

Article

Olive Pomace-Derived Compost: Phytotoxicity Assessment and Relevance for Soil Systems

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Abstract

Olive pomace (OP) contains phytotoxic compounds that can impair plant growth and soil quality. Composting provides an effective method for detoxifying olive pomace (OP) and improving its suitability for agricultural use. Therefore, this study investigated the phytotoxic effects of raw olive pomace filtrate (OPF) on seed germination in radish (*Raphanus sativus* L.) and barley (*Hordeum vulgare* L.), as well as the impact of composted olive pomace (COP) on their growth. Seeds were exposed to OPF at concentrations of 0% (control), 1%, 3%, 5%, 10%, 20%, and 100%. Additionally, three composting treatments were evaluated: R1 (control: OP + barley straw), R2 (OP + barley straw + urea), and R3 (OP + barley straw + sheep litter). Results showed that OPF at concentrations of 10%, 20%, and 100% significantly reduced seed germination, with complete inhibition at concentrations > 10%. The COP treatments showed different physicochemical properties, such as R2 exhibiting better nutrient availability (C/N = 19, oil content = 0.04%). R3 had the highest concentrations of K (40,430.2 mg/kg) and P (6022.68 mg/kg). Results also indicated that R1 significantly reduced radish dry biomass production compared to barley, although R2 performed slightly better than R1 and R3. The findings highlight the need for proper compost stabilization to minimize the phytotoxicity and improve the agricultural potential of COP for improving soil health.

Keywords: agricultural potential; compost stabilization; olive pomace filtrate; photosynthetic pigments; physicochemical properties; seed germination



Academic Editors: Remigio Paradelo Núñez and Antonio Gelsomino

Received: 6 August 2025

Revised: 9 September 2025

Accepted: 29 September 2025

Published: 30 September 2025

Citation: Javed, Q.; Bouhadi, M.; Palčič, I.; Anđelini, D.; Cvitan, D.; Major, N.; Lukić, M.; Goreta Ban, S.; Ban, D.; Heath, D.; et al. Olive Pomace-Derived Compost: Phytotoxicity Assessment and Relevance for Soil Systems. *Soil Syst.* **2025**, *9*, 107. <https://doi.org/10.3390/soilsystems9040107>

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1. Introduction

The Mediterranean region is the world's largest producer of olive oil, accounting for approximately 70% of global production [1]. Within the European Union, production is dominated by Spain (63%), Italy (17%), Greece (14%), and Portugal (5%), together accounting for 99% of the EU's total production [2]. Across these four countries, 11.6 million hectares of land are dedicated to olive cultivation. Olive oil production is deeply rooted in the history, economy, and culture of the Mediterranean region, making it a cornerstone of agricultural activity [3]. In response to growing global demand, production has steadily increased, supported by advances in extraction technologies that have significantly improved yield efficiency [4,5]. However, this expansion produces large quantities of byproducts, mainly olive pomace (OP) [3,6,7].

Olive pomace is the solid residue remaining after olive oil extraction, consisting of a mixture of olive skin, pulp, crushed seeds, and residual oil [8]. Depending on the extraction method, OP can account for up to 35–40% of the total olive weight [9]. While OP contains valuable organic matter, its high concentrations of polyphenols, tannins, and organic acids [10,11] could cause serious environmental issues, such as soil degradation [12], water pollution, and microbial imbalances, if not properly managed [13]. Furthermore, the presence of potentially phytotoxic compounds limits its direct application as a soil amendment, as it can negatively impact seed germination and plant development [14,15]. Moreover, olive mill waste from the two-phase system, containing about 60–70% of moisture, can have phytotoxic and antimicrobial characteristics due to its acidic pH (4.2 to 5.6), higher EC levels (2.1 to 7.2 mS/cm), high C/N (43 to 94), the presence of phenolic substances (0.33 to 0.81% d.w.), remaining oils (0.98 to 8.12% d.w.) and various organic acids [16].

Studies have shown that the phenolic compounds in OP can exhibit phytotoxic effects, interfere with nutrient availability, increase soil salinity, and create unfavorable growing conditions for plant species [17,18]. For example, fresh OP can inhibit the germination of sensitive crops such as lettuce (*Lactuca sativa* L.) [19], tomato (*Solanum lycopersicum* L.) [20], and wheat (*Triticum aestivum* L.) due to the presence of polyphenols that interfere with root and shoot elongation [19]. Similarly, research indicates that barley (*Hordeum vulgare* L.) experiences reduced germination rates and stunted seedling growth when exposed to raw OP [21], and radish (*Raphanus sativus* L.), known for its fast germination, has shown significant root inhibition when grown in soil amended with untreated OP [22]. High concentrations of phenolic compounds can also suppress the growth of leguminous plants such as *Vicia faba* L. [23]. Therefore, understanding the extent of its phytotoxic effects due to its total phenolic content, high salinity (EC), and ammonium–nitrogen content is essential for evaluating its potential applications in agriculture.

The observed phytotoxicity of OP also highlights the need for its effective treatment before its application to agricultural soils. One promising method is composting [24,25]. Composting is a biological degradation process in which microorganisms break down organic materials into a stable, nutrient-rich product [26]. During this process, harmful polyphenols and organic acids can be degraded or transformed, reducing their toxicity and enhancing soil fertility [27,28]. Composted OP offers benefits, such as improved soil structure and fertility, increased microbial activity, and enhanced plant productivity [25,29]. However, raw OP is unsuitable for composting due to its low nitrogen content and high C/N ratio. However, it does have a high organic matter content (92.1%) and levels of K (7 to 39 g/kg d.w.), while lower contents of other macronutrients, such as N (9.5 to 11.7 g/kg d.w.), P (700 to 2200 mg/kg d.w.), Ca (1.7 to 9.2 g/kg d.w.), Mg (810 to 3800 mg/kg d.w.) and Fe (78 to 1462 mg/kg d.w.), which strongly supports its use as a fertilizers [30,31].

Additionally, raw OP exhibits low porosity caused by its high moisture content and other physical and chemical properties [32]. Therefore, it is recommended to mix fresh OP with organic structural materials, such as wheat straw, olive leaves, pruning residues, or green waste, to improve aeration in the initial composting mixture [33]. Moreover, the high C/N should be adjusted using manure or urea as an N source, thus ensuring a balanced carbon-to-nitrogen ratio for efficient organic matter breakdown [34,35]. Despite these recommendations, the extent to which composting reduces the phytotoxicity of OPF on plant germination and growth remains a key research question requiring further investigation.

