Research Article

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Effect of management of organic wastes on inactivation of *Brassica nigra* and *Fusarium oxysporum* f.sp. *lactucae* using soil biosolarization

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Abstract

BACKGROUND: Soil biosolarization is a promising alternative to conventional fumigation. Volatile fatty acids (VFAs) produced in the soil through fermentation of amended organic matter can affect pest inactivation during biosolarization. The objective was to determine how soil amended with organic wastes that were partially stabilized through either composting or anaerobic digestion affected the inactivation of *Brassica nigra* (BN; a weed) and *Fusarium oxysporum* f. sp. *lactucae* (FOL; a phytopathogenic fungus).

RESULTS: The mortality of BN seeds in the biosolarized soil was 12% higher than in the solarized soil, although this difference was not significant. However, a significant correlation between BN mortality and VFA accumulation was observed. The number of FOL colony-forming units (CFU) in solarized samples at 5 cm was 34 CFU g^{-1} of soil, whereas in the biosolarized samples levels were below the limit of quantification. At 15 cm, these levels were 100 CFU g^{-1} for solarized samples and < 50 CFU g^{-1} of soil for the biosolarized samples. Amendment addition positively affected the organic matter and potassium content after the solarization process.

CONCLUSION: The organic waste stabilization method can impact downstream biosolarization performance and final pest inactivation levels. This study suggests that organic waste management practices can be leveraged to improve pest control and soil quality.
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Supporting information may be found in the online version of this article.

Keywords: solarization; pest control; compost; digestates; volatile fatty acids; soil quality

1 INTRODUCTION

Soil fumigation is a widely used agricultural practice for inactivating a wide range of soil pests. The negative environmental and health consequences of conventional fumigants such as methyl bromide, chloropicrin or 1,3-dichloropropene require usage restrictions and regulation, and motivate consumer demand for alternatives that enable environmentally friendly crop production systems.[1–3]

Soil solarization and solarization with organic soil amendments, termed soil biosolarization (SBS), are promising disinfestation techniques that can compete with artificial agrochemical pesticide application.[4,5] Soil solarization induces hydrothermal inactivation of soil pests by covering moist soil with clear plastic tarp to promote passive solar heating.[6,7] Soil solarization application is limited by climate, time of year, treatment duration, soil depth, susceptibility of target pest organisms, and other factors.[7] SBS combines organic soil amendments with solarization to increase pesticidal activity. Some of the additional inactivation effects attributed to SBS include: (i) additional heat generation from biological activity,[8,9] (ii) accumulation of biotoxic compounds, such

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as volatile fatty acids (VFAs) or ammonia,[10–12] (iii) direct effects on pest organisms from fungi and/or bacteria present in organic soil amendments such as competition or predation[11,13,14] and (iv) generation of micro-anaerobic or anoxic conditions through fermentation in the soil that are inhibitory to obligate aerobic pests.[15] This last mechanism is directly related to anaerobic soil disinfestation (ASD). In SBS, the combined action of heat and VFA accumulation has shown promising effects in the control of different pests.[8,16,17] However, there is still a lack of information on the role of VFAs in inactivating different pests.

The release of these pesticidal compounds depends on how readily the organic matter (OM) amendments are bioconverted in the soil under SBS soil conditions. For example, application of organic amendment previously stabilized via anaerobic digestion (AD) did not release significant amounts of VFAs during SBS.[18] Alternately, non-stabilized amendments, such as tomato pomace or wheat bran, led to greater changes in soil temperature,[9,19] soil acidification, and VFA accumulation.[16,19,20] Stabilizing organic wastes prior to their soil application is common practice to avoid potential issues such as phytotoxicity[21] or human pathogen contamination.[22] Composting and anaerobic digestion of organic wastes are common alternative practices to landfill disposal of stabilized organic wastes.

The objective of this study was to characterize SBS performance using organic wastes that were partially stabilized through composting or anaerobic digestion. SBS efficacy was tested by measuring mortality for two agricultural pests. Pest inactivation data were regressed against VFA accumulation data to determine if a correlation existed. Moreover, changes to soil quality were examined following SBS. Specifically, soil OM content, plant macronutrient levels, and cation exchange capacity (CEC) were measured.

Two model pests were considered to assess SBS efficacy across two very different pest classes. In particular, *Brassica nigra* (BN),[23] a weedy forb, and the strain *Fusarium oxysporum* f. sp. *lactucae* (FOL), a soilborne fungal plant pathogen, were used. Weeds can cause losses in many agricultural crops and can harbour destructive insects and pathogens.[24] BN is an annual, cool-season forb native to the Mediterranean region of Europe. Prior research indicates that SBS amendment stability is related to BN seed mortality. In a previous study, SBS using labile tomato pomace and mature green waste compost as amendments increased BN inactivation from ∼25% in the non-amended soil to 100% in the amended soil after 8 days of SBS.[16] In another study, SBS of soil amended with stable digestates from thermophilic anaerobic digestion increased BN seed mortality from ∼40% in the non-amended, solarized soil to ∼71% in the digestate-amended, solarized soil.[18] The fungal pathogen FOL causes a disease of lettuce known as *Fusarium* wilt or *Fusarium* root rot, and affects lettuce production throughout the world.[25] This pathogen is seed-transmitted and now it is established in soils where lettuce is grown and causes serious economic losses.[26] Soil solarization and SBS using dried pellets of *Brassica carinata* A. Braun as amendment have significantly reduced *Fusarium* wilt in soils.[27] However, this study did not elucidate soil factors that may be involved in their inactivation.

2 MATERIALS AND METHODS

2.1 Organic amendment preparation

The feedstocks and conditions [carbon:nitrogen (C:N) ratio, inoculum and moisture level] used for anaerobic digestion and composting processes were selected based on a prior laboratory study where they were shown to be effective stabilization conditions. The primary substrates were model green and food wastes prepared to avoid the confounding effects of heterogeneous composition and particle size that are often encountered in municipal food waste.[28] They were mixed at a ratio (g/g dry basis) of 67:23 to achieve a C:N of 27. The green waste components included leaves, grass, prunings and trimmings, and branches and stumps, as described elsewhere.[29] This composition was based on the California 2008 Statewide Waste Characterization Study.[29] Dog food was selected as a model food waste based on its compositional similarity to food wastes measured in a municipal waste Hill's Pet Nutrition, Inc. management facility in Dubai (data not shown). The main composition of the food waste was: 22% protein, 14% fat, 50% carbohydrate and 13% crude fiber (Oral Care Adult Dog Food; Hill's® Science Diet®, Hill's Pet Nutrition, Inc. Topeka, KS, USA). For consistency, the inoculum used in the AD and composting processes was a thermophilic liquid digestate from an anaerobic digester located in Sacramento (CleanWorld Inc., Sacramento, CA, USA). The digester system processed solid organic wastes from food processing and food waste from local restaurants and groceries. Liquid effluent was sampled from the methanogenesis tank (operated at 55 °C) and contained 4 to 5% solids.

AD was conducted in batch digesters containing 1 to 2 L of sludge incubated at 55 °C. The digesters were comprised of glass bottles with caps that permitted headspace gas to leave the digester through check valves (catalog #80103; Qosina, Edgewood, NY, USA) without risk of oxygen contamination from retrograde airflow. Digesters were loaded with 7.5% (dry weight basis) model waste and 92.5% thermophilic liquid digestate (wet basis). The composting experiments were also performed in batch reactors of variable volume (250–1000 mL) similar to those described elsewhere.[20] To maintain aerobic conditions, reactors were supplied with air at a rate of 20 mL min^{-1} and were incubated at 55 °C and at ∼67% moisture content (wet basis) in an incubator. The inoculum for composting experiments was also thermophilic liquid digestate at a rate of 8% (dry weight basis). When necessary, distilled water was added to the reactors to maintain constant moisture.

