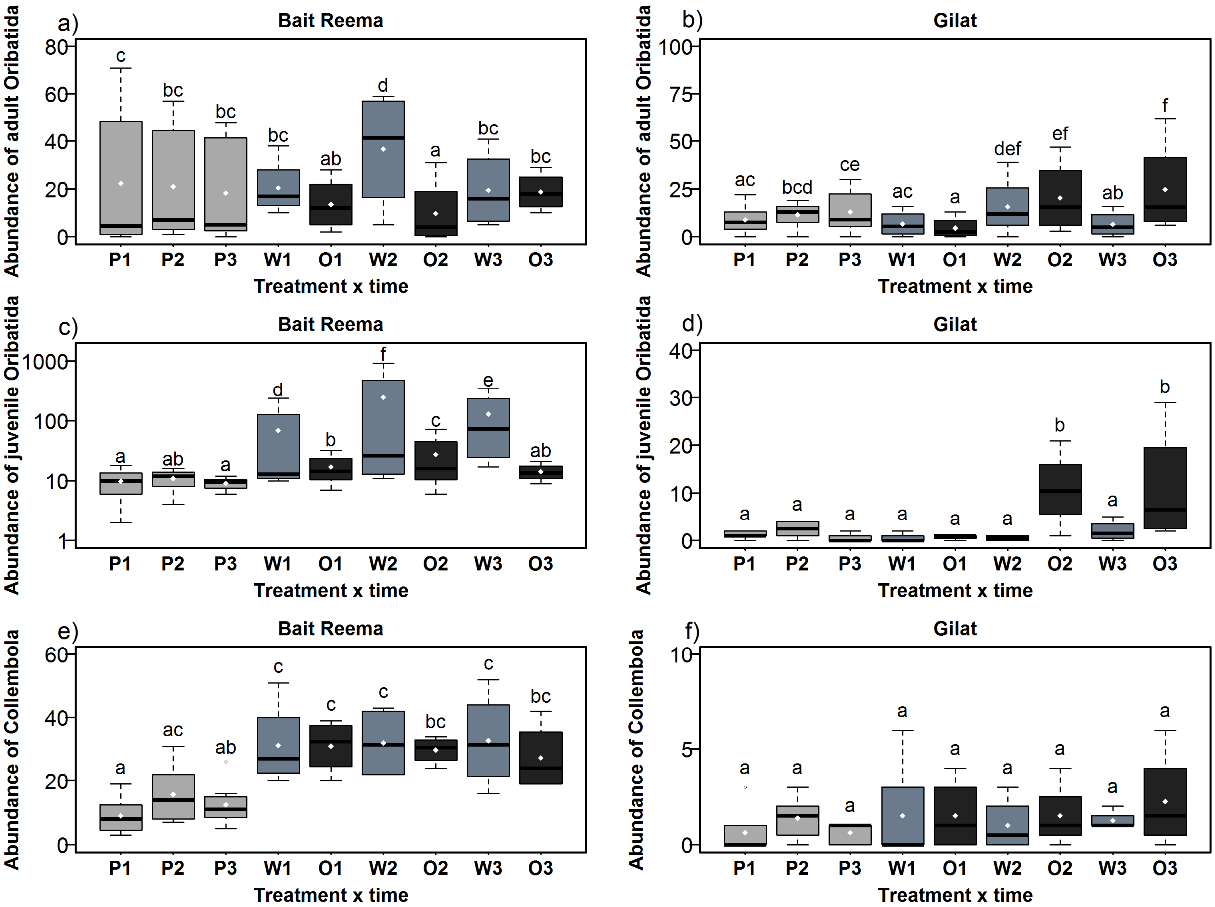

Figure 2. Box-and-whisker plots of Oribatida and Collembola abundances in different treatment groups and time in Bait Reema and Gilat. P, pre application plots; W, water-treated plots; O, OMW-treated plots at the 1st, 2nd and 3rd sampling interval. White dots show mean values. Mean values followed by the same letter are not statistically different.