To evaluate compost maturity, plant-based bioassays, such as seed germination tests and calculation of germination index (GI), are widely used across different composted materials, including olive pomace compost [36]. Common test species include garden cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus* L.), fenugreek

(*Trigonella foenum-graecum* L.), and barley (*Hordeum vulgare* L.), selected for evaluation of compost maturity because of their rapid germination [37]. GI values above ~90% are generally considered non-toxic and indicate compost maturity. For example, Diacono et al. [38] reported GI values exceeding 90% for cress with olive pomace compost extracts, while Ameziane et al. [25] observed high germination rates for fenugreek seeds under similar conditions, confirming compost stability and absence of phytotoxicity. These bioassays complement physicochemical indicators (e.g., C/N ratio, EC) and provide a reliable, biologically relevant measure of compost maturity [39].

This study focuses on two aspects: OPF and composted olive pomace (COP), where the authors solve this problem in two ways. The first approach is to verify the reliability of this study and demonstrate the necessity of OP composting. Secondly, it aims to verify the toxicity of composted OP as a fertilizer solution and compare it with fresh olive pomace filtrate to demonstrate the reduction in pomace toxicity through composting. In this study, radish and barley were used as bioassay indicators of compost maturity. Both radish and barley are sensitive to stress conditions such as salinity and the presence of phytotoxic compounds, making them suitable indicators of compost phytotoxicity and nutrient availability. Additionally, their relatively short growth cycles allow for rapid assessment of compost maturity and its potential effects on plant growth [37]. Therefore, the objective of this study was to investigate the potential effects of raw and COP on seed germination and early plant growth parameters (biomass production) in radish and barley, to assess compost maturity and the reduction in phytotoxicity through composting. Further, it investigated the effect of composting OP with different amendments, including barley straw, urea, and sheep litter, to assess composting as a suitable soil amendment for agricultural use and effective management of olive oil production waste. The study is significant for its contribution to waste valorization, soil amendment development, and sustainable agriculture. It will also provide scientific evidence on the phytotoxicity thresholds of OP and compare the effectiveness of various composting treatments, which can inform future strategies for agricultural reuse of organic waste in a circular economy framework.

2. Materials and Methods

2.1. Experiment 1: Evaluation of Olive Pomace Phytotoxicity Using Germination Test

Radish (*Raphanus sativus* L.; cultivar: saxa) and barley (*Hordeum vulgare* L.) seeds were selected as bioindicators for the germination experiment. Fresh OP samples were collected from a local two-phase olive mill (Istria, Croatia). The OP was filtered using filter paper (Munktell 21/N, Ahlstrom, Germany), and the resulting filtrate was diluted with deionized water to prepare a series of OP filtrate (OPF) test concentrations: 0% (control), 1%, 3%, 5%, 10%, 20%, and 100%. Distilled water was used as the control treatment for seed germination, and the germination index (GI) was calculated relative to this control. The average ($n = 4$) chemical characteristics represent the average composition of the undiluted (100%) OPF, which were as follows: pH: 4.6 ± 0.2 ; electrical conductivity: 12.7 ± 0.3 mS/cm; total C: 40.0 ± 1.2 g/L; total N: 0.4 ± 0.2 g/L; total phenols: 483 ± 53 mg/L; $\text{NO}_3\text{-N}$: <0.079 mg/L; and $\text{NH}_4\text{-N}$: 0.93 ± 0.05 mg/L.

2.2. Germination Index

The germination assay was conducted in 9 cm diameter plastic Petri dishes, each lined with filter paper as the substrate. Dishes were labeled according to treatment and moistened with the corresponding concentration of OPF. The radish and barley seeds were first rinsed with deionized water and then surface-sterilized in a 2% sodium hypochlorite (NaClO) solution for 15 min to eliminate potential microbial contaminants. The seeds

were then thoroughly rinsed with distilled water to remove any traces of NaClO. Twenty sterilized seeds were placed in each Petri dish, and the filter papers were kept moist with the corresponding treatment solutions throughout the experimental period. We used 20 seeds for both radish and barley to maintain uniformity across treatments and ensure sufficient replication for statistical analysis. Although barley seeds are larger, the 9 cm Petri dishes provided enough space for seed placement without overlap, and preliminary trials confirmed that this density did not restrict germination. Each treatment was replicated four times. A blank filter paper served as the control. All seeds were incubated at 25 °C for 24 h. We aimed to assess the early germination response to fresh and composted olive pomace filtrates rather than complete seedling development. Using a uniform 24 h incubation period for both radish and barley allowed us to directly compare their responses under identical conditions and avoid bias introduced by species-specific germination times. The germination index (GI) was calculated using the method described by Baruah et al. [40] as follows:

$$\text{Germination index (GI) (\%)} = \frac{\text{number of seeds germinated}}{\text{total number of seed}} \times 100 \quad (1)$$

2.3. Experiment 2: Compost Production, Growth Test, and Assessment of Compost Maturity Composting

Three bioreactors were used to compost olive pomace (OP), which are custom-made vertical cylindrical drums with a storage capacity of half a ton, designed to simulate controlled aerobic composting conditions. Each bioreactor is equipped with sensors to monitor temperature and oxygen levels. The temperature probes were placed at different depths, and oxygen probes were positioned near the top of each reactor (Figure 1). The airflow was 0.4 m³/h to ensure aerobic conditions. Temperature was recorded at 30 min intervals. The complete temperature dynamics and oxygen concentration are provided in Figures S1 and S2. Each bioreactor was then prepared as follows: Bioreactor-1 (R1 used as control) contained 120 kg of fresh OP (76.9%) and 36 kg of barley straw (23.1%; total 156 kg); Bioreactor-2 (R2) used the same OP (76.9%) and straw amounts (23.0%) with an additional 861 g of urea (0.1%; total 156.86 kg); Bioreactor-3 (R3) included 120 kg of OP (72.3%), 36 kg of straw (21.7%), and 37 kg of sheep litter (6.0%). Treatments varied in the type of nitrogen source added, i.e., no added nitrogen source, urea, and sheep litter, reflecting typical composting scenarios. Barley straw was added as a constant baseline component. The composting process lasted for 5 months, during which the contents were turned every three weeks to maintain aerobic conditions and promote microbial activity. For chemical analysis, 1000 g of raw OP was oven-dried at 60 °C for three days (UF160, MEMMERT, Schwabach, Germany), then equilibrated at room temperature to a constant weight. Finally, the samples were ground to pass through a 1 mm mesh sieve (RETSCH-Allee 1-5, 42781 Haan, Germany) and then homogenized.

