Research Article

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Weed seed inactivation in soil mesocosms via biosolarization with mature compost and tomato processing waste amendments

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Abstract

BACKGROUND: Biosolarization is a fumigation alternative that combines passive solar heating with amendment-driven soil microbial activity to temporarily create antagonistic soil conditions, such as elevated temperature and acidity, that can inactivate weed seeds and other pest propagules. The aim of this study was to use a mesocosm-based field trial to assess soil heating, pH, volatile fatty acid accumulation and weed seed inactivation during biosolarization.

RESULTS: Biosolarization for 8 days using 2% mature green waste compost and 2 or 5% tomato processing residues in the soil resulted in accumulation of volatile fatty acids in the soil, particularly acetic acid, and >95% inactivation of *Brassica nigra* and *Solanum nigrum* seeds. Inactivation kinetics data showed that near complete weed seed inactivation in soil was achieved within the first 5 days of biosolarization. This was significantly greater than the inactivation achieved in control soils that were solar heated without amendment or were amended but not solar heated.

CONCLUSION: The composition and concentration of organic matter amendments in soil significantly affected volatile fatty acid accumulation at various soil depths during biosolarization. Combining solar heating with organic matter amendment resulted in accelerated weed seed inactivation compared with either approach alone.

Keywords: tomato pomace; soil acidification; volatile fatty acids; passive solar heating; compost; sustainable agriculture; integrated pest management

1 INTRODUCTION

Chemical fumigation is a well-known agricultural practice for effectively inactivating a wide range of soil pests. In particular, soil fumigation with methyl bromide, a broad-spectrum, halogenated hydrocarbon fumigant, has been widely used in agriculture for more than 50 years.[1] However, the global use of methyl bromide has been gradually phased out under the 1989 Montreal Protocol[2] owing to its negative environmental impact, particularly with regard to stratospheric ozone depletion.[1,3] With the reduced availability of methyl bromide application, and the resultant increased cost to end users, other fumigants such as chloropicrin, 1,3-dichloropropene and metam sodium/potassium have gained prominence. While these fumigants are less damaging to the ozone layer than methyl bromide, they present their own environmental and human health hazards, such as carcinogenesis and mutagenesis.[4] Furthermore, there has been steadily increasing consumer demand for food produced without industrial pesticide inputs.[5] As a result, there is a need for soil fumigation alternatives.

Soil solarization is a chemical-free fumigation alternative that relies on passive solar heating of moist soil mulched with clear plastic tarp to inactivate pests.[6] Various solarization implementation strategies, heat transfer models and soil microbial community effects have been investigated.[7–11] Despite these efforts to model, optimize and translate solarization to commercial agriculture, it has not been widely adopted and is currently only used for high-value horticultural crops, mostly under organic production.[12] Long treatment times (more than 3 weeks) and reliance on conducive weather to accumulate passive solar heating in the soil have been cited as factors that discourage use of solarization by growers.[13,14] To overcome these limitations,

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solarization has been combined with soil amendments, including crop residues[15] and composts,[16] to increase pesticidal efficacy. This modified form of solarization is termed biosolarization.[13] The organic matter amendment component of biosolarization stimulates microbial activity in the soil to enhance pest inactivation and reduce dependence on weather and climatic conditions. In essence, biosolarization is an amalgam of the biocidal processes that occur during conventional solarization and those of the similar, non-temperature-dependent strategy known as biological or anaerobic soil disinfestation (ASD).[17] Biosolarization combines the high temperature stress of solarization with the strongly reductive conditions and biocidals generated during ASD, which result from fermentation of organic matter amendments in saturated soil.[17,18] However, it should be noted that different pest inactivation mechanisms, such as biological soil heating,[19,20] may occur if aerobic or microaerobic soil conditions are present.

Biosolarization research has explored the use of a number of organic matter amendments in the soil to control a variety of pests, including nematodes, fungi, bacteria and weeds. Soil amendments such as fresh sheep manure,[21] Japanese radish residues, mixtures of sheep and chicken manure,[13,22] *Brassica* crop residues and pellets, sugar beet vinasse and olive pomace, have been tested and reported to be effective for biosolarization.[11,23–25] While these studies have provided information regarding the end result of biosolarization, less is known regarding biological, chemical and physical conditions in the soil during biosolarization and how they relate to pest inactivation kinetics during treatment.

Prior studies have investigated soil temperature and microbial activities that are relevant to biosolarization. Laboratory studies have considered the effect of soil temperature on weed seed inactivation. This research showed rapid inactivation of weed seeds from a variety of species at ≥50 °C.[26] However, at temperatures ≤40 °C, which may be encountered deeper in the soil during solarization, weed seed inactivation was less consistent and varied by species.[26] Regarding soil microbial metabolic activity during biosolarization, previous studies have measured levels of adenosine triphosphate and activities of dehydrogenase, phosphatase, urease and β-glucosidase enzymes following treatment.[13] Although these indicators of microbial activity are useful for gauging microbial vitality in the soil, they are not directly responsible for pest inactivation. When soil oxygen is limiting, anaerobic fermentation products such as volatile fatty acids (VFAs) are likely to have a direct impact on pest inactivation during biosolarization. VFA production in soil following organic matter amendment has been observed in wet soils[27,28] and in anaerobic soil disinfestation studies.[17,29] Prior research involving laboratory microcosms has demonstrated relationships between temperature, amendment concentration and treatment time on weed seed inactivation in soils amended with *Allium* crop residues.[30] To date, biosolarization studies have not combined analysis of soil heating and acidification with measurement of weed seed inactivation kinetics in a field setting, which is required to explore interactions between soil amendments, soil heating, VFA accumulation and treatment time that may affect biosolarization efficacy.

In this study, soil heating, VFA accumulation and weed seed mortality were determined in a mesocosm-based biosolarizaion field trial using mature green waste compost and industrial tomato processing pomace (TP) (the waste skins and seeds from commercial tomato paste production) as soil amendments. These materials have been shown to be effective amendments for inducing fermentation and VFA production under biosolarization soil conditions while avoiding lingering phytotoxicity in the soil after treatment.[19,20] In the present study, biosolarization with varying levels of compost and TP amendments in the soil was used to test the feasibility of inactivating seeds of two weedy forbs commonly found in California agriculture: *Brassica nigra* (black mustard) and *Solanum nigrum* (black nightshade). Furthermore, the kinetics of weed seed inactivation was measured during treatment. To gain a better understanding of the relationship between soil amendments and generation of weed-seed-inactivating conditions in the soil during biosolarization, changes in soil temperature, pH and VFA content were measured in response to various solar heating and soil amendment treatments.