The higher abundance and diversity in Bait Reema compared to Gilat can be explained by several factors. Aridity in combination with high proportions of sand [45,46], low organic matter, total carbon and nitrogen contents in Gilat [47] affects edaphic mesofauna abundance negatively. Microhabitat diversity like litter and humus complexity [48,49] are expected to trigger diversity and abundance positively. It can be expected to be higher in the extensive and near-natural management in Bait Reema than under the influence of frequent irrigation [50] and intensive agriculture [51] as is the case for Gilat. On the other hand, the lower abundance and diversity in Gilat could derive from the soil type and the extraction method: Behan-Pelletier [52] suggested that behavioral extractors like the Berlese-Tullgren funnel extract microarthropods insufficiently in arid soils. Nevertheless, the abundance in Gilat is comparable to other studies conducted in the region where Oribatida dominated the soil community as well and *Zygoribatula* sp. was the most abundant species [53].

3.2. Effects of OMW and Water Application on Soil Properties

Although the OMW applied in Bait Reema and Gilat had the same origin, the development in soil properties due to OMW application differed qualitatively and quantitatively between the two locations.

For a better understanding of the location-dependent impact of OMW application on the soil microarthropod communities, these differences will be discussed first.

In Bait Reema, OMW application increased the water content (Figure 3a). This is largely due to the regular irrigation and the coarser soil structure in Gilat, leading to leaching and prompt water redistribution in the pore system. The OMW application resulted in a clear acidification by more than 0.8 pH units in Bait Reema to pH ≈ 7.3 and by ≈ 0.3 pH units in Gilat to pH ≈ 8.5 (Figure 3b). However, only in Bait Reema did this difference remain significant throughout the three-week period. The non-persisting acidification in Gilat may be explained by irrigation-induced leaching effects. Long-term acidification effects of OMW application in Gilat and Bait Reema are unlikely due to the soils' significant carbonate content (Table 2). This is in line with other studies [6,21], which did not report acidification effects either.

The application of OMW resulted in increased soluble Mg^{2+}, Ca^{2+} and K^{+} as well as Cl^{-} and PO_4^{3-} contents with respect to control (Figure 3c, d, f–h) in both locations. Although the applied OMW was the same for both sites, the qualitative time development of soil composition and persistence of effects differed between the sites. In Gilat, both rise and decline in the soluble K^{+}, Mg^{2+} and Ca^{2+} contents after OMW application was sharp and occurred within one week of each other. In Bait Reema, the contents of these soluble cations declined more slowly and the extent of the increase was significantly lower than in Gilat. Significant surpluses compared with the pre-application contents were detected for chloride while a phosphate increase was detected only in Gilat (Figure 3). Notably, OMW application resulted in strong nitrate depletion (Figure 3).

Interestingly, the content of soluble calcium even decreased in Bait Reema by 205 $mg \cdot kg^{-1}$ with respect to pre-application conditions for both OMW and water application, whereas it increased in Gilat by 462 $mg \cdot kg^{-1}$ (Figure 3). An explanation could be a partial assimilation by plants, though it is improbable for this period of year (August), or a stronger cation adsorption on clay minerals in Bait Reema due to the higher clay content and higher CEC_{eff} than in Gilat. In general, the increased mineral nutrient content in both fields upon OMW application is consistent with previous findings [14,54] but may not be effective in the long term. Chelation of these ions by soil or OMW-derived chelating agents like polyphenols can bind these ions [55]. Cabrera, *et al.* [8] found exchangeable K^{+} contents increasing in a clayey soil throughout a study period of three years. In that study, K^{+} accounted for about 30 % of all exchangeable cations; in our field study, this share was 7.6 % in Bait Reema and 8.9 % in Gilat (Table 3).

Soil total carbon, LOI, SPC and DOC contents (Figure 4) showed a similar pattern as the mineral nutrients. All parameters increased after OMW application and decreased again from the second week on. The extent of the rise was always higher in Bait Reema compared to Gilat. OMW application significantly reduced the $SUVA_{254\ nm}$ of DOC (Figure 4), which indicates that the increase in UV inactive organic matter (*i.e.*, easily degradable sugars, proteins, amino acids [56]) due to OMW application overbalances the antagonistic effect of the UV-active phenolic compounds on $SUVA_{254\ nm}$. Interestingly, neither C_{tot}, DOC, SPC nor LOI reached their initial values within three weeks after OMW application, suggesting an incomplete degradation of phenolic OMW constituents in the three-week period. Thus, OMW application changed amount, quality and composition of the organic matter in soil after OMW application for at least three weeks.