Total nitrogen (TN) was determined using the Kjeldahl method (VELP-Scientifica, Usmate Velate, Italy), following the method described by Bremner and Mulvaney [41]. Total organic carbon (TOC) was determined using a solid sample combustion unit (Shimadzu Corporation, Kyoto, Japan) as described by Moschou et al. [42]. pH (1:2.5 H₂O) and electrical conductivity (EC) (1:5 H₂O) were measured using ISO standard procedures [16]. Dry matter (DM) was determined following U.S. Environmental Protection Agency (EPA) [43], and organic matter (OM) via ignition at 750 °C for 4 h [44]. Total phenolic content (TPC) was measured using the Folin–Ciocalteu method as described by Singleton et al. [45]. Gallic acid was used as a standard to prepare a calibration curve (0–200 mg L^{−1}). Briefly, 0.5 mL of sample extract was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. The reaction mixture was incubated for 30 min at room

temperature ($\approx 25\text{ }^{\circ}\text{C}$) in the dark, and absorbance was measured at 765 nm. The residual oil content was determined according to Brkić et al. [46].



Figure 1. Visual presentation of bioreactors.

The levels of Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, and Zn were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Shimadzu, Japan) following microwave-assisted digestion (ETHOS UP, MILESTONE, ICP-OES, Shimadzu, Kyoto, Japan), as described by Černe et al. [47]. The analytical accuracy was verified using the compost reference material MARSEP 225 (WEPAL, Wageningen, The Netherlands), with results falling within $\pm 10\%$ of certified values.

2.4. Growth Test

The maturity of COP was assessed using the relative plant growth test following Itävaara et al. [48], which defines toxicity as “the ratio of the total biomass dry matter per control biomass dry matter,” where the compost is considered toxic if growth is $< 80\%$, compared to commercial growing substrate. The method involved first sieving the compost (4 mm mesh) to ensure uniform particle size. Once homogenized, approximately 200 g of the sieved compost was mixed with a commercial growing substrate in a 1:1 ratio (v/v). The commercial growing substrate is used as the reference material to compare the growth responses against compost treatments. Once prepared, the radish and barley seeds (five per pot) were sown in the pots with the compost medium, with four replicates per treatment. The pots used for growing the plants had a length of 15 cm and a diameter of 12 cm. The pots were maintained in a growth chamber under controlled conditions: a 16-h/8 h light/dark photoperiod, a $25\text{ }^{\circ}\text{C}/16\text{ }^{\circ}\text{C}$ day/night temperature cycle, and 60% relative humidity. The continuous fluorescent light intensity of $300\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ was used for seedlings, and when the seedlings started growing, we increased it to $800\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$.

After 15 days, the biomass of both the root and shoot (both fresh and dry) was recorded, corresponding to the two-leaf stage for both radish and barley, to assess early growth responses to the treatments. The relative plant growth was determined according to Equation (2).

$$\text{Relative plant growth} = \frac{\text{total biomass dry matter to each treatment}}{\text{dry biomass matter in control}} \times 100 \quad (2)$$

2.5. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) in SPSS (version 22.0; SPSS Inc., Chicago, IL, USA). Post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test at a significance level of $p < 0.05$ to assess differences among olive pomace flour (OPF) concentrations (0%, 1%, 3%, 5%, 10%, 20%, and 100%) and composting treatments (R1, R2, and R3). The same procedure was used to compare the physicochemical properties of composted olive pomace (COP) across treatments. Before conducting one-way ANOVA, the assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk test and Levene's test, respectively. Additionally, the authors used a Pearson correlation analysis in SPSS to identify significant relationships between soil physicochemical parameters and elemental content. Data visualization was performed using OriginPro 9 (64-bit; OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Effect of Olive Pomace Filtrate on Seed Germination

The results showed a significant, dose-dependent inhibition of seed germination in radish and barley when OPF concentration increases ($p < 0.05$; Figure 2). In the control group, 18 radish seeds germinated, compared to 17, 12, and 4 seeds at 1%, 3%, and 5% OPF, respectively, representing reductions of 5.6%, 33.3%, and 77.8%. Complete inhibition (100% reduction in germination) was observed at 10%, 20%, and 100% OPF concentrations (Figure 2a). A similar trend was observed in barley: 14 seeds germinated in the control, whereas only 10 and 9 seeds germinated at 1% and 3% OPF, corresponding to decreases of 28.6% and 35.7%, respectively. No germination occurred at OPF concentrations of 5% and above, indicating complete inhibition (Figure 2a).