2 MATERIALS AND METHODS

2.1 Field preparations

The field site was located at the Kearney Agricultural Research and Extension Center in Parlier, CA (36.6° N, 119.5° W; elevation 97 m a.s.l.). The field site was left fallow from July 2012 until the start of the field trial, with some cool-season weed cover during winter months. The site was prepared as previously described.[20] Briefly, the site was irrigated, dried, plowed in two directions with a disc harrow and tilled with a rotovator, then smoothed with an orchard float. The site was irrigated 5, 3 and 1 day before the field experiment using solid set sprinklers. An additional irrigation was performed immediately before application of plastic film to the field, to bring total pre-experiment soil wetting to approximately 6.5 cm of water. This was adequate to bring the soil moisture to field capacity (~11% wet basis) at the depths sampled in this study. The experimental site was surrounded by a field fence to prevent disruption by intruding fauna.

2.2 Soil amendments and mesocosm preparation

Tomato pomace (TP) was previously collected from an industrial tomato paste production facility in Dixon, California, during the 2014 processing season. Mature green waste compost (GWC) generated from yard clippings was obtained from a commercial composting site in Zamora, California, in 2015. Both materials were air dried and then stored in sealed plastic containers indoors under ambient conditions until use. The TP and GWC had C/N ratios of 17 and 20 respectively. Additional relevant material properties such as water-holding capacity, pH and organic matter content can be found in other published work.[31] Prior work has demonstrated that the mature green waste compost used in this study is highly stable and does not induce respiration over time periods relevant to biosolarization when amended into soil by itself.[19] As a result, the compost can be viewed as an inoculum to introduce lignocellulolytic microorganisms into the soil rather than as a source of digestible organic matter. Instead, the tomato pomace acted as a source of digestible organic matter for soil and compost microorganisms. To facilitate uniform soil amendment at mesocosm scale, dried TP was processed in a laboratory blender to reduce the particle size to less than 1 mm ahead of soil amendment.

To prepare amended soil for the field trial, dry topsoil (Hanford sandy loam) was collected from the upper 0–15 cm of the field site the day prior to the field trial. Soil was sieved through a 3.18 mm screen to remove large rocks and organic matter particulates. Sieved soil was combined with varying levels of compost and tomato pomace (Table 1) and then mixed until uniform. Relatively high amendment levels were used to test the feasibility of using tomato pomace for biosolarization in a field setting. Water was added to achieve 80% field capacity for each mixture.[19]

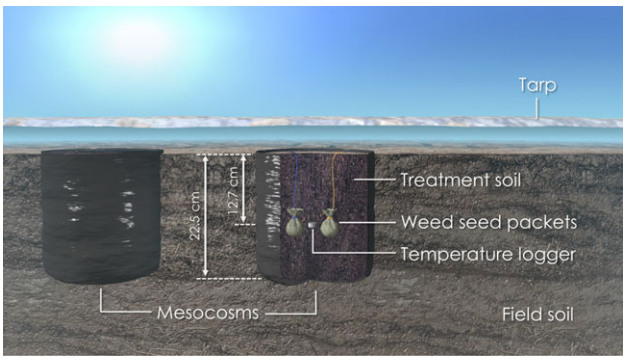

Figure 1. Transverse cutaway of a field plot, illustrating two soil mesocosms as they would appear when embedded in the soil during biosolarization. The cutaway of the mesocosm reveals the arrangement of weed seed packets and temperature dataloggers within the soil.

Wetted mixtures were sealed in plastic bags and incubated at 4 °C overnight to permit moisture equilibration.

Mesocosms containing amended soil served as experimental units for the biosolarization field trial. All mesocosms were prepared in 3.8 L black plastic soil grow bags (New England Hydroponics, Southampton, MA) with drainage holes to facilitate moisture and gas exchange with the soil in the field. Mesocosms were partially filled with wetted soil mixture, and a miniature temperature sensor and data logger (Thermochron iButtons model 1922 L; Embedded Data Systems, Lawrenceburg, KY) was placed at the center of the mesocosm such that it was located at a depth of 12.7 cm from the top of the grow bag. Two permeable nylon mesh packets of weed seeds were also placed at this depth within each mesocosm (Fig. 1), each containing either 30 seeds of *B. nigra* or 50 seeds of *S. nigrum* and 2.46 mL of the appropriate soil mixture to provide direct contact with seeds.[32] The baseline germination rates of the seed stocks were 75 and 43% for *B. nigra* and *S. nigrum* respectively. Additional soil mixture was added to fill the soil bags completely. Lengths of string were attached to each seed packet and exposed at the surface of filled mesocosms to permit retrieval of the packets at chosen intervals. Control mesocosms for room temperature incubation were prepared as described, except the temperature loggers were omitted. A 50 g soil mixture sample from each mesocosm was taken during preparation and stored at −20 °C for subsequent measurement of VFA content and pH.

2.3 Field plot design and biosolarization

The field site was divided into five plots. Each plot measured 1.8 by 8.5 m. Plots were arranged linearly with a 1.8 m buffer between each plot. Ten soil mesocosms (one of each amended soil treatment described in Table 1 plus three of each treatment used for measurement of seed inactivation kinetics) were buried in carefully excavated holes along the midline of each plot. Embedded mesocosms were positioned such that the center point of every mesocosm was 0.6 m from that of each flanking mesocosm, and at least 0.9 m from the nearest plot borders to minimize edge effects. The arrangement of the mesocosms was randomized in each plot to minimize positional effects. Each buried mesocosm was covered with a thin layer (approximately 1 cm) of field soil to conform to the level surface of the field plot. Strings attached to weed seed packets in the mesocosms remained above the soil surface to permit retrieval during biosolarization (Fig. 1).

Biosolarization was initiated on 9 July 2015 by covering each freshly irrigated plot with 0.7 mil transparent plastic film ('Huskey Film Sheeting'; Poly-America, Inc., Grand Prairie, TX) and burying the sheet edges in the soil along plot borders. Biosolarization lasted 8 days. During biosolarization, weed seed packets were removed at 1 and 5 days post-initiation for certain treatments to measure inactivation kinetics (Table 1). Before removal of weed seed packets, weighted boards were placed around target mesocosms to isolate the mesocosm headspace and prevent oxygen contamination of the plot. A small incision was created in the film above each mesocosm, and the seed packets were gently removed by pulling on the attached strings. Film incisions were sealed using transparent packaging tape.

A duplicate set of mesocosms was incubated indoors to act as non-solar-heated controls. The control mesocosms were stored in a temperature-controlled building that was maintained at 27/22 °C (day/night). Control mesocosms were loosely covered with a plastic tarp to minimize moisture loss. Weed seed packets were periodically removed from appropriate mesocosms for measurement of weed seed inactivation kinetics as described previously.