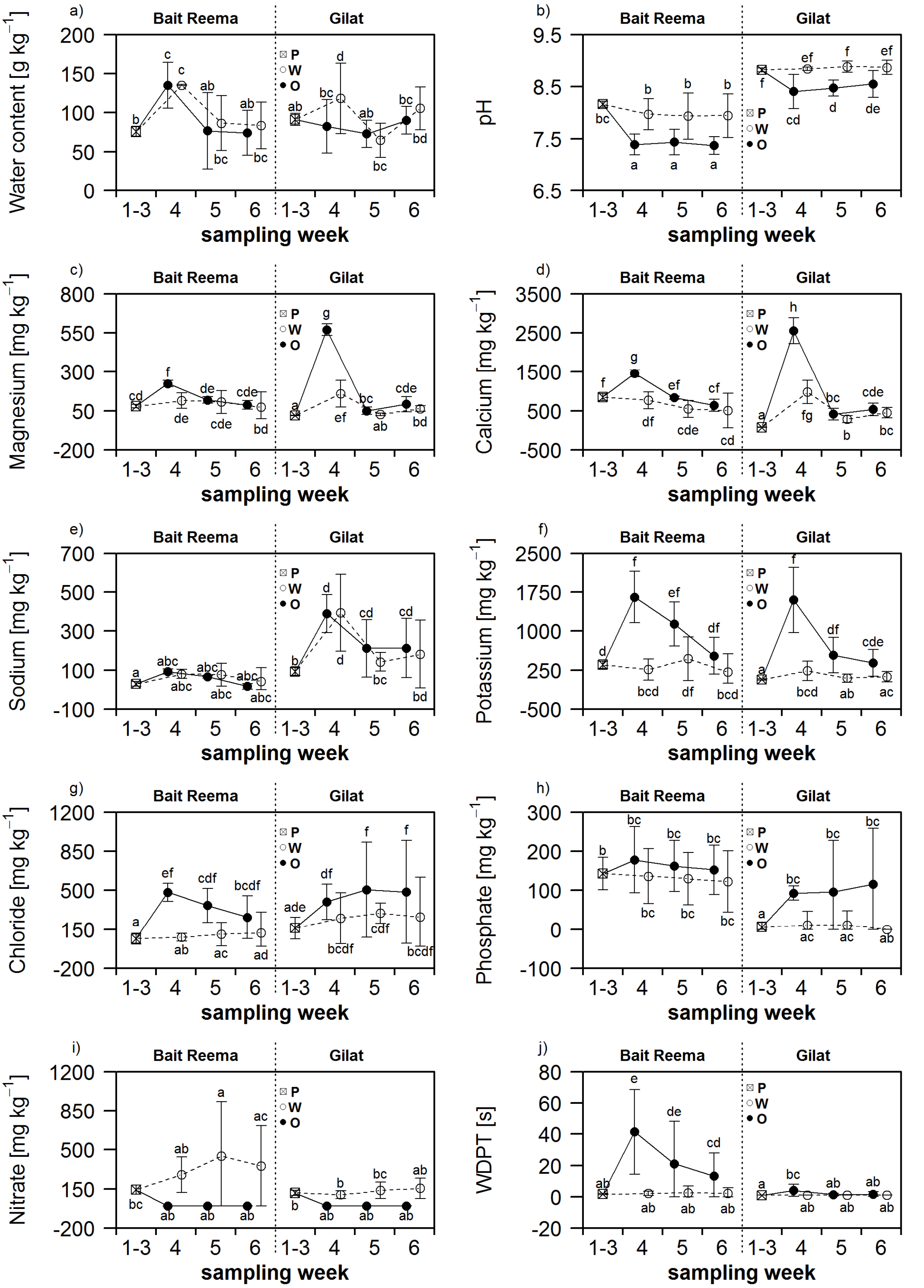

Figure 3. Evolution of water content, pH, concentration of nutrient cations as well as anions and chloride and wettability following amendment with OMW and water during six weeks of sampling in Bait Reema and Gilat. P, data before the application (were averaged after finding no statistical differences between three sampling weeks); W, water application; O, OMW application. Mean values followed by the same letter are not statistically different. Error bars show ±95 % confidence intervals.