For both digestion processes, incubation was stopped after 7 days. Our prior studies have shown that after this time, maximum methane (for AD) and carbon dioxide (for composting) production rates were achieved but substrates were not completely stabilized (Supporting Information Figure S1). After the partial anaerobic digestion, the anaerobic digestates were separated into solid and liquid digestate phases using a sieve of mesh size 1 mm. The solid partially stabilized anaerobic digestate (PSAD) and the partially stabilized compost (PSC) were left to air dry and the partially stabilized liquid digestate (PSLD) was kept at 4 °C until soil application.

2.2 Stability measurements

The initial stability of the non-amended and amended soils was directly estimated by measuring microbial respiration of samples using a previously described, bioreactor-based respirometry method.[9,20] Briefly, 250-mL aerated bioreactors (20 mL air min^{-1}) filled with 80 g (dry weight) of sample were incubated at 55 °C for 160 h. CO_2 content in the air leaving the reactor was continuously monitored, permitting calculation of the CO_2 evolution rate (CER; mg day^{-1} g $soil^{-1}$), as previously described.[9,20] Cumulative CO_2 evolution (cCER; g CO_2 g $soil^{-1}$) was determined by integrating CER over time and fitting the observed data to a saturation model.[21]

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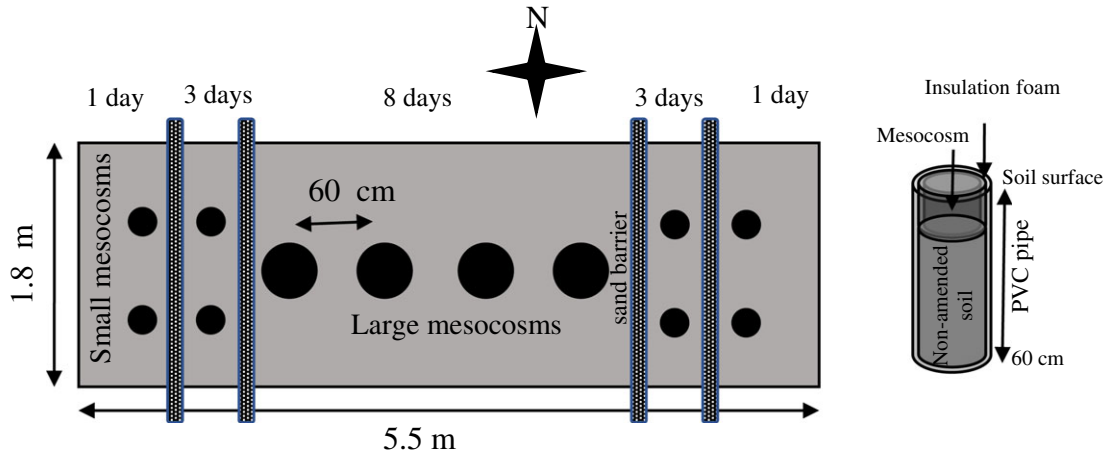

Figure 1. Schematics of the field plot and mesocosm arrangement (left) and of the PVC pipe with the mesocosms integrated (right).

2.3 Field preparation

The field plot was prepared at the UC Davis Plant Pathology Research Farm (Davis, CA, USA; 38.521028, −121.760755; elevation 18.5 m above sea level). The field was planted annually with lettuce cultivars (iceberg, romaine or leaf)[30] and was left fallow over the winter of 2015. Prior to the experiment, the field was rototilled to kill and incorporate the naturally occurring weeds. An orchard float was used to flatten the fields and then the field was drip irrigated to assure the water front was >60 cm deep. The experimental units in field studies were large (15 cm diameter and 20 cm height; 0.8 cm thick) and small (5 cm diameter and 20 cm height; 0.3 cm thick) mesocosms constructed from polyvinyl chloride (PVC) pipes. To isolate the mesocosms from the surrounding unamended field soil, 60-cm (22 and 7.6 cm internal diameter for large and small mesocosms, respectively) PVC pipes were embedded in the field (Figure 1). The pipes allowed gas and water exchange between the mesocosms and the natural soil through the bottom of the mesocosms. To ensure isolation, 1.9-cm-thick and 0.95-cm-thick foam rubber was wrapped around the large and small mesocosms, respectively. The top 20 cm of soil in the columns was removed to accommodate the mesocosms.

The field site contained five replicate plots (1.8 m width and 5.5 m length) and was arranged to include a 2-m buffer between plots. The plots were oriented lengthwise from west to east. Each plot had four columns for large mesocosms where each mixture was randomly allocated in the center of the plot (Figure 1). At the sides of the plot, eight columns for small mesocosms were used for the VFA kinetics study (Figure 1).

2.4 Soil mesocosm preparation

Soil was obtained from the UC Davis Plant Pathology Research Fields. The texture of the soil was sandy clay loam (47%, 27% and 26% sand, silt and clay, respectively), the OM content was 2.64% and the water retention at 0.33 atm of pressure (field capacity) was 21.90% (wet basis). Mesocosms were prepared with soil mixed with PSC, PSAD and PSLD. Soil without amendment was used as a control [untreated soil control (UTC)]. The amendment rate for PSC and PSAD was 2.5% (dry weight basis) whereas for PSLD the amendment rate was 15.38% (wet weight basis). This was the equivalent amount of PSLD to produce enough PAD to amend the soil at 1% (dry weight basis). The 1% PAD equivalent loading was selected because the high water content of PSLD made the soil supersaturated when an amendment rate of 2.5% PAD equivalent was used. Soil mixtures were packed in the mesocosms that were embedded in the PVC pipes of the field. Large mesocosms were used to monitor soil temperature, VFA levels, inactivation of BN and FOL, and soil properties after 8 days of solarization. Smaller mesocosms were extracted from the field and used for measuring VFA accumulation kinetics in the soil during SBS. A perforated stainless-steel sheet was attached to the bottom of each mesocosm and covered with weed barrier fabric to prevent soil loss but still allowing for drainage and gas exchange with the deeper layer of soil in the field soil. For controls lacking solar heating, soil mixtures were prepared in 250-mL polystyrene containers loosely covered to prevent drying for incubation at room temperature (RT; 23 ± 1 °C). RT samples were loaded with weed seeds to serve as controls to assess temperature effects on pest inactivation. Soil mixtures were wetted through capillarity to above their respective field capacities while equilibrating overnight at 4 °C. Compact temperature sensors and data loggers (Thermochron iButtons model 1922 L; Embedded Data Systems, Lawrenceburg, KY, USA) were placed at 7 and 15 cm depths in each mesocosm. A permeable nylon mesh packet with 30 seeds of BN and 2.46 mL of the appropriate soil mixture to provide direct contact with seeds[31] was embedded at 15 cm in the PVC mesocosms and at the center of the RT samples.

2.5 Field experiment

As the plots and mesocosm were already wet, after mesocosm set-up in the field, 1 h of irrigation was enough to re-wet the surface of the plot. Plots were then tarped with 0.7-µm transparent plastic (Husky Film Sheeting; Poly-America, Inc., Grand Prairie, TX, USA) and the edges were buried to ensure a proper seal. For the kinetic study, the small mesocosms were removed after 1 and 3 days of solarization by opening a hole in the tarp. To minimize contamination of the tarp headspace with ambient air during the kinetic sampling, bags filled with wetted gravel and sand were placed along the length of the plot to isolate mesocosms by sampling time point (Figure 1). The extracted small mesocosms were divided into upper (0–7 cm) and lower (14–20 cm) layer samples, transferred to a Ziploc® bag (S. C. Johnson & Son, Inc. Racine, WI, USA) and stored at -20 °C in the freezer for analysis. After 8 days of solarization, the tarp was removed and the large mesocosms were extracted from the soil for analysis and were also separated into upper (0–7 cm) and lower (14–20 cm) layers for further analyses.