At the end of the 8 day treatment period, the plastic film was removed from the field site and mesocosms were gently exhumed. Both biosolarized and non-solar-heated control mesocosms were transported at ambient temperature in an air-conditioned automobile for approximately 3.5 h to the laboratory. Biosolarized mesocosms were then sectioned into three 7.5 cm layers for subsequent analysis of properties by soil depth. Temperature loggers and remaining weed seed packets were retrieved, and sectioned soil samples were stored at −20 °C until further analysis.

2.4 Soil pH and volatile fatty acid content measurement

The moisture content of soil mixtures was measured gravimetrically by determining the change in sample mass after desiccation in a drying oven at 105 °C. Soil samples were extracted by combining soil with water at a 1:1 mass ratio. pH values were measured in soil and water mixtures using a InLab®Routine Pro ISM, 3-in-1 pH sensor (Mettler-Toledo, Columbus, OH). After settling, the supernatant was filtered through a Titan3 PTFE membrane syringe filter with a 0.2 µm pore size (Thermo Fisher Scientific Inc., San Diego, CA). Filtered extracts were mixed with an equal volume of 5 mM sulfuric acid in distilled, deionized water.

Formic, acetic, propionic, isobutyric and butyric acid contents in soil extracts were analyzed by high-performance liquid chromatography (model UFLC-10Ai; Shimadzu, Columbia, MD). Extracts were run through an Aminex HPX-87H column (300 × 7.8 mm; Life Science Research, Hercules, CA) to separate VFAs using 5 mM sulfuric acid in distilled, deionized water as the mobile phase. The mobile phase flow rate was kept at 0.6 mL min^{-1} for 37 min. The absorbance at 210 nm in the column effluent was detected using an SPD-M20A photodiode array detector (Shimadzu). VFA standards were prepared from analytical-grade formic, acetic, propionic, isobutyric and butyric acids (Sigma-Aldrich Corp., St Louis, MO). Dilutions of these VFAs ranging from 32.5 to 1000 mg L^{-1} were run alongside the experimental extracts and used to determine VFA concentrations in samples. The retention times for the standards were 14.1, 15.4, 18.8, 20.2 and 21.8 min for formic, acetic, propionic, isobutyric and butyric acid respectively. Measured VFA concentrations were normalized according to the moisture content of each extracted sample to yield concentration per unit dry weight of soil.

Table 1. Soil amendment treatments used in the biosolarization field study

Mixture[a] (% dry weight)	Description	Associated experiments
100% Soil	Non-amended soil control	Soil heating and acidification Final weed seed inactivation Weed seed inactivation kinetics
93% Soil + 2% GWC + 5% TP	Inoculated soil with high organic matter level	Soil heating and acidification Final weed seed inactivation
95.5% Soil + 2% GWC + 2.5% TP	Inoculated soil with moderate organic matter level	Soil heating and acidification Final weed seed inactivation Weed seed inactivation kinetics
95% Soil + 5% TP	Uninoculated soil with high organic matter level	Soil heating and acidification Final weed seed inactivation
97.5% Soil + 2.5% TP	Uninoculated soil with moderate organic matter level	Soil heating and acidification Final weed seed inactivation

[a] Mixtures comprise soil, mature green waste compost (GWC) and tomato pomace (TP).

2.5 Weed seed mortality measurement

After removal from mesocosms, weed seed packets were cut open and the contents placed in a kitchen strainer suspended over a 500 mL plastic beaker. Seeds were rinsed with distilled water to remove soil particles and removed from the strainer with soft forceps. Seeds were then placed in 100 × 15 mm petri dishes on Whatman No. 1 filter paper moistened with 1.4 mL of distilled water. Petri dishes were maintained in a growth chamber on a cycle of 8 h at 20 °C in darkness and 16 h at 30 °C in light, and remoistened as needed. Germination percentages for the contents of each weed seed packet were determined 14 days after removal from mesocosms. Seeds were counted as germinated if the radicle had emerged to a length of 3 mm. After 14 days, non-germinated seeds with visible evidence of putrefaction or mold growth were discarded, and all non-germinated seeds with intact seed coats were evaluated for viability by tetrazolium staining.[33] Seeds were incubated for 24 h at 30 °C in 1% (wt/vol) triphenyl tetrazolium chloride, and then seed coats were removed and embryos were examined for staining patterns.

2.6 Data analysis

Cumulative temperature of soil during biosolarization was quantified by determining the area beneath plots of temperature versus time data (i.e. degree-day values). Degree-day values were estimated by using the trapezoidal rule to approximate the integral of temperature versus time for each mesocosm. The cumtrapz command in MATLAB software (v.R2012a; MathWorks, Natick, MA) was used to conduct the approximation.

For soil acidification and weed seed inactivation responses, main and interaction effects for biosolarization process variables were detected using multiway ANOVA. Comparison of mean responses among treatments was conducted via one-way ANOVA with Tukey's honest significant difference *post hoc* test. A family-wise error rate of 0.05 was used for all comparisons. Linear regression was used to correlate weed seed inactivation with soil VFA levels, with a critical *P*-value of 0.05 used to determine whether the slope was significantly non-zero. Statistical analyses were performed using JMP-pro software (v.12.0.0; SAS, Cary, NC).

3 RESULTS

3.1 Soil temperature and moisture content during biosolarization

For all field treatments, soil temperature at 12.7 cm depth gradually increased during the first 4 days of biosolarization (Fig. 2A). Daily peak temperatures were achieved between 15:00 and 18:00 h. The greatest peak temperature observed was in the non-amended soil, which reached 45.57 ± 0.82 °C on the final day of treatment (Fig. 2B). This peak temperature was significantly greater than that observed for soil amended with compost and high levels of organic matter (2% GWC and 5% TP) ($P < 0.001$), which exhibited the lowest peak temperature at 43.92 ± 0.56 °C. There were no significant differences in peak temperature among other treatments. Degree-day values provided an indication of cumulative temperature differences between treatments during the biosolarization period. Soil containing 5% TP had the greatest degree-day value at 282.73 ± 1.04 °C-day and was significantly greater than all other treatments (Fig. 2B). Soil amended with compost inoculum and a high organic matter level (2% GWC and 5% TP) had the lowest value at 273.56 ± 1.42 °C-day.

The initial moisture contents of the amended soils before placement in the field (Table 2) reflected differences in the water-holding capacity for each amendment treatment.[19] The moisture content of the soil following biosolarization was measured across three depth ranges for each mesocosm. Within each treatment, no significant differences in moisture content were observed for the depths tested. Soil amended with compost inoculum and a high organic matter level (2% GWC and 5% TP) had the greatest mean moisture content (0.16 g water g^{-1} dry weight) and the non-amended control soil had the lowest mean moisture content (0.10 g water g^{-1} dry weight). Similar moisture content values were observed for the non-solar-heated (NSH) samples (Table 2).