OMW application furthermore led to a significant increase of WDPT to 41 s in Bait Reema, indicating slight water repellency, and to 4 s in Gilat, which is still classified as wettable (Figure 3). WDPT remained elevated in Bait Reema while it did not differ significantly from pre-application levels in Gilat at the end of the three-week period. The increase in soil water repellency through OMW application can be explained with its high organic content, consisting to a large extent of fatty acids and other amphiphilic molecules [57]. Its decrease with time indicates degradation of these hydrophobizing compounds [4,58] or leaching. Although stronger water repellency was expected for Gilat because coarser soils are more prone to develop water repellency compared with finer structured soils [57,59], the opposite was observed. It is possible that the irrigation in Gilat suppressed the development of water repellency and increased transport of hydrophobic compounds [60]. Also, Steinmetz *et al.* [61] found a significant effect of irrigation towards water repellency. The stronger persistence of the elevated WDPT in Bait Reema indicates that hydrophobizing compounds like grease, oil and OMW organic matter remained in the upper soil layer [62] and that water repellency has the potential to persist even after one single OMW treatment.

3.3. Effects of OMW and Water Application on Soil Arthropod Communities

OMW did not reduce the abundance of Oribatida with respect to pre-application levels in either Bait Reema or Gilat. In Gilat, OMW application even significantly increased juvenile and adult Oribatida abundance two and three weeks after OMW treatment (Figure 2), whereas water application alone had no effect. Collembola abundance in Gilat showed no effect after application with OMW or water (Figure 2). In contrast, in Bait Reema water application alone increased the abundance of adult and especially juvenile Oribatida significantly after two weeks (Figure 2). This moisture-induced emergence was suppressed in the plots where OMW was applied, but Collembola abundance was increased by both water and OMW application. Analysis of the community indices (Shannon-Wiener diversity index and Pielou's evenness index) failed to reveal significant differences between the treatments (Table 4). But in both study sites, the diversity of Oribatida increases after OMW application, indicating a community shift. Non-metric multidimensional scaling ordination (Figure 5) for Oribatida and Collembola abundance in Bait Reema revealed distinct assemblages in OMW and water-treated plots compared with plots before the application (P). Fitting of environmental parameters showed significant ($p < 0.01$) correlations only with pH. A subsequent test using PERMANOVA confirmed pH as significant parameter to explain the ordination ($p = 0.003$, d.f. = 1). Interestingly, the changes in diversity and community composition are significant despite the short sampling period and the single OMW application.

Thus, both inhibitory and beneficial effects of OMW application on Oribatida and beneficial effects on Collembola have been observed, dependent on the time after the first OMW-soil contact and the location, and these effects led to community shifts as shown for Bait Reema. These findings will be discussed in the light of the initially formulated hypotheses addressing the interplay between water availability and toxic effects (hypothesis i), the interplay between toxic and beneficial effects of OMW constituents (hypothesis ii) and the relevance of phenolic compounds for the toxic effects (hypothesis iii).