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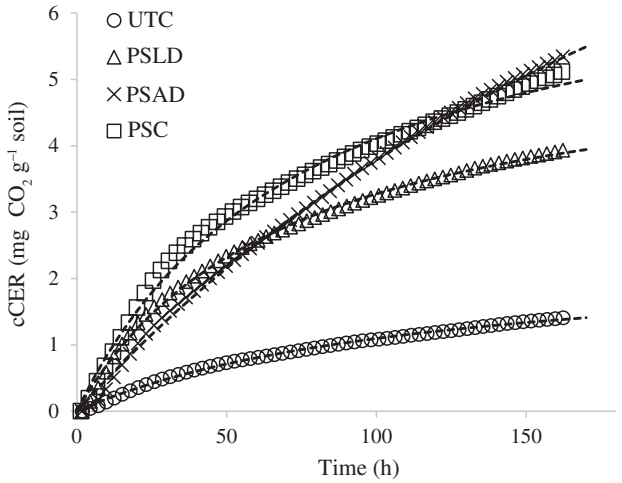

Figure 2. Mean cumulative CO_2 evolution (cCER) of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD) incubated in aerobic conditions at 55 °C ($n = 3$). Symbols correspond to the observed values and dashed lines to the saturation model.

2.6 Weed inactivation analysis

BN weed seed packets were removed from the mesocosms and RT samples after 8 days of treatment. Germination percentages for each sample were determined after incubation in Petri dishes in a growth chamber for 14 days on a cycle of 8 h at 20 °C in darkness and 16 h at 30 °C in light.[16,32] After 14 days, all non-germinated seeds with intact seed coats were evaluated for viability by tetrazolium staining to differentiate dead seeds from dormant seeds.[33] The baseline germination rate of the seed stock was 95%.

2.7 *Fusarium oxysporum* f. sp. *lactucae* inactivation analysis

The field plot was previously inoculated with FOL and homogeneous FOL distribution was expected in the sampled soil. FOL levels in each sample before solarization were compared with FOL levels after 8 days of solarization and after incubation at RT. Field inoculation levels were quantified used a dilution plating method developed to specifically detect this FOL strain, as described previously.[30] For each sample, 5 g of soil was suspended in 200 mL of 1% sodium hexametaphosphate solution and stirred for 5 min. Then, 10 mL of the supernatant was transferred into 90 mL of 0.1% water agar and stirred another 5 min. Then, 400 µL was transferred and spread onto each of 12 plates containing Komada's selective medium.[34] Inoculated plates were left to incubate at RT under fluorescent light continuously for 10 to 11 days. After incubation, FOL colonies were identified based on morphology.[35]

2.8 Analysis of electrical conductivity, pH and volatile fatty acids

The electrical conductivity (EC), pH and VFA content were measured on water extracts from soil. The extracts were prepared as 1:1 (weight) mixtures of soil and distilled water that were allowed to equilibrate for 30 min. Duplicate extracts were prepared and analyzed for each soil sample. For VFA analyses, a 1-mL aliquot of the supernatant was sampled after centrifugation for 10 min at 10000 ***g*** and filtered through a 0.2-µm filter [Titan-3; 17-mm filter blue 0.2-µm polytetrafluoroethylene (PTFE) membrane; Thermo Fisher Scientific Inc., San Diego, CA, USA] into a high-performance liquid chromatography (HPLC) vial. Centrifuged samples were re-homogenized by vortexing for 30 s to analyze EC and pH using a conductivity meter (Mettler Toledo, Columbus, OH, USA) and a pH meter (Mettler Toledo, Columbus, OH, USA) calibrated according to the manufacturers' guidelines.

Acetic, propionic, formic, butyric and isobutyric acids were measured using an HPLC-UFLC-10Ai (Shimadzu, Columbia, MD, USA) equipped with an Aminex® HPX-87H (300 x 7.8 mm) column (Life Science Research, Education, Process Separations, Food Science, Hercules, CA, USA) and an SPD-M20A diode (Shimadzu, Columbia, MD, USA) array detector set at 210 nm. The HPLC conditions are described elsewhere.[19]

2.9 Soil analysis

To determine the impact of amendments and biosolarization on soil properties, the OM, extractable ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), potassium, ortho-phosphate (PO_4-P), and CEC were measured. These properties were analyzed using standard methods described elsewhere.[36] Briefly, the soil OM was analyzed by the loss on ignition method. OM was oxidized at high temperature in a muffle furnace and semiquantified by the gravimetric weight change after oxidation of soil. Extractable NO_3-N was analyzed using the ion selective electrode method. Nitrate was extracted from soils using an aluminum sulfate solution and subsequently determined using a nitrate ion-specific electrode. Extractable NH_4-N was extracted from soil using 2.0 N KCl and determined by spectrophotometric techniques. Extractable phosphorus (P) was analyzed using the dilute acid-fluoride Bray and Kurtz method. Bioavailable PO_4-P was extracted using a dilute acid solution of pH 2.60 (0.025 M HCl and 0.03 M NH_4F). Phosphorus content was determined spectrophotometrically at 882 nm at an acidity of 0.19 M H_2SO_4 by reacting with ammonium molybdate using ascorbic acid as a reductant in the presence of antimony. Exchangeable potassium (K), calcium (Ca), sodium (Na) and magnesium (Mg) were analyzed by the ammonium acetate method. Ammonium acetate solution was used to displace the bases and the concentration was determined using atomic emission spectrometry. Finally, CEC was quantified by the ammonium replacement method. Cation exchange sites were saturated with ammonium and the excess was removed with ethanol and then replaced with protons from HCl acid. The ammonium in the final leachate was determined with an ALPKEM (Alpkem Corporation, Clackamas OR, USA) rapid flow analyzer.

2.10 Statistical analysis

Degree-day values were calculated by using the trapezoidal rule to approximate the integral of soil temperature versus time data using 0 °C as the baseline in R-studio (Version 0.98.1103; RStudio, Boston, MA, USA). To study factorial effects, two-way analysis of variance (ANOVA) tests were performed to determine main and interaction effects for experimental factors on various soil responses. Mean responses for treatments within a given experiment were compared using one-way ANOVA and Tukey's honest significant difference (HSD) post hoc test. Bivariate analysis was performed to establish Pearson correlation coefficients (r) between continuous variables. The significance threshold level for both analyses was $P = 0.05$. Statistical analyses were performed using JMP-IN software (version Pro 12; SAS, Cary, NC, USA).

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Table 1. Summary of temperature responses (T_{max}, T_{mean}, T_{min} and $\Delta T = T_{max} - T_{min}$; °C) and moisture contents (%H_T1, wet basis) for solarized mesocosms. Temperature responses represent the average over the treatment duration for each plot except for those denoted by _T1, which indicate values averaged over the first complete day of the experiment for each plot. Moisture content values correspond to measurements taken at the beginning of the experiment. Samples included the top (H; 0–7 cm) and bottom (L; 14–20 cm) layer of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD)