3.2 Soil acidification and volatile fatty acid production during biosolarization

Soil pH was measured prior to biosolarization for the various amendment treatments. Non-amended control soil had the highest pH (7.21 ± 0.03). Soil amended with varying levels of GWC and TP all showed more acidic pH values than non-amended soil (Fig. 3). Changes in soil pH after biosolarization varied by amendment treatment. Multiway ANOVA of pH data from biosolarized soils revealed that GWC level, TP level and soil depth all had significant negative main effects on pH ($P < 0.0002$ for all). Additionally, there were significant interaction effects between GWC and TP levels ($P < 0.0001$) and between GWC level, TP level and soil depth ($P = 0.008$). These interaction effects could be seen as pH generally

Table 2. Moisture content of soil mixtures before and after treatment[a]

Mixture (% dry weight)	Moisture content at start of biosolarization (g water g^{-1} DS)	Moisture content after biosolarization (g water g^{-1} DS)[b]	Moisture content for non-solar-heated samples following incubation (g water g^{-1} DS)
100% Soil	0.18 ± 0.08	U – 0.09 ± 0.01 M – 0.11 ± 0.04 L – 0.11 ± 0.01	0.13 ± 0.03
93% Soil + 2% GWC + 5% TP	0.25 ± 0.05	U – 0.15 ± 0.01 M – 0.15 ± 0.04 L – 0.17 ± 0.01	0.16 ± 0.01
95.5% Soil + 2% GWC + 2.5% TP	0.23 ± 0.12	U – 0.12 ± 0.01 M – 0.14 ± 0.01 L – 0.14 ± 0.01	0.20 ± 0.02
95% Soil + 5% TP	0.16 ± 0.02	U – 0.15 ± 0.07 M – 0.15 ± 0.01 L – 0.13 ± 0.04	0.15 ± 0.01
97.5% Soil + 2.5% TP	0.16 ± 0.01	U – 0.14 ± 0.06 M – 0.12 ± 0.01 L – 0.13 ± 0.01	0.15 ± 0.01

[a] Values are given as mean ± standard deviation ($n = 5$).
[b] U, upper layer of 0–7.5 cm depth; M, medial layer of 7.5–15 cm depth; L, lower layer of 15–22.5 cm depth.

decreased with greater soil depth in amended soils but remained stable at all measured depths in non-amended soil (Fig. 3). Moreover, GWC addition led to greater acidification when 5% TP amendment was used but had no significant effect on acidification in the presence of 2.5% TP. Soil amended with 2% GWC and 5% TP exhibited the greatest acidification following biosolarization (Fig. 3), with values falling to approximately pH 5.4 at depths of 7.5 cm and beyond. Soil inoculated with 2% GWC but with a lower TP amendment of 2.5% showed smaller changes in pH. For this treatment, the pH in the uppermost soil layer increased to 7.97 from an initial value of 6.57 before biosolarization. Similar, but more exaggerated, trends were observed in soil containing moderate or high organic matter (2.5% and 5% TP) but lacking GWC inoculation. For these treatments, even more alkaline pH values were obtained in the uppermost soil layer and the acidification at lower soil depths was less drastic. In total, no amended soils had significantly lower pH than the non-amended control soil within the uppermost 7.5 cm. At medial depths spanning 7.5–15 cm below the surface, only soil amended with compost inoculum and a high organic matter level (2% GWC and 5% TP) yielded significantly lower pH compared with the non-amended control. At the deepest layer tested, ranging from 15 to 22.5 cm depth, soils amended with 2% GWC and either 2.5% or 5% TP both showed significantly lower pH compared with the non-amended control soil (Fig. 3).

The pH values of non-solar-heated control mesocosms were not analyzed by depth because the nature of their incubation promoted spatially uniform heating (in contrast to the temperature gradient experienced by biosolarized mesocosms owing to one-dimensional heat transfer from the surface). As a result, soil pH values were measured only at the medial layer of non-solar-heated control mesocosms. In general, changes in pH for the non-solar-heated control mesocosms mirrored those seen in biosolarized mesocosms at the equivalent depth. Similarly to biosolarized soils, only soil amended with 2% GWC and 5% TP showed significantly decreased pH compared with non-amended soil (Fig. 3).

Levels of certain VFAs, specifically formic, acetic, propionic, butyric and isobutyric acids, were measured in soils prior to biosolarization, at multiple depths following biosolarization and in non-solar-heated controls (Fig. 4). The total VFA level in each sample was calculated as the sum of the concentrations for each measured VFA. Logarithmic transformations were performed on total VFA level data to create homogeneous variance among groups and satisfy the assumptions of ANOVA. Multiway ANOVA of total VFA data in biosolarized treatments (Fig. 4A) revealed significant positive main effects for GWC level, TP level and soil depth ($P < 0.0001$ for all). Furthermore, there was a significant interaction effect between TP level and soil depth ($P = 0.015$). The interaction was most evident for GWC-amended soils with 5% TP, which exhibited markedly higher total VFA levels near the soil surface than those with 2.5% TP (Fig. 4A). Non-amended soil did not exhibit measurable background VFA levels. Likewise, non-amended soil did not show significant accumulation of VFAs above the baseline following biosolarization or incubation at room temperature. In contrast, amended soils showed varying initial VFA levels, depending on the treatment (Figs 4B to F), suggesting that VFA production began quickly during the overnight equilibration period prior to embedding mesocosms in the field. Soil amended with 2% GWC and 5% TP showed the greatest initial levels of formic, acetic, propionic and isobutyric acids. These levels were either maintained or altered during biosolarization, depending on the VFA and the soil depth. Soil containing 2% GWC and 5% TP maintained the greatest levels of formic, acetic, propionic and isobutyric acids following biosolarization or incubation at room temperature. For this treatment, acetic acid was the most abundant of the measured VFAs, and the levels increased with soil depth. A similar trend was observed for propionic acid, the second most abundant VFA. GWC addition had a significant effect on the composition of VFAs in biosolarized and non-solar-heated soils. For soils containing 5% TP, GWC addition enhanced accumulation of formic, acetic, propionic and isobutyric acids during treatment, although the significance of the effect was more pronounced for different soil depths and heating regimens for each VFA. Notably, butyric acid was only detected in TP-amended soils when GWC was absent.