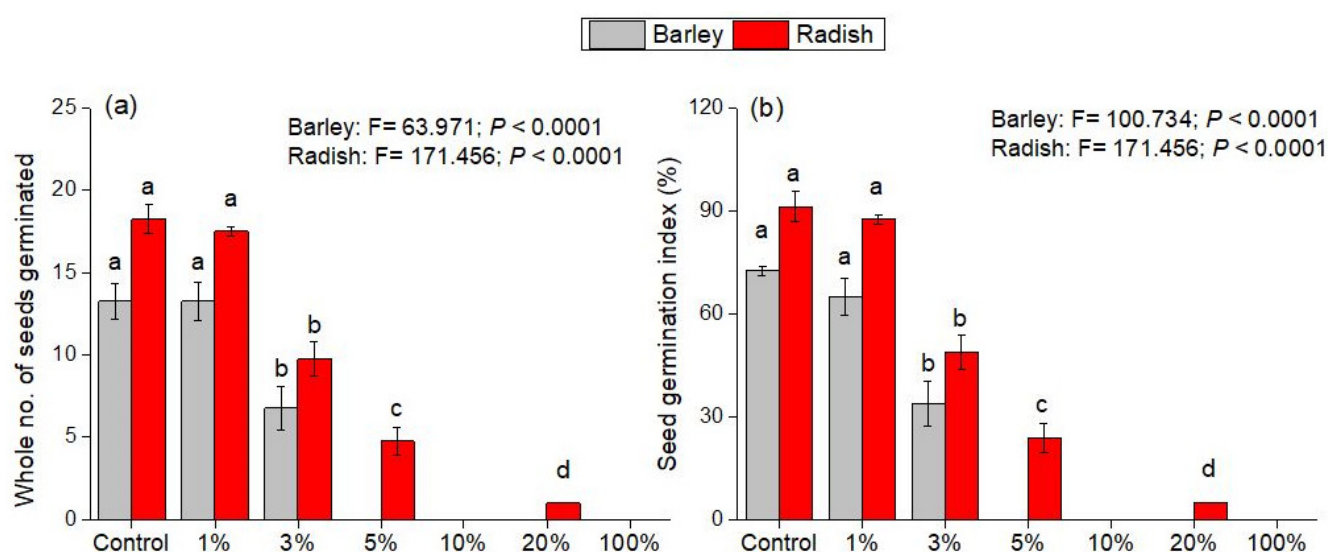


Figure 2. Effect of different OP filtrate concentrations on seed germination (a), and germination index (b). The data are presented as mean $\pm$ standard error ($n = 4$). Different letters above the error bars indicate statistically significant differences between treatments ($p < 0.05$) as determined by Tukey's test.

Similarly, the germination index (GI) for radish declined with increasing OPF concentrations, with values of 91.25%, 87.50%, 48.75%, and 23.75% at 0% (control), 1%, 3%, and 5% OPF, respectively. The GI further declined to 0% at 10% OPF, slightly increased to 5% at 20%, and dropped again to 0% at 100% OPF. For barley, the GI decreased from 72.5% (control) to 65% and 33.75% at 1% and 3% OPF, respectively. Complete inhibition (0%) occurred at values greater than 5% OPF (Figure 2b).

3.2. Composting Characteristics

The initial physicochemical properties of the composting substrates, including straw, raw OP, and sheep litter, demonstrated marked variability. Raw OP exhibited the highest OM (96.8%) and a relatively high oil content (1.9%), with a low pH (4.96) and a high TPC of 12.18%. In contrast, sheep litter was more alkaline (pH 7.15), with a high electrical conductivity (8162 $\mu\text{S}/\text{cm}$) and a significantly higher ammonium–nitrogen concentration (10,303 mg/kg). Straw, although high in OM (93.8%), had the lowest TN content (0.30%) (Table 1).

Table 1. Physicochemical properties of the individual material before mixing, i.e., straw, raw olive pomace, and sheep litter.

Material	Analysis	Measured Parameters												
		pH	EC [$\mu\text{S}/\text{cm}$]	Ash [%]	Organic Matter [%]	TPC [%]	TN	TC	C/N	Dry Matter [%]	Moisture Content [%]	Oil Content [%]	$\text{NO}_3\text{-N}$ [mg/kg]	$\text{NH}_4\text{-N}$ [mg/kg]
Straw	Mean	5.54	122	6.16	93.8	4.72	0.30	37.7	127	/	/	/	/	/
	N	5	5	5	5	5	5	5	5	/	/	/	/	/
	SE	0.08	4	0.04	0.04	0.31	0.02	1.3	6.8	/	/	/	/	/
Raw olive pomace	Mean	4.96	2692	3.18	96.8	12.18	0.97	48.4	50	33.0	67.0	14.79	1.9	<0.38
	N	5	5	5	5	5	5	5	5	5	5	5	5	5
	SE	0.01	89	0.04	0.04	0.33	0.03	0.2	1.6	0.5	0.5	0.15	0.3	/
Sheep litter	Mean	7.15	8162	18.05	81.9	4.91	2.51	34.4	14	43.8	56.2	/	0.6	10,303
	N	5	5	5	5	5	5	5	5	5	5	/	5	5
	SE	0.05	199	0.35	0.3	0.16	0.18	0.3	1.0	0.8	0.8	/	0.2	634

Values are presented as mean $\pm$ SEM (standard error of the mean). All data, except pH, EC, DM, total phenolic content, and residual oils, are expressed on a dry-weight basis. N = number of replicates.; TPC corresponds to total phenolic content; EC corresponds to electrical conductivity; TN and TC correspond to total nitrogen and total carbon. The mark “/” used in the table represents values that were not detected in our measurements.

The composting treatments in the three bioreactors (R1, R2, and R3) exhibited distinct differences in their physicochemical properties (Table 1). R1 had the highest moisture content (51.7%) and C/N ratio (31). In contrast, R2 and R3 showed lower C/N ratios (19 and 17, respectively). OM content was highest in R1 (85.4%) and R2 (85.8%), whereas R3 had a lower percentage (80.4%). EC was significantly higher in R3 (9632 $\mu\text{S}/\text{cm}$) compared to R2 (5958 $\mu\text{S}/\text{cm}$) and R1 (4670 $\mu\text{S}/\text{cm}$), indicating a higher salt content in R3. The pH values ranged from slightly acidic to neutral in R1 (6.93) and R2 (7.13), while R3 was more alkaline (7.74). Total nitrogen was highest in R3 (2.11%), followed by R2 (1.96%), and lowest in R1 (1.22%), reducing the nitrogen available for microbial activity. The TPC was similar across treatments, with slightly higher values in R3 (3.42%), while the oil content was highest in R1 (0.09%), followed by R2 (0.04%) and R3 (0.05%) (Table 2). Nitrate–nitrogen ($\text{NO}_3\text{-N}$) levels remained <0.40 mg/kg across all treatments, while ammonium–nitrogen ($\text{NH}_4\text{-N}$) was highest in R2 (396 mg/kg), followed by R1 (293 mg/kg) and R3 (222 mg/kg) (Table 2).

Table 2. Physicochemical properties of composted olive pomace.