	Degree-day	T_{mean} (°C)	T_{max} (°C)	T_{min} (°C)	ΔT (°C)	T_{mean}_T1 (°C)	T_{max}_T1 (°C)	T_{min}_T1 (°C)	ΔT_T1 (°C)	%H_T1
UTC-H	$257.83 \pm 4.7^{a\dagger *}$	$33.87 \pm 0.63^{a*}$	46.52 ± 0.74^{ab}	23.50 ± 0.82^{b}	23.02 ± 1.04^{a}	33.03 ± 0.19^{a}	45.50 ± 0.64^{ab}	22.25 ± 0.73^{a}	23.25 ± 1.33^{a}	25.71 ± 1.58^{a}
PSC-H	262.84 ± 7.86^{a}	34.54 ± 1.04^{a}	47.65 ± 1.06^{a}	23.81 ± 1.14^{b}	23.84 ± 1.05^{a}	33.84 ± 1.04^{a}	47.17 ± 0.91^{a}	22.52 ± 1.16^{a}	24.65 ± 0.68^{a}	26.53 ± 2.24^{a}
PSAD-H	$263.60 \pm 6.92^{a*}$	$34.64 \pm 0.92^{a*}$	48.46 ± 1.52^{a}	23.48 ± 0.48^{b}	24.98 ± 1.08^{a}	33.91 ± 0.99^{a}	47.49 ± 1.41^{a}	22.42 ± 0.75^{a}	25.07 ± 0.78^{a}	27.87 ± 1.85^{a}
PSLD-H	$261.81 \pm 3.77^{a*}$	$34.34 \pm 0.50^{a*}$	44.39 ± 1.62^{b}	25.87 ± 0.55^{a}	18.52 ± 2.05^{b}	33.11 ± 0.92^{a}	43.15 ± 2.01^{b}	24.26 ± 0.54^{b}	18.89 ± 2.09^{b}	20.55 ± 2.46^{b}
UTC-L	251.79 ± 2.36^{a}	32.92 ± 0.29^{a}	38.18 ± 0.49^{ab}	27.86 ± 0.43^{b}	10.32 ± 0.69^{a}	31.54 ± 0.45^{a}	37.73 ± 0.60^{ab}	25.94 ± 0.62^{b}	11.79 ± 0.83^{a}	25.18 ± 0.98^{a}
PSC-L	256.33 ± 4.90^{a}	33.45 ± 0.62^{a}	38.44 ± 0.86^{a}	28.52 ± 0.40^{ab}	9.92 ± 0.54^{a}	32.23 ± 0.49^{a}	38.15 ± 0.73^{a}	26.92 ± 0.33^{ab}	11.23 ± 0.53^{a}	26.20 ± 0.84^{a}
PSAD-L	255.73 ± 3.85^{a}	33.44 ± 0.52^{a}	38.66 ± 0.78^{a}	28.40 ± 0.42^{ab}	10.27 ± 0.61^{a}	32.08 ± 0.68^{a}	38.13 ± 0.82^{a}	26.55 ± 0.64^{ab}	11.58 ± 0.56^{a}	26.40 ± 2.05^{a}
PSLD-L	253.44 ± 2.98^{a}	32.98 ± 0.41^{a}	37.05 ± 0.72^{b}	28.91 ± 0.24^{a}	8.14 ± 0.62^{b}	31.52 ± 0.60^{a}	36.37 ± 0.81^{b}	27.24 ± 0.55^{a}	9.13 ± 0.66^{b}	24.43 ± 1.49^{a}

†Values that do not share a letter indicate significant differences based on the Tukey–Kramer HSD test within the treatment at the same depth ($P < 0.05$).
*Significant differences based on the Tukey–Kramer HSD test between the upper and lower layer values for the same amendment treatment within each column ($P < 0.05$).

3 RESULTS

3.1 Stability of the amended soil

Laboratory incubation of the non-amended and amended soils in bioreactors at 55 °C for 160 h showed differences in the cumulative respiration curves (Figure 2). The estimated steady-state maximal cCER mean and standard deviation values for S, PSC, PSAD and PSLD were 2.38 ± 0.17, 7.23 ± 0.62, 15.45 ± 1.13 and 5.56 ± 0.11 mg CO_2 g soil^{-1}, respectively. The cCER values for PSC and PSLD were significantly higher than those for the non-amended soil and significantly lower than those for the PSAD treatment ($P < 0.05$).

3.2 Temperature evolution of solarized soils

Within each soil depth layer, the degree-day values did not show significant differences between the four solarized treatments (Table 1). The degree-day value for the upper layer of the mesocosms was significantly higher ($P < 0.05$) than that for the lower layer for the S, PSAD and PSLD samples. The mean maximum temperature (T_{max}) during the experiment ranged between 44 and 48 °C and between 37 and 39 °C in the upper and lower layers, respectively (Table 1). The mean temperature in the top layer was significantly ($P < 0.05$) higher (~1 °C) than that in the lower layer for the S, PSAD and PSLD samples. Differences in T_{max} between the upper and lower layers were not significant for PSC samples. In the upper layer, the differences between the maximum and minimum temperatures (ΔT) were approximately 24 °C for the S, PSC and PSAD samples and they were significantly lower (~18 °C; $P < 0.05$) for the PSLD samples. In the lower layer, ΔT was ~10 °C for S, PSC and PSAD and significantly lower (8 °C; $P < 0.05$) for PSLD.

To assess whether differences in the temperature variation could be attributed to the moisture content of the soil, the temperature data recorded during the first 24 h (starting at midnight of the night when the experiment started, T1) and the moisture of the samples collected the same day were examined (Table 1). A significant positive correlation between the percent water content (%H; wet basis) and the ΔT in the first 24 h (ΔT_T1) was observed for all the samples for both the upper and lower layers ($P < 0.001$ and $P = 0.010$ for the upper and lower layers, respectively). Moreover, sample PSLD showed lower T_{max} and higher T_{min} than the rest of the samples, meaning that the ΔT_T1 of PSLD was significantly ($P < 0.05$) lower than the ΔT_T1 of S, PSC and PSAD for both the upper and lower layers. PSLD samples also had lower moisture content than the other samples; however, these differences were only significant ($P < 0.05$) in the upper layer.

3.3 Evolution of volatile fatty acids

3.3.1 Kinetics of volatile fatty acid accumulation in soil

Formic, propionic, and acetic acid VFAs were detected during the experiment (Figure 3 and Table 2). During solarization, VFAs were not observed or were observed at trace levels in the non-amended soil (< 3.5 μg g^{-1}). Formic acid showed the lowest concentration (<16 μg g^{-1}). In the upper solarized layer, formic acid reached peak levels in PSC and PSLD samples after 1 day of solarization (~10–12 μg g^{-1}) and in PSAD after 3 days (~5 μg g^{-1}). In the lower solarized layer, formic acid in PSLD samples peaked after 1 day of solarization, whereas PSC and PSAD samples showed the highest level of formic acid after 8 days of treatment (~15 μg g^{-1}). Propionic acid in the upper solarized layer showed the highest level after 1 and 3 days of solarization for PSC (~83 μg g^{-1}) and PSAD (~20 μg g^{-1}), respectively. Propionic acid in PSLD showed the highest level at the beginning of the experiment (~175 μg g^{-1}) and then it decreased to <12 μg g^{-1} in the upper solarized layer. In the lower solarized layer, PSC and PSAD showed an accumulation of propionic acid during the experiment and after 8 days it reached similar levels to those found in PSLD, which showed a stable concentration of ~100 μg g^{-1}. Acetic acid levels were the highest among the VFAs measured. In the upper solarized layer, acetic acid in PSC and PSAD peaked after 3 days of solarization (~38 μg g^{-1}), whereas for PSLD, it gradually decreased from ~112 to 10 μg g^{-1} during the experiment. Finally, the lower solarized layer showed the most significant accumulation of acetic acid and after 8 days of solarization the levels were ~238, ~464 and ~595 μg g^{-1} for PSLD, PSC and PSAD, respectively.