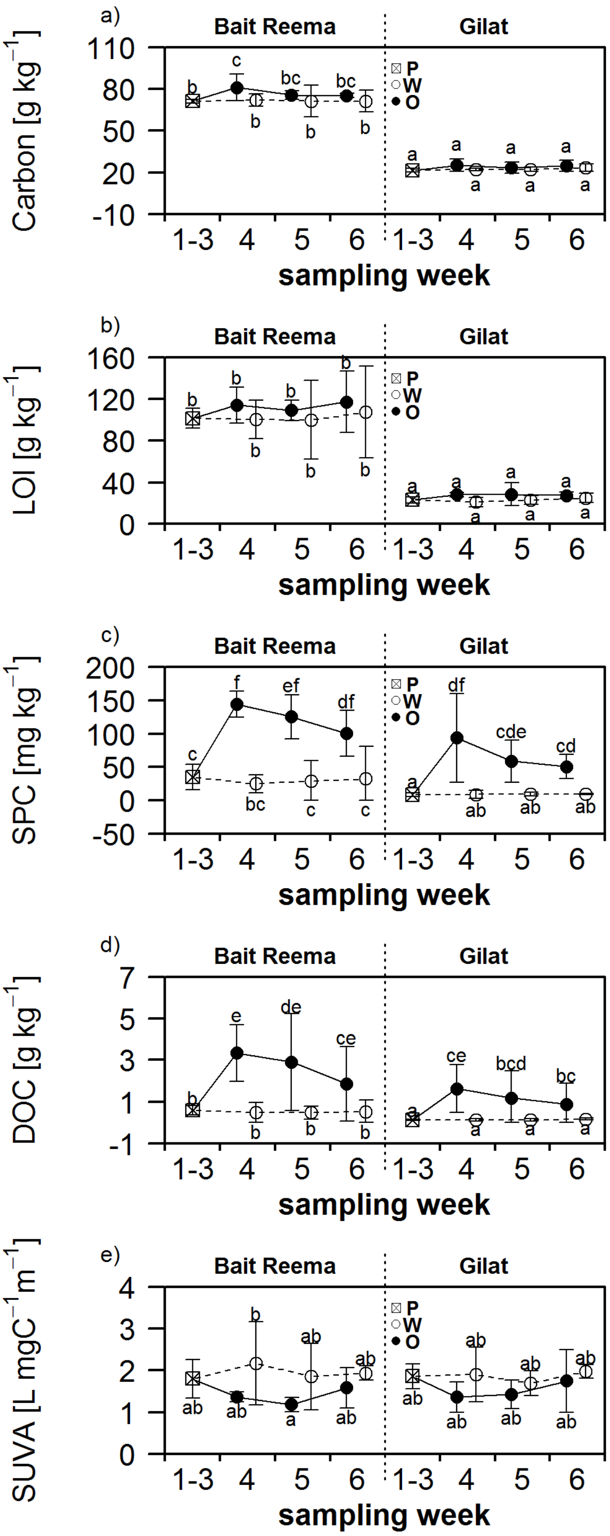

Figure 4. Evolution of soil carbon content, loss on ignition (LOI), soluble phenolic content (SPC), dissolved organic carbon (DOC) and specific UV-adsorption at 254 nm ($SUVA_{254\ nm}$) following amendment with OMW and water during six weeks of sampling in Bait Reema and Gilat. P, data before the application (were averaged after finding no statistical differences between three sampling weeks); W, water application; O, OMW application. Mean values followed by the same letter are not statistically different. Error bars show ±95 % confidence intervals.

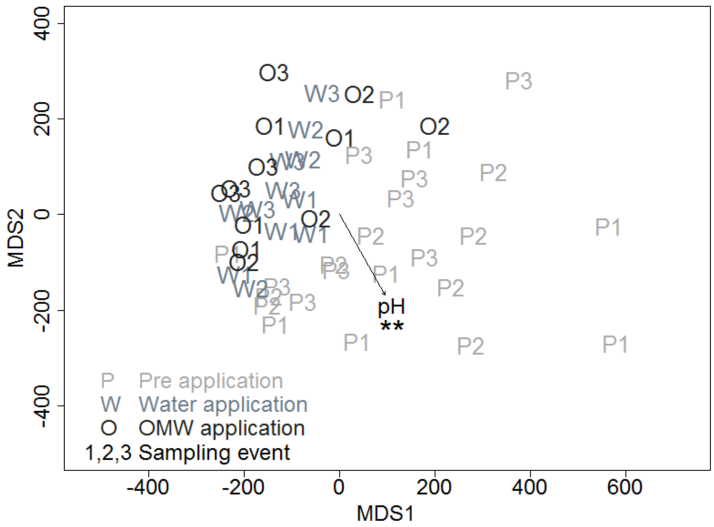

Figure 5. Non-metric multidimensional scaling based on the Oribatida and Collembola community in Bait Reema. Arrow of the explanatory variable pH points to the direction of the increasing gradient, the length is proportional to the correlation between the variable and the ordination. Ordinations are based on Bray-Curtis dissimilarity after Wisconsin double standardization of the community matrix. Asterisks code significant relationships (** $p < 0.01$) between community composition and environmental parameters. P, pre application; W, water application; O, OMW application at the 1st, 2nd and 3rd sampling interval.