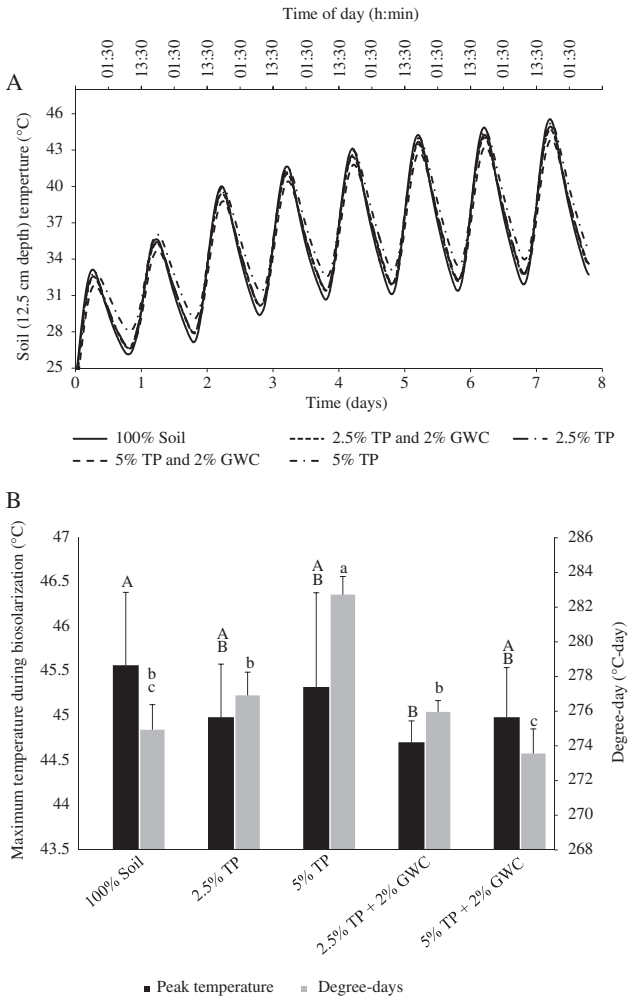

Figure 2. Soil temperature parameters during biosolarization. (A) Soil temperature at 12.7 cm depth during biosolarization. (B) Peak and cumulative temperature values during biosolarization. The treatments are as follows: soil control – 100% non-amended soil; 2.5% TP and 2% GWC – soil amended with 2.5% tomato pomace and 2% green waste compost; 2.5% TP – soil amended with 2.5% tomato pomace; 5% TP and 2% GWC – soil amended with 5% tomato pomace and 2% green waste compost; 5% TP – soil amended with 5% tomato pomace. For each response, values that do not share a letter are significantly different ($P \leq 0.05$). Values represent the mean of five replicate mesocosms for each treatment. Error bars indicate one standard deviation.

3.3 Weed seed inactivation

Weed seed mortality was measured at several time points during biosolarization. The data revealed that there were marked differences between amendment treatments in terms of the rate and ultimate level of weed seed inactivation for both *B. nigra* and *S. nigrum*. Inactivation kinetics data for inoculated soil with moderate organic matter amendment (i.e. 2% GWC + 2.5% TP) showed that the biosolarized soil resulted in more rapid seed inactivation and higher overall mortality levels for both weed species relative to the unheated controls and heated, non-amended soil (Fig. 5). Specifically, by the fifth day of treatment, biosolarized soil amended with compost inoculum and moderate levels of organic matter provided nearly complete inactivation of both *B. nigra* and *S. nigrum* (93 and 98% mortality respectively). This was significantly higher than the mortalities observed at the same time point for both non-solar-heated treatments and non-amended, solarized soil ($P < 0.001$ for all comparisons). Mortality increased to 99% for both weed species in biosolarized soil containing compost and organic matter amendments by the conclusion of the 8 day treatment period. In contrast, the final mortality of *B. nigra* and *S. nigrum* seeds ranged from 36 to 60% and from 72 to 81%, respectively, in all other treatments (Fig. 5).

Final weed seed inactivation data also showed differences in weed seed inactivation, depending on the amendment treatment and incubation conditions. Multiway ANOVA showed significant positive main effects for solar heating and TP level on *B. nigra* mortality ($P < 0.0001$). There was also a significant interaction effect between GWC level, TP level and solar heating ($P = 0.008$). For *S. nigrum*, the only significant main effect was solar heating ($P = 0.0005$). As with *B. nigra*, a significant interaction among the three biosolarization factors was detected ($P = 0.04$). These three-way interaction effects could be seen in the data, as GWC and TP amendment had more variable effects on seed mortality in the absence of solar heating while all amended soil treatments performed similarly when solar heated (Fig. 6). For biosolarized soil, near complete weed seed inactivation was obtained at 8 days of treatment for 2.5% and 5% TP, regardless of whether GWC inoculum was added (Fig. 6). These levels were significantly higher than those observed for both weed species in non-amended, solarized soil control treatments ($P < 0.001$).

More variable results were seen for non-solar-heated amended soils. When incubated at room temperature, increasing weed seed mortality was observed with increasing TP level in soils inoculated with GWC (Fig. 6). For non-solarized soil with 2% GWC and 5% TP, 98–100% mortality was achieved for both weed species, which was significantly greater than the mortality observed in non-amended, non-solarized soil. Non-solarized soil containing TP but not GWC exhibited variable results, with 2.5% TP yielding greater weed seed mortality than 5% TP for both weed species.

Linear regression analyses of seed mortality data versus total VFA levels in the soil were conducted for solar-heated and non-solar-heated soils. For both *B. nigra* and *S. nigrum*, significant positive relationships were detected between the total concentration of VFAs in the soil and seed mortality ($P = 0.05$, $R^2 = 0.16$ and $P = 0.006$, $R^2 = 0.28$ for *B. nigra* in heated and non-heated soils respectively; $P = 0.02$, $R^2 = 0.22$ and $P = 0.014$, $R^2 = 0.24$ for *S. nigrum* in heated and non-heated soils respectively). Low coefficient of determination values resulted from variable seed inactivation at lower VFA levels and constant inactivation (i.e. 100% mortality) at higher VFA levels.