3.3.2 Analysis of volatile fatty acids and pH of the initial and final incubated samples

Table 2 shows the mean and standard deviation for the pH and VFA levels measured in the initial samples, the samples incubated at RT and the samples from the upper and lower soil layers after 8 days of solarization. Two-way ANOVA showed that treatment (or amendment type; $P < 0.001$; Table S1) and temperature (categorized as type of incubation: RT and lower and upper solarized layers;

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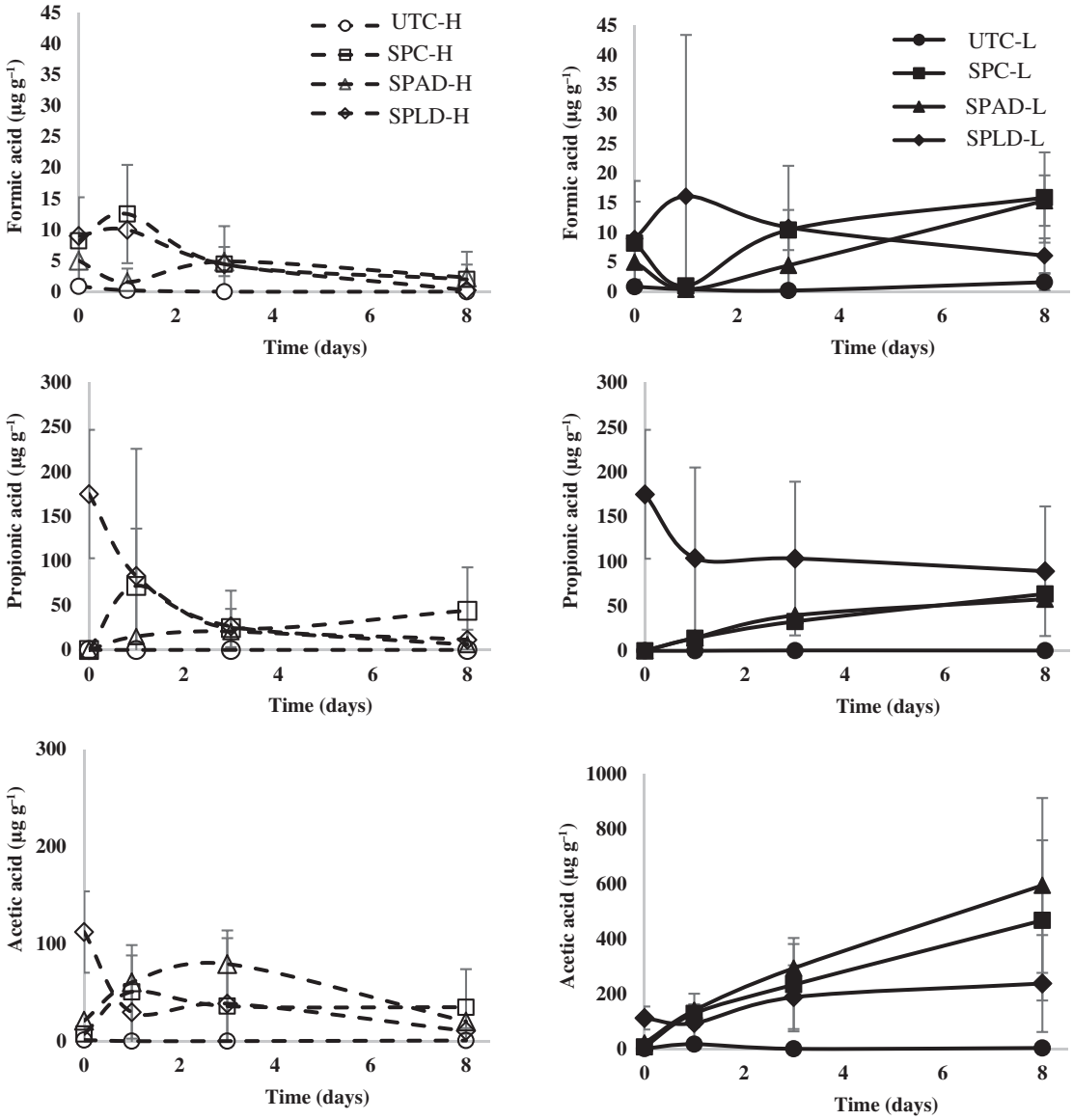

Figure 3. Kinetics of the mean concentration of formic, propionic and acetic acid in the upper (H; left) and lower (L; right) layers of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD). Bars represent the standard deviation ($n = 5$).

$P < 0.001$) and their interaction had significant impacts on formic, propionic and acetic acid accumulation ($P < 0.01$). This interaction effect meant that amended and solarized samples showed higher VFA levels than non-solarized and/or non-amended samples. The non-amended soils did not show significant differences for any of the measured VFAs between the treatments (Table 2). For PSC samples, formic acid was present at significantly higher levels in the lower solarized layer than in the upper solarized layer ($P = 0.004$). Propionic acid was not significantly different within PSC samples (Table 2). Acetic acid showed the highest accumulation ($P < 0.05$) in the lower solarized layer. For PSAD samples, formic acid accumulation was significantly higher at RT than in the upper solarized layer ($P = 0.039$). The propionic acid level was significantly higher ($P < 0.05$) in the lower solarized layer than in the RT-incubated and upper solarized layer samples. As for PSC, acetic acid accumulation was significantly higher in the lower solarized layer of the PSAD samples ($P < 0.05$). Finally, PSLD did not show significant differences in formic acid accumulation within samples. PSLD resulted in significantly higher levels of propionic acid at the beginning of the experiment compared with the 8-day RT-incubated and upper solarized layer samples ($P < 0.05$). Finally, the acetic acid level in the lower solarized layer was significantly higher ($P < 0.05$) than that in the upper solarized and RT-incubated samples.

The two-way ANOVA showed that pH values were significantly affected by the amendment type, incubation temperature and their interaction ($P < 0.001$; Table S1). The non-amended soil did not show significant differences among the temperature incubation treatments (Table 2). The PSC treatment showed the highest pH for the samples incubated at RT and the upper solarized layer ($P < 0.05$). For PSAD treatments, the lowest pH was observed in the lower solarized layer ($P < 0.05$). Finally, for PSLD treatments, the lower solarized and RT-incubated samples showed significantly lower pH values than the initial sample. Moreover, a negative correlation ($P < 0.01$) was found between the pH values and the sum of measured VFAs for the PSC and PSAD samples.

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Table 2. Mean and standard deviation of the volatile fatty acid (formic, propionic and acetic acids) concentration and pH of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD) at the beginning of the experiment (T0), after 8 days of incubation at room temperature (RT) and after solarization in the top (H; 0–7 cm) and bottom (L; 14–20 cm) layers

Amendment	Temperature regime	Formic acid(μg g^{-1})	Propionic acid (μg g^{-1})	Acetic acid(μg g^{-1})	pH
UTC	T0	0.86 ± 1.18 a(A)†	<d.l. a(B)	0.98 ± 2.19 a(B)	7.89 ± 0.11 a(B)
	RT	1.29 ± 1.65 a(B)	<d.l. a(C)	2.10 ± 1.68 a(B)	7.95 ± 0.12 a(AB)
	H	<d.l. a(A)	<d.l. a(A)	0.54 ± 0.54 a(A)	8.04 ± 0.03 a(B)
	L	1.62 ± 1.41 a(B)	0.18 ± 0.41 a(B)	3.33 ± 1.86 a(B)	8.06 ± 0.07 a(B)
PSC	T0	8.20 ± 7.02 ab(A)	<d.l. b(B)	7.20 ± 6.28 b(B)	7.67 ± 0.04 c(C)
	RT	10.66 ± 2.86 ab(AB)	15.36 ± 3.70 ab(AB)	81.35 ± 20.16 b(AB)	7.83 ± 0.03 b(B)
	H	1.99 ± 4.44 b(A)	44.07 ± 48.58 ab(A)	34.76 ± 39.23 b(A)	8.05 ± 0.08 a (B)
	L	15.91 ± 7.64 a(A)	63.56 ± 42.27 a(AB)	467.47 ± 291.43 a(A)	7.68 ± 0.12 c(A)
PSAD	T0	4.99 ± 3.75 ab(A)	<d.l. b(B)	20.95 ± 4.37 b(B)	7.76 ± 0.03 b(BC)
	RT	19.20 ± 16.50 a(A)	21.37 ± 11.71 b(A)	155.96 ± 106.75 b(A)	7.77 ± 0.10 b(B)
	H	2.29 ± 2.07 b(A)	6.06 ± 8.35 b(A)	20.14 ± 32.29 b(A)	7.98 ± 0.06 a(B)
	L	15.39 ± 4.26 ab(A)	57.75 ± 31.03 a(AB)	594.86 ± 318.29 a(A)	7.51 ± 0.11 c(A)
PSLD	T0	9.00 ± 9.72 a(A)	174.92 ± 72.05 a(A)	112.12 ± 41.84 ab(A)	8.53 ± 0.12 a(A)
	RT	1.80 ± 0.77 a(B)	7.41 ± 7.68 b(BC)	16.65 ± 13.66 b(B)	8.16 ± 0.27 b(A)
	H	0.25 ± 0.57 a(A)	11.53 ± 11.24 b(A)	10.42 ± 2.82 b(A)	8.33 ± 0.12 ab(A)
	L	6.08 ± 2.92 a(B)	88.93 ± 72.46 ab(A)	238.17 ± 176.36 a(AB)	8.18 ± 0.06 b(B)

d.l., detection limit.
†Within each column, values that do not share a letter are significantly different based on the Tukey–Kramer HSD test ($P < 0.05$). Lowercase letters compare temperature regimes within each amendment treatment; uppercase letters compare amendment treatments for a given temperature regime.