Tr.	Analysis	Ash [%]	Organic Matter [%]	Moisture [%]	Dry Matter [%]	EC [μS/cm]	pH	TC [%]	TN [%]	C/N	TPC [%]	Oil Contents [%]	NO ₃ -N [mg/kg]	NH ₄ -N [mg/kg]
Bioreactor-1	Mean	14.5 b	85.4 a	51.7 a	48.3 ab	4670 c	6.93 a	37.8 a	1.22 b	31 a	2.93 a	0.09 a	<0.40	293 b
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	0.60	0.60	1.004	1.00	413	0.08	0.10	0.02	0.70	0.31	0.01	/	26
Bioreactor-2	Mean	14.2 b	85.8 a	46.1 b	53.9 a	5958 b	7.13 a	37.7 a	1.96 a	19 b	3.05 a	0.04 b	<0.40	396 a
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	0.20	0.20	0.40	0.40	448	0.13	0.30	0.01	0.10	0.26	0.02	/	10
Bioreactor-3	Mean	19.6 a	80.4 a	46.3 b	53.7 a	9632 a	7.74 a	35.3 b	2.11 a	17 b	3.42 a	0.05 b	<0.40	222 b
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	0.10	0.10	0.40	0.40	112	0.05	0.10	0.02	0.20	0.30	0.02	/	10

Statistically significant differences between treatments ($p < 0.05$), as determined by Tukey's test, are indicated by different letters. Values are presented as mean $\pm$ SEM (standard error of the mean). All data, except pH, EC, DM, total phenolic content, and residual oils, are expressed on a dry-weight basis. N = number of replicates.; TPC corresponds to total phenolic content; EC corresponds to electrical conductivity; TN and TC correspond to total nitrogen and total carbon. The composition of bioreactors: Bioreactor-1 (control): 120 kg olive pomace (76.9%) + 36 kg barley straw (23.1%); total 156 kg; Bioreactor-2 (urea): 120 kg olive pomace (76.9%) + 36 kg barley straw (23.0%) + 0.861 kg urea (0.1%); total 156.86 kg; Bioreactor-3 (sheep litter): 120 kg olive pomace (72.3%) + 36 kg barley straw (21.7%) + 37 kg sheep litter (6.0%); total 193 kg.

Elemental analysis revealed significant variations among the treatments (Table 2). R2 had the highest levels of Ca (29,483.0 mg/kg) and S (4035.3 mg/kg), while R1 and R3 had comparatively lower levels. Conversely, R3 had the highest levels of Mg (2869.9 mg/kg), K (40,430.2 mg/kg), and P (6022.68 mg/kg), whereas R1 had the lowest levels: Mg: 1192.9 mg/kg; K: 30,843.1 mg/kg; P: 1498.07 mg/kg. Cadmium, Co, Cr, Cu, Mo, Ni, and Pb were below the detection limit. Moreover, there was no significant difference between the levels of Li in all bioreactors, while Mn (97.2 mg/kg) and Zn (119.5 mg/kg) levels were highest in R3, followed by R2 (81.0 mg/kg and 76.5 mg/kg), and lowest in R1 (53.3 mg/kg and 26.6 mg/kg) (Table 3).

Table 3. Elemental contents of composted olive pomace.

Tr.	Analysis	Ca [mg/kg]	Mg [mg/kg]	K [mg/kg]	P [mg/kg]	S [mg/kg]	Al [mg/kg]	Cr [mg/kg]	Cu [mg/kg]	Fe [mg/kg]	Li [mg/kg]	Mn [mg/kg]	Zn [mg/kg]
Bioreactor-1	Mean	21,714.2 c	1192.9 c	30,843.1 c	1498.07 c	3000.6 c	3272.5 a	<0.5	<0.5	1256.3 a	29.0 a	53.3	26.6 c
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	3235.5	19.9	433.8	686.8	73.0	53.4	0.0	0.0	182.6	0.8	5.5	3.0
Bioreactor-2	Mean	29,483.0 a	2235.5 b	38,794.4 b	2900.8 b	4035.3 a	1772.9 c	<0.5	<0.5	1108.5 b	29.2 a	81.0	76.5 b
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	1162.5	431.7	3219.7	32.3	444.1	20.9	0.0	0.0	101.1	0.6	15.5	22.7
Bioreactor-3	Mean	27,111.3 b	2869.9 a	40,430.2 a	6022.68 a	3849.8 b	2248.5 b	15.2	13.2	1231.4 a	24.0 b	97.2	119.5 a
	N	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	SE	900.1	32.7	773.9	132.1	503.0	50.4	6.2	5.4	31.2	2.0	7.7	2.2
EU Limit values for soil improvers ¹								100	100	/	/	/	300

Statistically significant differences between treatments ($p < 0.05$), as determined by Tukey's test, are indicated by different letters. Values are presented as mean $\pm$ SEM (standard error of the mean). All data are expressed on a dry-weight basis. N = number of replicates. The maximum limits for Cr and Cu are 100mg/kg and for Zn is 300mg/kg, according to ¹ Commission Decision of 3 November 2006 establishing revised ecological criteria and the related assessment and verification requirements for the award of the Community eco-label to soil improvers. The composition of bioreactors: Bioreactor-1 (control): 120 kg olive pomace (76.9%) + 36 kg barley straw (23.1%); total 156 kg; Bioreactor-2 (urea): 120 kg olive pomace (76.9%) + 36 kg barley straw (23.0%) + 0.861 kg urea (0.1%); total 156.86 kg; Bioreactor-3 (sheep litter): 120 kg olive pomace (72.3%) + 36 kg barley straw (21.7%) + 37 kg sheep litter (6.0%); total 193 kg.

Correlation analysis revealed significant relationships between soil physicochemical parameters, elemental contents, and treatments. EC showed a strong positive correlation with ash content ($r = 0.87^{**}$), pH ($r = 0.94^{**}$), Ca ($r = 0.78^{**}$), and Mg ($r = 0.90^{**}$). OM exhibited strong negative correlations with ash content ($r = -1.00$) and EC ($r = -0.87^{**}$). At the same time, TC was negatively correlated with the elemental contents of Ca, Mg, K, P, and S. Total nitrogen exhibited a negative correlation with Al and Fe. Calcium, Mg, K, and S were also interrelated, suggesting shared behavior. An inverse relationship was observed between Al and Fe and the nutrients N and P (Table S1). Among the treatments, R2 was strongly correlated with EC, whereas R3 showed strong correlations with Mg, K, and P. Overall, Ca, Mg, K, and S were highly interrelated across all treatments.