4 DISCUSSION

4.1 Soil temperature during biosolarization

Conventional soil solarization, i.e. not combined with organic amendment(s), relies primarily on passive solar heating of soil to induce the physical, chemical and biological changes in soil that result in inactivation of soil pests.[6,8,34] In terms of physical soil heating, prior research has modeled the complex heat transfer processes associated with solarization.[35] Temperature stress remains a key pest inactivation mechanism for biosolarization, and it may be augmented by an additional heating mechanism – biological heat generation. This additional heat source, produced from the exothermic metabolism of soil microbes as they consume amended organic matter, can elevate soil temperature beyond that achieved solely through solar heating. Such temperature elevation has been demonstrated in prior microcosm-based biosolarization studies using compost, wheat

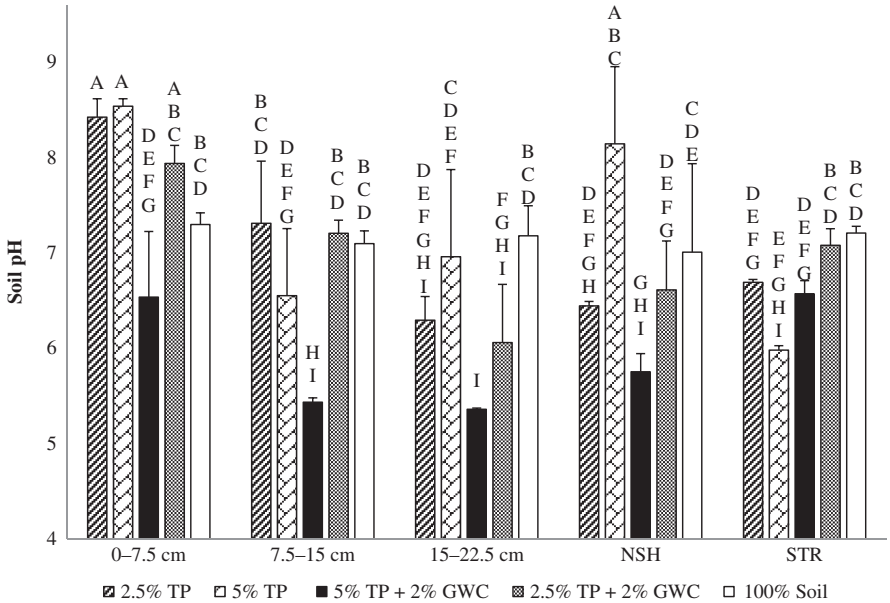

Figure 3. Soil pH prior to and following biosolarization with various soil amendments. Soils contained tomato processing residues (TP) and mature compost from yard clippings (GWC) at varying levels (% dry basis). Values are given for amendment treatments at the start of biosolarization (STR), various soil depths following biosolarization (0–7.5 cm, 7.5–15 cm and 15–22.5 cm) and non-solar-heated control samples incubated at room temperature (NSH). Values that do not share a letter are significantly different ($P \leq 0.05$). Error bars represent one standard deviation ($n = 5$).

bran and manure amendments.[20,22] Soil amended with mixtures of GWC and TP gave significant peak temperature elevations due to biological heating, although the effect was only observed when the soil was not completely anaerobic.[19]

Notably, no significant biological temperature elevation of the soil was observed during biosolarization in the present field study. None of the biosolarized soil treatments showed increased peak temperatures, or cumulative temperature elevations, with respect to non-amended, solarized soil. In fact, soil amended with compost inoculum and a high organic matter level (2% GWC and 5% TP) yielded a significantly lower peak temperature compared with non-amended soil. The weather during the field trial may have contributed to this result. Despite biosolarizing during what is traditionally the warmest period of the year, an uncharacteristic cool weather system was present during the first half of the field trial. The CIMIS weather monitoring station at KARE reported that the high temperatures during the first four days of the field trial were 27.5, 29, 30.4 and 32.3 °C respectively. In contrast, the final four days had high temperatures of 33.5, 33.8, 33.9 and 35.8 °C.[36] This was reflected in the soil temperature profiles during biosolarization, where the peak temperature at 12.7 cm depth only exceeded 43 °C from the fifth day onward. The cooler weather along with and more anaerobic conditions expected at the depth of the temperature loggers, which attenuated passive solar heating, may also have retarded microbial activity in the soil, such that biological heat generation was insufficient to produce a measurable temperature increase beyond that obtained through passive solar heating. Furthermore, the decreased peak temperatures observed in soils amended with GWC may have stemmed from the greater water-holding capacity afforded by the compost. Moisture content data showed that GWC-amended soil contained more water than other treatments after biosolarization. Given similar heat input, this additional water would result in lower temperature elevation in compost-amended soil compared with non-amended soil owing to the high specific heat capacity of water. These results highlight the need to consider the climate and weather along with changes in soil water-holding capacity due to soil amendments when seeking to maximize temperature elevation during biosolarization.

4.2 Soil pH and VFA level changes during biosolarization

The chemical compounds produced in soil during biosolarization are expected to boost pest inactivation efficacy. Factors produced by amending soil with a sufficient quantity of appropriate organic amendment(s) should add pest inactivation power to the more modest thermal, chemical and biological inactivation action afforded by soil solarization. The use of multiple, biocidal mechanisms can potentially accelerate inactivation of pest organisms and overcome a limitation of conventional soil solarization by reducing reliance on favorable weather conditions. Change in soil pH can be an important indicator of the biochemical state of the soil and is relevant to pest inactivation. Past research has shown that different types of plant biomass variably alter the soil pH upon amendment into soil.[37] Several studies have used nitrogen-rich soil amendments, such as fresh leaves and stems of *Diplotaxis tenuifolia L.* (wild rocket) and *Thymus vulgaris* (thyme),[38] anaerobically digested pig slurry[28] and either fresh or semi-composted mixtures of animal manure and dry chicken litter, to create neutral to alkaline soil conditions that can be associated with pest inactivation.[7] On the other hand, acidification of soil and associated accumulation of VFAs following soil amendment with wheat bran or liquid swine manure have also been shown to control several soil pests.[39,40] Moreover, the composition of VFAs in the soil can have a significant effect on the magnitude and kinetics of inactivation for various soil pests.[39,41–44] However, the mechanism through which VFAs inhibit pests is not fully understood.[45,46] While VFA production has previously been studied in the context of anaerobic soil disinfestation,[47] less is known regarding VFA production and composition during partially or completely aerobic biosolarization. In this study, we measured pH and VFA profiles by depth in soils amended with the previously untested combination of TP