3.4 Pest inactivation

3.4.1 *Inactivation of* Brassica nigra *seeds*

Results of the tetrazolium testing confirmed that all non-germinated seeds were inactivated, showing no issue with dormancy. BN mortality in the control soil incubated at RT was 9.07% and in the amended soils incubated at RT the mean mortality was <5%. For the solarized samples, the non-amended soil had a mean mortality of 18.4% and in the amended samples it was 34%. Two-way ANOVA with solar heating and soil amendment as factors showed that only solar heating ($P < 0.001$) was a significant main effect for BN inactivation. The HSD Tukey test comparing all the samples showed that BN inactivation had significantly higher mortality in the solarized amended samples (PSC, PSAD and PSLD; $P < 0.05$; Figure 4) than in the RT-incubated samples.

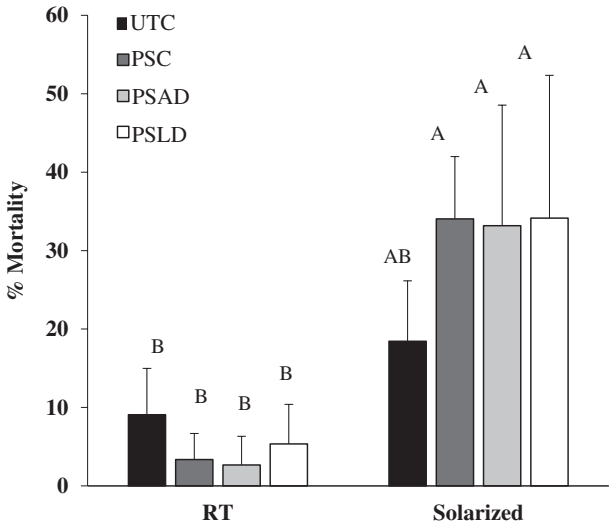

Figure 4. Seed mortality for *Brasicca nigra* seeds in the room temperature (RT) and in the solarized non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD). Bars represent the standard deviation of the mean ($n = 5$). Different letters indicate significant differences based on the Tukey–Kramer test between samples ($P < 0.05$).

3.4.2 *Inactivation of* Fusarium oxysporum *f. sp.* lactucae

Two-way ANOVA with temperature regime and soil amendment as factors showed that temperature regime ($P < 0.001$) and amendment type ($P = 0.004$), but not their interaction, had significant main effects for colony-forming unit (CFU) values. In general, all solar-heated treatments showed decreased FOL CFU levels compared with the initial levels observed immediately after amendment or those measured following RT incubation (Figure 5). Additionally, within each temperature regime, all amendment types exhibited lower FOL CFU levels compared with the non-amended control following the 8-day treatment duration. One-way ANOVA was used to determine the significance of differences among mean CFU counts from differing temperature regimes within each amendment treatment. Both solarized, non-amended soil layers showed significantly lower FOL numbers ($P < 0.05$) than the RT samples. The top solarized layer showed significantly lower CFUs ($P < 0.05$) than the initial soil. PSC samples showed a significant decrease in CFUs for RT-incubated and both solarized layers ($P < 0.05$). PSAD samples showed a significant decrease in FOL after incubation at RT ($P = 0.003$) and both solarized layers showed significantly fewer CFUs than samples incubated at RT. Both solarized samples showed significantly lower FOL than the initial soil ($P < 0.05$). Regarding the amendment type effect, the PSLD sample showed significantly lower CFUs than the non-amended soil ($P = 0.01$) for the initial samples and non-amended soil had significantly higher CFUs ($P = 0.02$) than PSLD-treated soil after incubation at RT. Additionally, one-way ANOVA was used to compare mean CFU counts between the various amendment treatments

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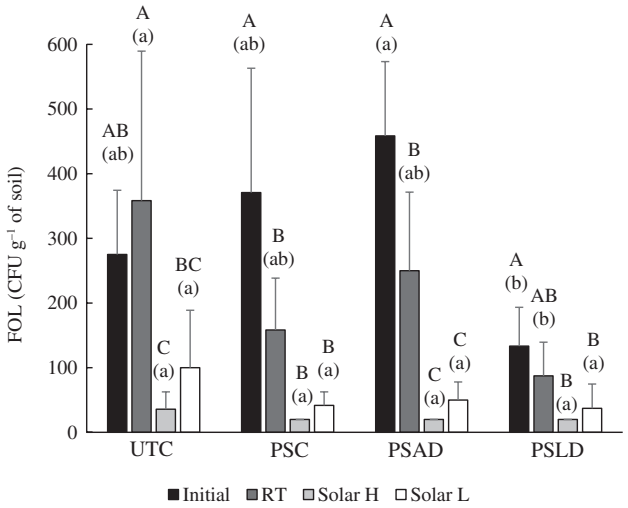

Figure 5. *Fusarium oxysporum* f. sp. *lactucae* (FOL) colony-forming units (CFUs) of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD) before the experiment and after incubation at room temperature (RT) or solarization (H, 0–7 cm; L, 14–20 cm depth). Bars represent the standard deviation of the mean ($n = 5$). Different uppercase letters indicate significant differences based on the Tukey–Kramer test between temperature regimes within a given amendment treatment ($P < 0.05$). Different lowercase letters indicate significant differences based on the Tukey–Kramer test between amendment treatment within a given temperature regime ($P < 0.05$).

within a given temperature regime. Significant differences were only observed between PSAD and PSLD ($P < 0.05$) for the initial samples and between the non-amended soil and PSLD ($P < 0.05$) after incubation at RT.