3.3.1. Hypothesis I: Interplay between Water Availability and Toxic Effects

The water addition clearly increased Oribatida abundance in Bait Reema, which is in line with the observation that reproduction in edaphic communities can be triggered by soil moisture [53,63]. According to Mitchell [64], mite larvae appear when moisture conditions and food resources are more favorable in the soil, and development from egg to adult is faster in warmer climate conditions. A completion of these life cycles is known for many species of Oribatida to last eight days up to three weeks [52]. Therefore, an increased occurrence of juvenile Oribatida is likely after water application in Bait Reema. The increase in Collembola abundance due to either OMW application or water application in Bait Reema suggests that the beneficial effect of water addition overbalances the potential toxic effect of the OMW constituents, which could be due to stronger reaction on moisture conditions or due to lower toxic effects of OMW constituents towards Collembola than towards Oribatida. Nevertheless, in Gilat, where the water content was not altered by the application of OMW or water, moisture-triggered emergence of Oribatida was not expected and was not observed.

Only in Bait Reema, where reproduction could be triggered by the increased water content, did OMW application not enhance the abundance of Oribatida and thus reproduction and emergence of juveniles. Similar to our findings, Skubala and Gulvik [65] found significantly lower abundances of juvenile Oribatida in non-reclaimed toxic mine dumps compared to non-toxic dumps. This suggests a reproduction toxicity of OMW compounds towards Oribatida rather than acute effects on mortality. Another explanation could be that eggs or later development stages are held within the females' oviducts until environmental conditions are appropriate for laying. It is known that various soil amendments affect adult Oribatida abundances [66], but until now few studies have analyzed the differences of effects of soil amendments or other deteriorations on juvenile and adult stages. Juvenile stages are more sensitive to changes in soil chemistry and are more suitable to monitor effects of environmental changes [67]. Thus, more attention should be drawn to these stages in future studies.

3.3.2. Hypothesis II: Interplay between Toxic and Beneficial Effects of OMW Constituents

Whereas the emergence of Oribatida in Bait Reema was due to water addition, their increased abundance in Gilat during the second and third week after OMW addition is rather a consequence of increased availability of nutrients, increased organic matter content and quality as well as the lack of toxic effects discussed above. An increased pool of organic matter will improve living conditions for Oribatida and Collembola [52] and for microorganisms. The observed reduction in $SUVA_{254\ nm}$ indicates the presence of an increased portion of UV-inactive, labile and biodegradable compounds including carbohydrates and proteins [56], which generally can enhance biological activity. The consequently increased nitrate consumption explains the observed depletion of nitrate. Also increased phosphorous and potassium contents are known to positively influence soil fauna [68] and induce migration [69]. The lack of increased emergence of Oribatida in Bait Reema two and three weeks after OMW application indicates the persistence of the toxic effects throughout the study period, probably in combination with water deficit outbalancing beneficial effects such as the additional organic matter.

3.3.3. Hypothesis III: Interplay between Stress Factors

The observed inhibitory effects on Oribatida reproduction could be due to the polyphenolic substances present in the OMW. They are toxic towards spider mites which live on the undersides of leafs and plants [70] and they are reported to cause toxicity towards soil fauna in general [18]. This is further underlined by the fact that phenolic compounds can serve as defense substances of plants against herbivore attack [71]. The reason for the lack of suppressive effects in Gilat could be that the content of water-soluble phenolic compounds in soil was lower in Gilat than in Bait Reema; this is most probably due to the dilution provided by irrigation in Gilat [72].