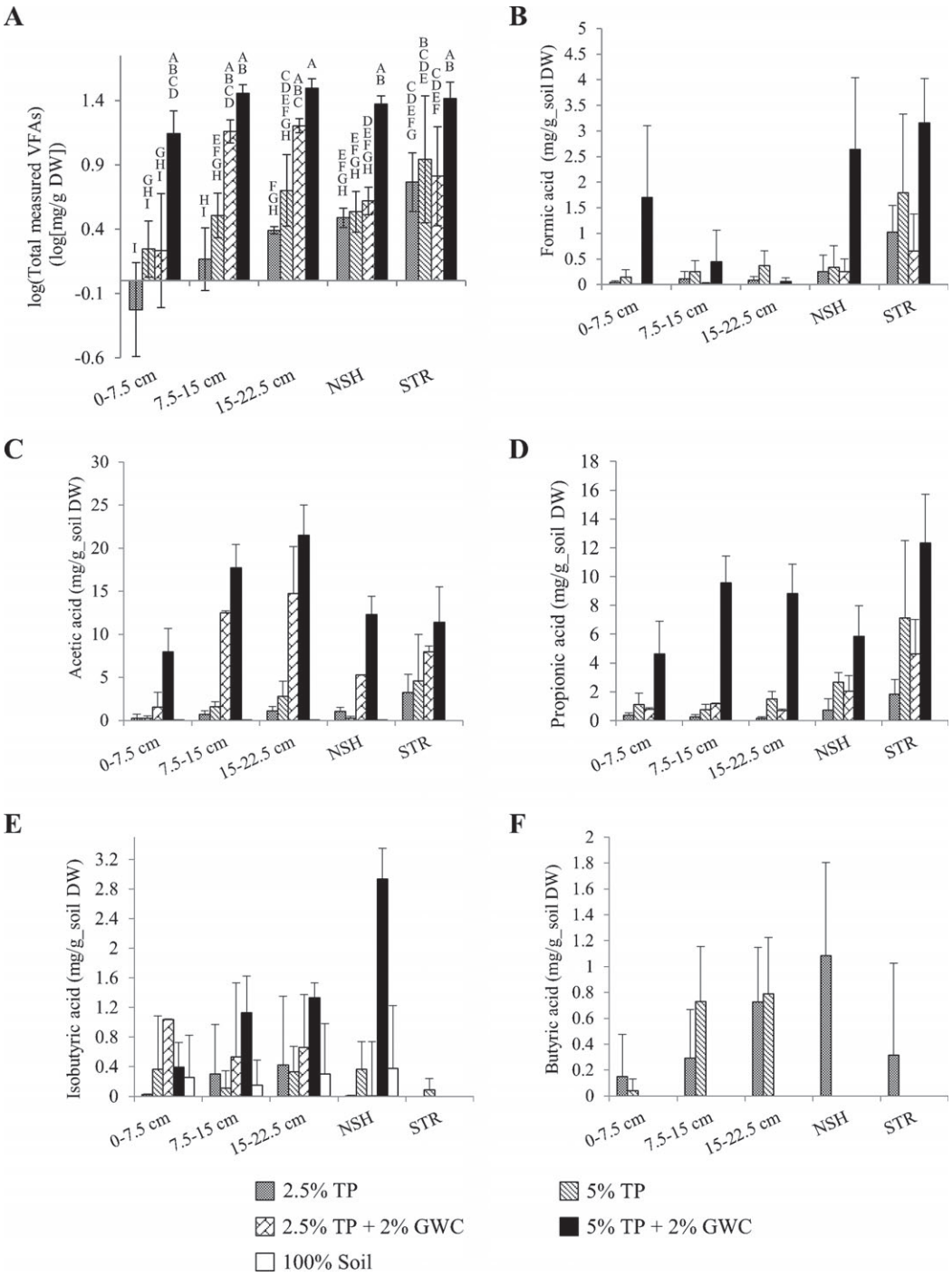

Figure 4. Volatile fatty acid (VFA) levels in soil prior to and following biosolarization with various soil amendments. Soils contained tomato processing residues (TP) and mature compost from yard clippings (GWC) at varying levels (% dry basis). The total VFA levels (A) were estimated by summing the levels of the individual VFAs measured (B to F). Values are given for amendment treatments at the start of biosolarization (STR), various soil depths following biosolarization (0–7.5 cm, 7.5–15 cm and 15–22.5 cm) and non-solar-heated control samples incubated at room temperature (NSH). Data for the 100% soil treatment are excluded from the total VFA figure because the absence of VFAs in these samples did not permit logarithmic transformation. Total VFA values that do not share a letter are significantly different ($P \leq 0.05$). Error bars represent one standard deviation ($n = 5$).

and GWC. The data indicated that the presence of GWC and the level of TP both influenced VFA composition and concentration in the soil, and that these effects varied by soil depth. In general, pH values in soils containing GWC and either 2.5 or 5% TP decreased during biosolarization, or incubation at room temperature, at depths below 7.5 cm. In light of previous research with the same soil source, showing that soil acidification during biosolarization occurs primarily under anaerobic conditions,[19] it can be assumed that 7.5 cm represents the approximate depth at which the oxygen concentration drops low enough to trigger anaerobic microbial activity. This could be due to limitations in oxygen diffusion from the surface and oxygen depletion from microbial activity that is spurred by the soil amendments and soil heating. In production agriculture, however, it is expected that this critical depth will vary