3.3. Effect of Composting Treatments on Plant Growth

A significant variation ($p < 0.05$) in dry biomass and relative growth of radish and barley was observed across all treatments compared to the commercial potting medium (Figure 3), indicating that the composting strategy has a marked effect on plant growth. For example, the dry biomass of radish shoots decreased by 81.65%, 64.71%, and 76.46% under R1, R2, and R3 treatments, respectively, relative to the control (Figure 3a). These findings suggest varying degrees of residual phytotoxicity or nutrient availability among the composted materials. R2 produced the highest radish biomass among the treatments, outperforming R1 and R3.

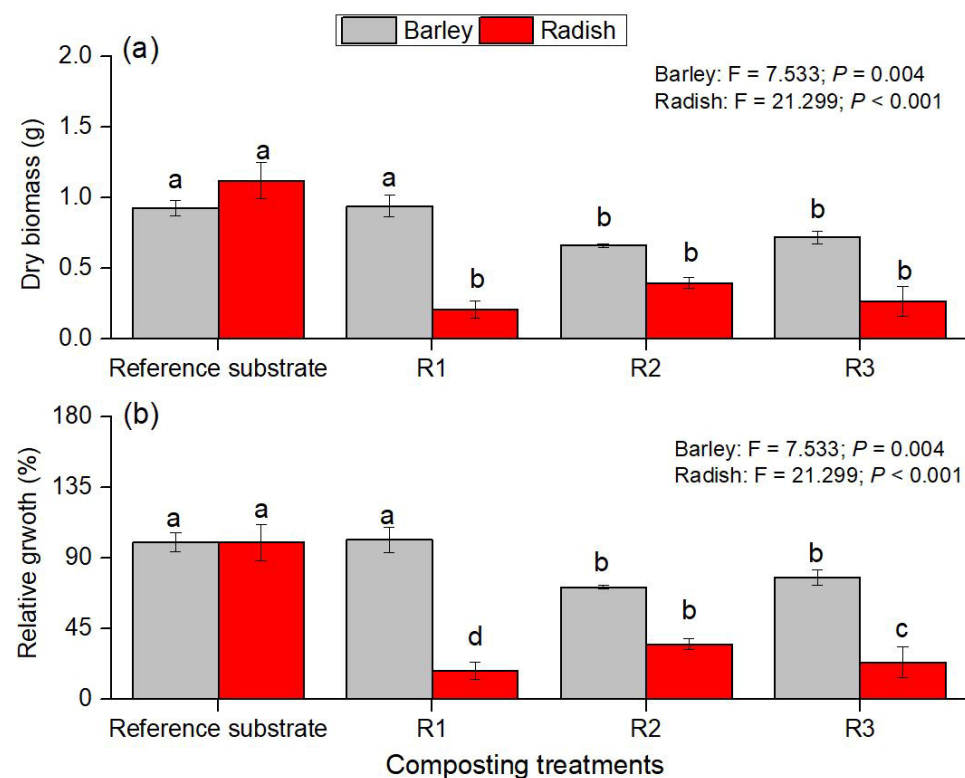


Figure 3. The effect of composting treatment on shoot dry biomass (a) and relative growth (b) of radish and barley. R1 indicates Bioreactor-1, R2 indicates Bioreactor-2 and R3 indicates Bioreactor-3. The data are presented as mean $\pm$ standard error ($n = 4$). Different letters above the error bars indicate statistically significant differences between treatments ($p < 0.05$) as determined by Tukey's test.

In contrast, barley dry biomass showed the most significant reduction under R2 and R3, with declines of 28.64% and 22.52%, respectively, compared to the reference substrate, whereas no reduction was observed under R1 (Figure 3a). For barley, R1 yielded the highest biomass relative to the other treatments. Composting treatments also significantly

influenced the RGR of radish, which reduced from 100% in the reference substrate to 18.35%, 35.28%, and 23.53% in R1, R2, and R3, respectively. For barley, the RGR values were 101.70% (R1), 71.35% (R2), and 77.48% (R3), relative to the control (Figure 3b), indicating improved or maintained growth under R1 and moderate inhibition under R2 and R3.

4. Discussion

4.1. Phytotoxicity of Olive Pomace

The findings of this study demonstrate the phytotoxic effects of OPF on radish seed germination. As OPF concentration increases, GI decreases while the IGI increases significantly ($p < 0.05$). Complete inhibition of germination at higher concentrations (10%, 20%, and 100%) suggests that the elevated total phenolic content (TPC, 3.42% in R3), high salinity (EC 9632 $\mu\text{S}/\text{cm}$ in R3), and the presence of organic acids were primary contributors to seed germination inhibition, with ammonium–nitrogen ($\text{NH}_4\text{-N}$, 0.93 ± 0.05 mg/L) playing a secondary role, corroborating previously reported inhibitory effects on plant development [49]. Similarly, Tüzel et al. [30] observed salinity-induced stress in tomato seedlings grown in OP-amended soils, where exceeding the EC threshold of 2.5 mS/cm resulted in reductions in shoot length, stem diameter, biomass accumulation, and overall plant growth. In particular, the EC of treatment R3 (9.632 mS/cm) was far above the 2.5 mS/cm threshold proposed by Tüzel et al. [30], a level that can induce osmotic stress by limiting water uptake and cause ion toxicity (e.g., Na^+ and Cl^- accumulation), ultimately reducing seed germination and crop performance.

Previous olive pomace composting studies have demonstrated that adding bulking agents such as straw or manure significantly moderates salinity and improves compost quality. For example, Ameziane et al. [25] composted olive pomace with poultry droppings or cattle manure and tracked pH and EC dynamics, finding lower final EC values and better stabilization compared to untreated pomace. Similarly, research on the application of composted olive pomace in soils has revealed that high EC levels (e.g., >12 mS/cm) can arise with large-dose amendments and may lead to detrimental soil salinity unless pre-treatment or dilution strategies are implemented [50]. Compared with these approaches, our findings underscore both the utility of olive pomace compost and the limitations posed by super-threshold EC when no mitigating amendment is used. Furthermore, phenolic compounds are particularly problematic, as they can compromise cell membrane integrity, impede water uptake, and inhibit enzymatic activities critical to the germination process [50].