3.5 Analysis of soil properties

To assess the impact of SBS on soil properties, the OM, EC, NH_4^+, NO_3^-, PO_4-P, K and CEC levels of the initial samples and the upper (0–7 cm) and lower (14–20 cm) layers of the solarized mesocosms were analyzed (Table 3). The two-way ANOVA showed significant effects ($P < 0.05$) for amendment type, temperature regime, and their interaction on the EC, OM and PO_4-P (Table S2). Both factors significantly affected ($P < 0.05$) the K levels, although they did not show an interaction effect. Amendment type significantly affected the CEC and sodium (Na) levels ($P < 0.05$), and had a significant interaction effect with temperature regime for Na level ($P < 0.05$). Finally, the temperature regime significantly impacted the NO_3^- level ($P < 0.05$). The addition of PSC and PSAD significantly increased the OM content ($P < 0.05$) before any incubation treatments were applied. After solarization, the lower solarized layer of the non-amended soil showed significantly lower OM content than PSC, PSAD and PSLD ($P < 0.05$), whereas, in the upper layer, differences were only significant between the non-amended soil and PSC and PSLD samples. The non-amended and PSLD soils presented significantly lower OM content in both solarized layers than the initial state ($P < 0.05$). For PSAD, only the higher layer showed a significantly lower OM content ($P < 0.05$) whereas for PSC, it was the lower solarized layer that showed significantly lower OM content ($P = 0.04$). The addition of the three amendments also significantly increased the initial EC ($P < 0.05$). After solarization, EC levels continued to be significantly higher ($P < 0.05$) in the amended soils in the lower and upper layers, except for PSAD in the upper layer, which showed similar values to the non-amended, solarized soil. NH_4^+ was not detected in any of the samples. The NO_3^- concentration was similar in all the samples at the beginning of the experiment and after solarization in both layers. However, in general, the levels of NO_3^- in the non-amended soil, PSC and PSAD samples were significantly reduced ($P < 0.05$) after solarization. PO_4-P levels at the beginning of the experiment were slightly lower for PSLD samples, although this difference was only significant between PSLD and PSAD ($P = 0.038$). After solarization, the non-amended soil and PSC showed a significantly ($P < 0.05$) higher PO_4-P concentration than PSAD and PSLD in the upper soil layer. However, the lower soil layer did not show significant differences among the treatments after solarization. K levels were increased significantly by amendment addition ($P < 0.05$), with the highest levels observed in the PSC and PSLD treatments. This trend was also observed in the upper solarized layer. Concentrations of K for PSAD and PSLD samples in the lower solarized layer were significantly higher than in the non-amended soil ($P < 0.05$). Initially, the amendment addition did not increase Ca levels significantly. Compared with the UTC and PSAD upper solarized layer, PSC showed significantly ($P < 0.05$) higher Ca levels and PSLD significantly lower levels ($P < 0.05$). Mg level was significantly increased ($P < 0.05$) in PSC and PSAD samples by the amendment addition. PSLD samples showed a significantly ($P < 0.05$) lower Mg concentration than the other samples in the upper solarized layer. Initially, Na levels were also increased by amendment addition ($P < 0.05$). The solarized, non-amended samples showed generally lower Na levels. Differences were only significant for PSC and PSLD in the upper layer and for PSAD in the lower layer. Finally, the initial CEC of the PSAD and PSC samples was significantly higher than in the non-amended soil ($P < 0.05$). After solarization, CEC remained significantly higher for PSC samples ($P < 0.05$) in the upper solarized layer. CEC differences between the treatments were not significant in the lower solarized layer.

4 DISCUSSION

Despite the significant differences in the respiration of amended soils, the observed degree-day values did not indicate significant differences between amended and non-amended soil. While soil respiration measurements were made in aerated soil bioreactors, the field mesocosms were probably more anaerobic as a result of the tarp excluding oxygen and the greater moisture content. Decreased exothermic activity under anaerobic conditions may have muted biological heating of the soil. Therefore, differences observed between non-amended and amended soil in the inactivation of the target pests cannot be attributed to an additional increase in temperature resulting from microbial exothermic metabolic activity as observed in other studies.[9] Differences in the porosity and water-holding capacity of the soil used in the present study compared with the sandy soil used in other work[9] may also have contributed to the lack of biological heating. Moreover, the correlation between moisture content and ΔT_T1 highlights the significant effect of moisture content on the thermodynamic properties of the soil. In addition, the slight difference in the mean temperature and degree-day values between the upper and lower layers (Table 1) also highlights that the complete inactivation of FOL in the upper layer (FOL levels below the quantification limit of the method) is probably attributable to maximum temperatures experienced in the upper layer. In addition to the impact on the thermodynamic properties, moisture content can also have a direct effect on soil soilborne pathogens,[37] and further specific studies are needed to elucidate this effect.

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Table 3. Organic matter content (OM; %), electrical conductivity (EC; µS cm^{-1}), nitrates (NO_3, mg kg^{-1}), phosphates (PO_4-P; mg kg^{-1}), potassium (K; mg kg^{-1}) and cation exchange capacity (meq 100 g^{-1}) of the non-amended soil (UTC) and soil amended with partial compost (PSC), partial solid digestate (PSAD) and partial liquid digestate (PSLD) before (T0) and after solarization at the lower level (L; 14–20 cm) and upper level (H; 0–7 cm) of soil mesocosms

		OM (%)	EC (µS cm^{-1})	NO_3 (mg kg^{-1})	PO_4-P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	CEC (meq 100 g^{-1})
Initial	UTC	2.64 ± 0.15 b(A)†	400 ± 34 c(B)	9.40 ± 3.71(A)	36.40 ± 5.37 ab(A)	353 ± 32c	1372 ± 171	1329 ± 157b	52.40 ± 5.73 c	37.62 ± 4.48 b
	PSC	3.64 ± 0.44 a(A)	931 ± 147 b	7.80 ± 2.17(A)	33.80 ± 3.27 ab	668 ± 26 a(A)	1524 ± 40(B)	1545 ± 36 a(A)	167.20 ± 8.84 b	45.30 ± 1.03 a(A)
	PSAD	3.42 ± 0.23 a(A)	746 ± 37b	10.40 ± 4.51 (A)	37.80 ± 8.29 a(A)	543 ± 65 b	1533 ± 34	1513 ± 20 a	151.60 ± 33.52 b	44.08 ± 1.16 a
	PSLD	2.92 ± 0.15 b(A)	1252 ± 303 a	6.80 ± 3.35	26.20 ± 6.38 b	670 ± 50 a	1489 ± 166	1378 ± 116 ab	241.00 ± 16.69 a	42.84 ± 3.94 ab
Solarized (H)	UTC	1.72 ± 0.37 b(B)	630 ± 120 b(A)	2.60 ± 0.89(B)	31.60 ± 4.88 a(A)	322 ± 3 c	1446 ± 33 b	1400 ± 34 a	79.80 ± 42.86 b	39.60 ± 0.97 b
	PSC	2.92 ± 0.51 a(AB)	1384 ± 327 a	2.00 ± 0.00(B)	32.80 ± 2.68 a	592 ± 2 a(B)	1602 ± 49 a(A)	1470 ± 65 a(A)	221.60 ± 58.76 a	44.90 ± 2.12 a(A)
	PSAD	2.22 ± 0.61 ab(B)	803 ± 305 b	3.00 ± 1.41(B)	25.40 ± 2.19 b(B)	441 ± 46 b	1451 ± 75 b	1391 ± 69 a	144.20 ± 63.15 ab	40.66 ± 2.42 b
	PSLD	2.58 ± 0.15 a(B)	1370 ± 184 a	3.60 ± 0.55	25.00 ± 1.58 b	554 ± 46 a	1320 ± 23 c	1272 ± 46 b	205.00 ± 37.27 a	38.54 ± 1.43 b
Solarized (L)	UTC	1.26 ± 0.21 c(B)	513 ± 63 b(AB)	3.20 ± 2.17(B)	20.60 ± 5.98(B)	312 ± 18 b	1361 ± 109	1298 ± 99	73.60 ± 11.19 b	36.98 ± 2.75
	PSC	2.65 ± 0.68 b(B)	1090 ± 191 a	4.25 ± 1.26(B)	27.50 ± 5.00	565 ± 31 ab(B)	1496 ± 38(B)	1374 ± 38(B)	186.50 ± 8.74 ab	41.78 ± 1.03(B)
	PSAD	3.90 ± 0.48 a(A)	1192 ± 292 a	3.20 ± 0.45(B)	28.20 ± 4.55(AB)	645 ± 300 a	1782 ± 623	1671 ± 628	276.40 ± 156.83 a	50.72 ± 19.35
	PSLD	2.12 ± 0.08 b(C)	1443 ± 186 a	5.20 ± 1.92	25.00 ± 2.35	666 ± 123 a	1527 ± 271	1363 ± 436	228.20 ± 65.42 ab	42.82 ± 11.03

†Within each column, values that do not share a letter are significantly different based on the Tukey–Kramer HSD test ($P < 0.05$). Uppercase letters compare temperature regimes within each amendment treatment; lowercase letters compare amendment treatments for a given temperature regime. When no significant differences were observed, letters have been omitted.