Also the increase in water repellency following OMW application was more distinct in Bait Reema than in Gilat. As hydrophobic films are discussed to suppress arthropods and act as physical barrier [73,74], it cannot yet be excluded that at least part of the suppression of Oribatida emergence is due to the reduction in water availability in the hydrophobic environment.

Therefore, our initial hypothesis that the phenolic compounds account for the toxic effects cannot be rejected, but other factors, like the development of soil water repellency, may additionally have a negative impact on soil biological activity. The clarification of these aspects thus needs further research. This is underlined by the interspecies variability in soil pH preferences of soil arthropods [75,76]. Acidity could be a stress factor especially under drought conditions [77], but the pH values are still above 7, and thus, this effect is not expected to be relevant in the two investigated sites. However, the reduction in soil sodicity can improve the conditions for Collembola and Oribatida, which are both known as acidophilic and many species show a median-preferred pH from 2.9 to 7.6 [75]. Additionally, irrigation can induce changes in community composition of both Collembola and Oribatida, although this effect is mainly observed after long-term experiments [53,63,78,79]. For example Cutz-Pool *et al.* [79] identified changes of pH, electrical conductivity and organic matter to trigger long-term community changes. These soil properties are known to at least partially not recover after repeated OMW application (e.g., [13]) and, therefore, affect communities on a long-term scale.

The OMW application resulted in changes in all these parameters, and it must therefore be excluded that also phenolic compounds or increased water repellency affect the Oribatida communities. More detailed studies are required to distinguish between these effects.

4. Conclusions

While qualitative and initial effects of OMW application on soil properties are independent of the location, the extent and time dependency of these effects are strongly determined by soil texture, soil properties and field management. The understanding of the effects of OMW application to soil and their severity requires increased consideration of soil moisture conditions, the sorptive capacity of the soil for nutrients and irrigation-derived leaching and dilution effects.

Generally, OMW has the potential to affect the soil microarthropod community, but the locational and managerial factors are decisive for the interplay between beneficial or suppressing effects of OMW application. Especially the moisture conditions determine how the microarthropod community reacts to the OMW application: Under the influence of irrigation, toxic compounds will be diluted and leached. As a result, their inhibitory impacts may not become effective under irrigation. Consequently the beneficial effect of nutrient addition on the arthropods may overbalance the OMW-induced toxicity after a few days as observed in our study. In contrast, OMW addition into un-irrigated soils may suppress Oribatida reproduction with lasting effects. Although this study suggests pH changes as the main trigger of the community shifts, the responsibility of phenolic compounds and potentially water repellency have to be considered as stress factors.

This pioneering study clearly shows that the effects of OMW application on soil biota need to be better understood in order to be able to judge its potential impact on biological soil functions, and, even more important: the understanding requires consideration of locational and managerial factors. Furthermore, OMW application impacts need to be understood on a long-term basis to be able to understand community changes and accompanied changes in nutrient mineralization, bioturbation and disease control. An important step towards greater comprehension will be the investigation of toxicity of OMW constituents towards soil arthropods and their effect mechanisms in targeted laboratory studies.

The dilution of OMW with water or the temporary irrigation after OMW application could attenuate toxic effects for the sake of positive effects on soil arthropod communities. Therefore, dilution of toxic compounds by rainfalls during winter and spring would be a cost-effective strategy for OMW application, but this will induce a currently unknown risk of leaching of the toxic compounds towards deeper soil layers and into the groundwater.

Generally, OMW application needs to be carefully adapted with regard to soil and climatic conditions, as well as cultivation scenarios to be able to profit from its potential fertilizing properties. Using a generalized application rate is, therefore, not a sustainable agricultural practice.

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Author Contributions

The work presented is a part of a Ph.D. thesis; the work is part of the TRILAT-OLIVEOIL project planned and conceptualized by G.E. Schaumann, M. Borisover, A. Marei and J. Shoqeir. The information and data were collected and summarized by M.P. Kurtz who took the lead on writing the manuscript. A. Dag, I. Zipori and J. Shoqeir supported the field work. A. Dag, I. Zipori and J. Shoqeir, C. Brühl, B. Peikert and G.E. Schaumann supervised the work and edited the manuscript.

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