The results also provide further evidence of the phytotoxic effects of the OPF. Supporting studies, such as that of Sciubba et al. [51] on olive mill wastewater (OMW), align with this paper's findings, showing significantly reduced germination rates in various plant species due to high TPC content and elevated COD. Likewise, research by Rusan et al. [52] also demonstrated that increasing olive-processing byproducts inhibited seed germination in barley, with the authors attributing this to the phytotoxicity of TPC and organic load. Additionally, Belaqqiz et al. [53] and Rusan et al. [52] highlight the adverse effects of olive mill residues on soil properties and plant development due to the accumulation of toxic compounds. Moreover, N in the form of $\text{NH}_4\text{-N}$ is found in high amounts (≥ 1.5 mM NH_4^+) and may also contribute to OPF's phytotoxicity. Elevated $\text{NH}_4^+\text{-N}$ levels are known to inhibit seed germination and root development due to ammonia toxicity, especially under acidic conditions typical of raw OPF [39,54]. The findings of this study support current research that raw OPF is unsuitable for direct application to agricultural soils, emphasizing the need to explore effective treatment methods to mitigate its adverse effects.

4.2. Effect of Olive Pomace Compost on Plant Growth and Its Applicability in Agriculture

The findings highlight the influence of various composting treatments on the growth of radish and barley plants. The variations observed among treatments (R1, R2, and R3) can be attributed to differences in EC levels, organic matter content, and nutrient-enriching additives such as urea and sheep litter, rather than straw. Straw, with its very high C/N ratio (127) and low nitrogen content (0.30%) (Table 1), served mainly as a structural bulking agent. However, it did improve porosity and aeration within the composting matrix but did not significantly alter the overall nutrient composition.

In contrast, sheep litter, characterized by a low C/N ratio (14), high total nitrogen content (2.51%), and elevated ammonium-N levels (10,303 mg/kg) (Table 1), played a more active role in nutrient supply and microbial stimulation in R3, thereby accelerating the degradation of OP. For example, R1 exhibited the highest moisture content (51.7%) and C/N ratio (31), suggesting a slower decomposition rate and potentially limited nitrogen availability for plants [55]. Reactors R2 and R3, with lower C/N ratios (19 and 17, respectively), likely achieved more advanced decomposition, making nutrients more accessible [56,57]. However, the high electrical conductivity in R3 (9632 $\mu\text{S}/\text{cm}$) indicates elevated salinity, which may be attributed to the intrinsic properties of sheep litter, which is rich in soluble salts and ammonium. During composting, the rapid mineralization promoted by its low C/N ratio released additional soluble ions, while the closed reactor system limited leaching. Furthermore, less frequent turning and suboptimal aeration may have contributed to the accumulation of ammonium and other salts, further increasing EC. High salinity could inhibit plant growth and nutrient uptake [58]. For instance, Shannon & Grieve [59] reported that for Chinese cabbage production, it was significantly reduced above an EC of 3200 $\mu\text{S}/\text{cm}$. In the reactors, the pH values ranged from slightly acidic (R1, pH 6.93) to neutral (R2, pH 7.13) and alkaline (R3, pH 7.74), with a pH (Table 2) sufficiently high to impact microbial activity and nutrient solubility [60]. Overall, the results suggest that straw functioned as a physical amendment, while urea and sheep litter were essential for improving compost maturity and nutrient availability, though excessive salinity from the latter requires careful management.

The $\text{NH}_4\text{-N}$ content was high across all treatments (R1: 293 mg/kg, R2: 396 mg/kg, and R3: 222 mg/kg) (Table 2), likely due to limiting oxygen availability, elevated temperatures, and hindering nitrification, the microbial process that converts $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ [48]. Additionally, fluctuating pH and high salinity might further inhibit nitrification. The compost is considered mature when the $\text{NO}_3\text{-N}$ to $\text{NH}_4\text{-N}$ ratio is typically greater than one [48], although in some cases, lower ratios may still indicate maturity [61]. In this study, the $\text{NO}_3\text{-N}$ levels remained <0.04 mg/kg, suggesting incomplete nitrification and potentially immature compost. Nevertheless, $\text{NH}_4\text{-N}$ (Table 2) can serve as a valuable source of nitrogen for plants. For instance, Di et al. [62] demonstrate that specific plant species grow better with $\text{NO}_3\text{-N}$ than with $\text{NH}_4\text{-N}$ as the sole nitrogen source, as high levels of $\text{NH}_4\text{-N}$ can induce $\text{NH}_4\text{-N}$ toxicity.

A correlation matrix (Table S1) reveals significant relationships among compost properties. For example, EC shows a strong positive correlation with ash content ($r = 0.87$) and a negative correlation with total carbon ($r = -0.95$), indicating that increased mineralization is associated with higher salinity, as observed in R1. Total nitrogen also positively correlates with Ca ($r = 0.94$) and negatively with the C/N ratio ($r = -0.99$), further supporting the idea that lower C/N ratios improve nitrogen availability [63]. The higher nitrogen content in R2 suggests that nitrogen contributes to plant growth [64], while R1 showed the lowest N availability and plant growth for both plants (Figure 3).

The impact of these properties is evident from the plant growth parameters. The dry biomass of radish significantly decreases across all composting treatments compared to

barley (Figure 3), likely due to each species' distinct physiological and biochemical adaptation mechanisms and tolerance to stress [65,66]. R2 has a comparatively smaller reduction in dry matter biomass, indicating more active microbial transformation and improved nutrient availability despite high $\text{NH}_4\text{-N}$ levels (396 mg/kg). These results align with those of Chaudhari et al. [67], who found that compost enhances soil properties and plant growth by increasing nutrient availability and microbial activity. In contrast, high $\text{NH}_4\text{-N}$ levels in R1 may have contributed to reduced plant growth, as elevated $\text{NH}_4\text{-N}$ is associated with toxic effects, including damage to chloroplast ultrastructure, photosynthesis, and hormonal imbalances, as well as inhibition of enzyme activities involved in ATP production [68,69]. Similarly, Guo et al. [70] noted that $\text{NH}_4\text{-N}$ as the only N source inhibits plant growth compared to $\text{NO}_3\text{-N}$ or a combination of both. The RGR of radish was higher in R2 and R3 compared to R1, despite R3 having a high total N (2.11%) and a slightly higher TPC (3.05%). This increase is likely due to growth inhibition caused by its high salinity and alkaline pH, which can reduce nutrient uptake. High salinity reduced water availability and induced nutrient imbalances, while alkaline pH further limited micronutrient solubility. At the same time, phenolic compounds exerted allelopathic effects by inhibiting root elongation, nutrient assimilation, and causing oxidative stress. Together, these stressors outweighed the nutritional benefits, resulting in reduced plant growth [71]. The observed reduction in biomass in R1 is consistent with Zulfiqar et al. [72], who demonstrated that TPC in compost can interfere with chlorophyll biosynthesis and reduce biomass production. Irin and Hasanuzzaman [73] also observed that high salinity could reduce biomass production, impairing plant stress tolerance and photosynthetic capacity. The lower nitrogen levels in R1 suggest that the compost is not sufficiently mature for sensitive plants such as radishes.