Cumulative CER data confirmed that application of partially stabilized amendments added to the soil labile OM. This available OM promoted VFA accumulation in the soil under biosolarization conditions. Soils amended with PSLD showed elevated VFA levels immediately following amendment. This was expected as VFAs are produced in the aqueous fraction of sludge during the anaerobic digestion process and were retained in the PSLD samples. PSLD showed significantly lower microbial activity, measured by the cCER, than the PSAD. This may be attributed to lack of available carbon or to a direct toxicity effect of the digestates as observed for FOL in the initial PSLD samples. PSC and PSAD had sufficient OM to significantly increase the OM content of the soil compared with the non-amended and PSLD samples ($P < 0.05$; Table 3). The OM was sufficiently labile to yield higher cCER values and support fermentation and significant VFA accumulation during solarization.

In general, VFA levels were lower in the upper layer of the soil compared with the lower layer, suggesting differences existed in the magnitude or mode of microbial activity in this layer. For instance, proximity to the surface may have supplied enough oxygen to limit anaerobic activity and VFA accumulation.[9] It may also have promoted volatilization and removal of VFAs in this layer. At the end of the experiment, acetic acid was the VFA with the highest concentration and results do not indicate whether a maximum concentration was reached or if it was still increasing. Acetic acid has often been used in herbicide formulations to control weeds.[38] Moreover, as found in previous studies,[16] a significant positive correlation was observed between the mortality of BN and the total VFA level when data from all treatments were pooled (Figure 6, solid line; $P < 0.001$; $r = 0.56$). Interestingly, when the percent mortalities of BN in samples incubated at RT and those that were solarized were correlated to the VFA content separately, the correlation between the total VFA and percent mortality of BN at RT (mean temperature of 25 °C) was significantly negative (Figure 6, dotted line; $P = 0.034$; $r = -0.47$), whereas solarized samples (mean temperature of 34 °C) showed a significant positive correlation (Figure 6, dashed line; $P = 0.015$; $r = 0.55$). These results suggest that, during SBS, increased temperature may induce thermal inactivation, create anaerobic stress by fostering increased soil fermentation, or enhance the pesticidal activity of fermentation products like VFAs. No correlation was found between pH and weed seed inactivation, which indicates that VFA accumulation during solarization may enhance weed seed inactivation without significantly affecting the pH. Also, the evolution, concentration, persistence, and activity of chemical compounds other than VFAs, along with microbial activity and other potential mortality factors in the amended soils, cannot be discounted in these findings.[5,17]

Optimal radial growth of FOL has been observed at 25 °C and 30 °C and temperature has already been shown to have a negative effect on FOL radial growth above 30 °C.[35] Therefore, the decrease of FOL in the solarized samples is not surprising. Moreover, the significant decrease of FOL in the upper layer, which depressed levels below the detection limit, is probably attributable to the high temperatures achieved in this layer during solarization. In the lower layer of the amended soils, FOL levels were < 50 CFU g^{-1}. It has been reported that the population range in soil adjacent to healthy plants was 5–27 CFU g^{-1} [25]. The significant decrease in FOL in the amended samples at RT confirms a positive effect of the amendments (Figure 5) on inactivation. The high temperature in the solarized treatments may have overriden the effect of the VFAs on FOL survival. Moreover, the PSLD samples showed lower CFU immediately after amendment, which may have been a consequence of the higher concentration of VFAs in the PSLD compared

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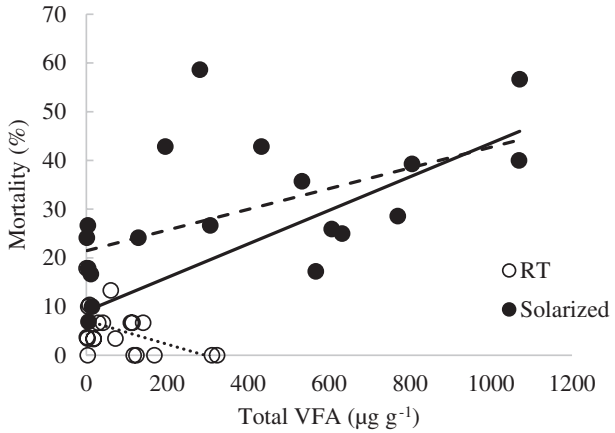

Figure 6. Correlation between the sum of all the volatile fatty acid concentrations (μg g^{-1} of soil) and the percent *Brassica nigra* mortality of the solarized (full dots) and room temperature (RT; empty dots) samples. The solid line includes correlation for all the samples, the dashed line includes only solarized samples and the dotted line includes only RT samples.

with other amendments. To assess the effect of the pH and VFA on FOL survival, their values were correlated with FOL CFUs removing from the analysis the top solarized layer. No significant correlation was observed, which may indicate that other parameters may be involved. In addition to VFA, metal ions such as iron (Fe^{2+}) and manganese (Mn^{2+}) have also been shown to contribute to *F. oxysporum* f. sp. *lycopersici* inactivation.[39] These metal ions can be released under the reducing conditions expected on amended soil covered with plastic tarp.[15] Furthermore, an increase in the ammonia concentration in the soil and pH are factors involved in the suppression of *Fusarium* wilt and other fungal plant pathogens such as *Verticillium dahliae*[40] or *Phytophthora capsici*,[41] especially when soil has been treated with nitrogen-rich organic amendments.[42,43] Ammonia was not detected in the soil analyses; however, a significantly higher pH ($P < 0.05$) was recorded in the initial PSLD samples (Table 2). The lower initial CFU count in these samples could thus be attributed to this effect and the higher amount of VFAs observed. In addition, it has been observed that native microbial communities in the soil contribute to the suppression of *Fusarium* spp.[44] As SBS has been shown to have a significant impact on the soil microbial community,[45] future studies are needed to determine how SBS-induced changes to the soil microbiota affect long-term FOL suppression.

Amendment addition positively affected the OM and K content after the solarization process, as observed in prior studies.[18,46] In addition to these specific impacts, other studies also observed that organic amendments followed by solarization led to higher values of total N, total P, several enzyme activities, microbial biomass C, potentially mineralizable N, water-soluble organic C and microbial functional diversity when compared with non-amended soils.[45,47] Biosolarization studies using thermophilic and mesophilic digestates showed a significant decrease in EC after solarization.[18] In this study, the increase in EC may suggest a need for irrigation after SBS application to avoid negative effects on crops if EC levels are not naturally recovered after SBS. SBS also decreased the NO_3^- concentration and this effect was also observed in prior studies.[18] This may be a negative outcome as NO_3^- is the preferred form of nitrogen for plants. However, in some cases NO_3^- leaching can result in underground water pollution[48]; for this reason, solarization may be a practice to reduce this risk. Moreover, other SBS studies using *Brassica* spp. as organic amendment showed NO_3^- accumulation. However, this study also showed that SBS has lower NO_3^- contamination potential than other practices, as a consequence of the lower irrigation of the soil during the process.[49]

5 CONCLUSIONS

SBS enhanced the inactivation of FOL and BN. Volatile fatty acid production corresponded with increased BN inactivation in the presence of solar heating. Conversely, solar heating was sufficient for achieving high FOL mortality regardless of soil amendment. However, incubation in amended soils showed lesser, but still significant, inactivation of FOL. In light of this, the interactions between digestate properties and FOL warrant further investigation. There is potential to optimize digestate amendment-based strategies to control FOL in the presence of sublethal soil temperatures, particularly by adjusting the amendment level and stability in tandem with the treatment duration. In addition to pest control, amended soils also benefitted from increased OM and potassium levels. However, the greater salinity observed in the amended soils should also be considered when selecting amendment rates for biosolarization. Despite being generated under differing conditions, the three organic amendments studied had similar impacts on SBS and soil properties. This similarity may indicate that the nature of the original feedstock and the level of amendment are more relevant than the process used to stabilize the OM. Further studies are needed to better understand the effect of different feedstocks on VFA accumulation, which have been shown to play a significant role in biosolarization, as well as production of other potential biopesticides.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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