The findings also agree with Kamal et al. [71], who reported that composts with high EC and $\text{NH}_4\text{-N}$ levels could inhibit plant growth by creating osmotic stress and impairing nutrient uptake. The organic matter and the C/N ratio of compost are crucial for plant growth, as a balanced C/N ratio enhances nitrogen availability for plants and promotes microbial activity, which is essential for nutrient cycling [74,75]. Similarly, Shan et al. [76] reported that composts with a lower pH and adequate nitrogen content were more effective at enhancing plant growth, as they minimized $\text{NH}_4\text{-N}$ toxicity and improved nutrient bioavailability.

The elemental content of COP is crucial for assessing its quality and suitability for agricultural applications [25,77]. For example, adequate element content can mitigate excess nitrogen, either negatively impacting (through a nutrient imbalance) or positively enhancing (by improving nutrient uptake) plant growth [75,78,79]. In this study, the elemental content (Table 3) varied across compost treatments. For example, Ca levels were highest in R2 (29,483.0 mg/kg), potentially mitigating the effects of ammonium toxicity by improving soil structure and reducing acidity, thereby improving the growth of radish and barley (Figure 2). However, R3, with the highest amounts of Mg (2869.9 mg/kg), K (40,430.2 mg/kg), and P (6022.68 mg/kg), exhibited high salinity (EC 9632 $\mu\text{S/cm}$), likely contributing to the greater growth inhibition in radish than barley. The physicochemical properties of compost also influence the availability, mobility, and stability of trace elements (Fe, Mn, Mo, Cr, Cu, Ni, Pb, and Zn) [80–83], which are essential for promoting its safe use as an organic fertilizer [81]. The imbalance in nutrient availability and elevated EC in compost may have led to osmotic stress, impairing plant uptake of essential nutrients, as reported in the literature [58]. However, high Fe (R3: 1231.4 mg/kg) and Mn (R3: 97.2 mg/kg) levels in R3 were unlikely to inhibit growth as they remained below toxic thresholds. Potentially toxic elements such as Cd, Co, Cr, Cu, Mo, Ni, and Pb in all composting treatments (R1, R2, and R3) are well below the limit set by the European Commission Decision [84] for soil improvers.

Overall, R2 demonstrated a smaller reduction in plant biomass, suggesting reduced phytotoxicity compared to R1 and R3. Ait-El-Mokhtar et al. [85] reported that composts with balanced nutrient content and lower EC levels positively influenced plant growth and productivity. The residual phytotoxicity in the composted material can be attributed to the TPC, elevated salinity [86], and $\text{NH}_4\text{-N}$ [68,69]. Additionally, trace metal concentrations can vary depending on the type of compost material and environmental conditions [87]. In this study, R2 prepared by mixing OP with urea was more promising than R1 and R2. For comparison, sewage sludge compost often contains a higher trace metal content than compost from vegetal residues [88,89].

The findings of the present study emphasize the critical role of composting parameters, such as C/N ratio, salinity, $\text{NH}_4\text{-N}$ content, and TPC, in determining the suitability of compost. While R2 (OP + straw + urea) showed comparatively better results among the three treatments (R1 and R3), all treatments negatively impacted biomass, underscoring the need for proper compost stabilization and treatment to reduce phytotoxicity and enhance its agricultural potential.

5. Conclusions

This study focuses on two key aspects: evaluating the phytotoxic effects of raw OP and assessing the impact of COP on evaluating its maturity on radish and barley seed germination and plant growth. The results indicated that increasing OPF concentrations significantly inhibited seed germination, with complete inhibition observed at concentrations of 10% or higher, likely due to the high phenolic content, organic acids, elevated EC levels, and high $\text{NH}_4\text{-NH}$ content. Composting treatments (R1, R2, and R3) resulted in distinct physicochemical properties, with R2 demonstrating relatively better nutrient availability due to a lower C/N ratio. In contrast, R3 exhibited adverse effects on plant growth, primarily attributed to the higher salinity (EC) and alkaline pH. All composting treatments significantly reduced radish dry biomass production compared to the reference substrate, though R2 showed comparatively better performance than R1 and R3. These findings underscore the need for optimization strategies, including adjustments to compost composition, improved stabilization, and targeted treatments to reduce phytotoxicity and enhance its agricultural value by promoting plant growth and improving soil health. Future research should focus on applying microbial or enzymatic treatments to reduce phenolic toxicity and assessing long-term impacts on soil functions under field conditions. Such efforts will be essential not only to enhance soil fertility, crop productivity but also to strengthen the role of composting in sustainable agriculture and soil system resilience under changing environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/soilsystems9040107/s1>, Figure S1: The complete temperature dynamics during the composting process from February to March across all bioreactors. CH-01, CH-02, and CH-03 represent the temperature sensors that were inserted at the top, middle, and bottom of the bioreactors; Figure S2: The oxygen concentration during the composting process from February to March across all bioreactors. B1 is bioreactor-1, B2 is bioreactor-2 and B3 is bioreactor-3; Table S1: Pearson's correlation coefficients between the physiochemical properties and elemental contents of olive pomace compost.

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

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the manuscript.

Acknowledgments: This work was supported by the Croatian Science Foundation under the project numbers HRZZ-IPS-2022-02-2099 and HRZZ-MOBDOL-2023-08-5800 and this work was supported by the Slovenian Research Agency (project No. J4-4561).

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

OP	Olive pomace
OPF	Olive pomace filtrate
COP	Composted olive pomace
R1	Bioreactor-1
R2	Bioreactor-2
R3	Bioreactor-3
GI	Germination index
RGR	Relative growth rate
TPC	Total phenolic content

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