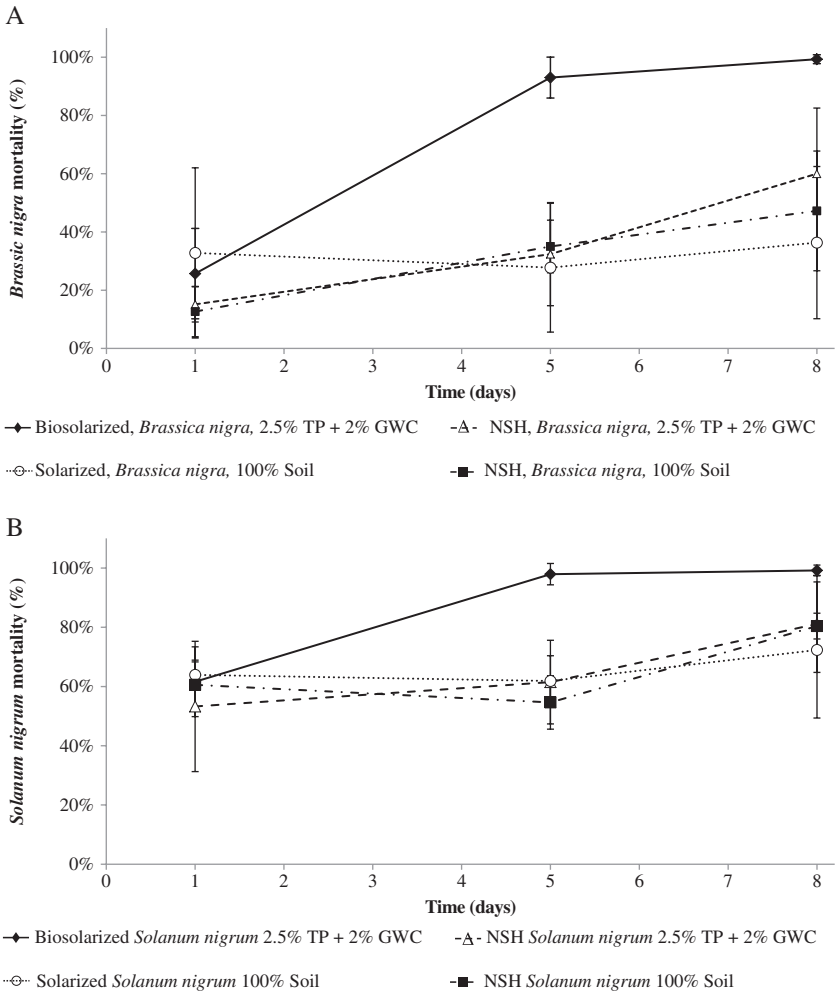

Figure 5. Mortality of weed seeds during biosolarization. Mortality kinetics is shown for seeds from (A) *Brassica nigra* (black mustard) and (B) *Solanum nigrum* (black nightshade). Data are given for non-amended soil and soil amended with green waste compost (GWC) and tomato pomace (TP) that were either solar heated in the field or non-solar-heated (NSH). Values are given as means ± standard deviation ($n = 5$). Lines connecting mean values within each treatment are given to ease interpretation of the data.

according to many factors, including soil type, moisture content, temperature, type of plastic film used, soil amendment properties and others. Previous studies have shown that application of mature compost from municipal solid waste often results in an increase in soil pH, whereas immature compost amendment leads to a pH decrease.[48] Given that mature GWC was used in the present field study, the addition of TP to the soil during biosolarization may be viewed as a destabilization of the compost. Although soil incorporation of immature composts generally is not encouraged in agriculture owing to a high risk of N leaching into groundwater, additional studies may help to determine whether amendment of immature GWC alone yields the same biosolarization results.

As might be expected, measured accumulation of VFAs largely aligned with changes in soil pH and occurred quickly; VFA contents in some amended treatments were greater initially than after biosolarization. The results indicate that, when relying on amended organic matter to induce VFA production during solarization, soil should be tarped immediately to maximize anaerobic conditions and retain organic acids. Measurement of VFA levels indicated that temperature, compost addition and tomato pomace loading influenced the concentration and composition of VFAs in the soil. The introduction of compost resulted in soil mixtures with much higher water-holding capacity than soils without compost. Saturated soils or those containing a greater amount of water have lower air-filled porosity and promote more rapid depletion of oxygen compared with soils with lower water content. The resulting reduced oxygen levels would facilitate anaerobic activity and production of organic acids. In prior studies that examined the impact of moisture content on production of organic acids during ensilage of tomato pomace, increasing accumulation of organic acids was observed as moisture content increased.[49] It is possible that the higher initial moisture content in treatments amended with green waste compost contributed to greater organic acid accumulation compared with treatments without compost.

Soil amended with 2% GWC and 5% TP exhibited the greatest accumulation of VFAs, particularly acetic acid and propionic acid. This was in contrast to non-amended soil, which contained only trace amounts of VFAs following solar heating. For soil amended with GWC and 5% TP, VFA levels generally increased with soil depth, in agreement with the notion that anaerobic microbial activity was greater at lower depths. Interestingly, although the concentrations of formic acid were relatively low, the data showed a unique trend where levels were greatest near the soil surface and

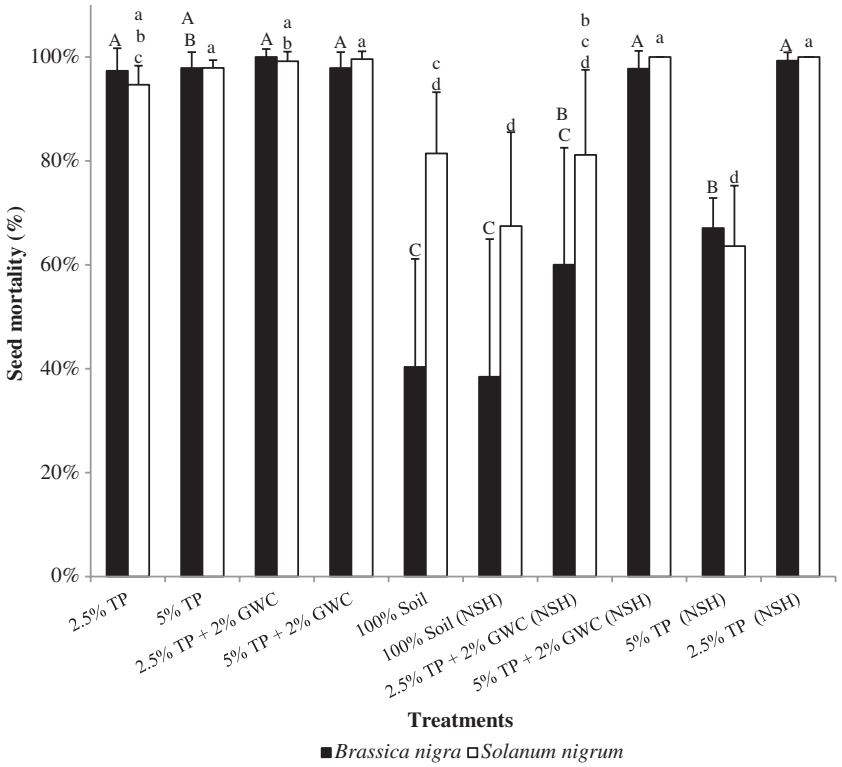

Figure 6. Weed seed mortality following 8 days of biosolarization for soils amended with varying levels of green waste compost (GWC) and tomato pomace (TP). Data are also given for non-solar-heated (NSH) control soil treatments. Values are given as means ± standard deviation ($n = 5$). Within each weed species, values that are not connected by the same letter are significantly different ($P \leq 0.05$).

decreased with increasing depth in soil containing 2% GWC and 5% TP. This may suggest that there was a low level of anaerobic activity in the uppermost soil layer by certain microbial taxa that thrived under the higher temperatures experienced near the surface. Notably, butyric acid accumulation was only observed in biosolarized soils amended with TP, in the absence of GWC. Together, these results indicate that GWC amendment introduced certain acidogenic, thermophilic micoorganisms to the soil that were unique from those endogenous to the soil. This agrees with that fact that VFA production is part of the composting process,[50,51] which also occurs at high temperatures comparable with those in the soil during biosolarization.[52] These data may enable biosolarization amendment strategies to produce specific VFAs according to the sensitivities of targeted pests. Although the present study focused on VFAs, other compounds produced in biosolarized soils may also contribute to pest inactivation. Additional research is needed to characterize fully the array of biocidal compounds that may be produced in response to different organic amendments and environmental conditions.

4.3 Weed seed inactivation

The pest inactivation efficacy of conventional solarization has been characterized for a wide array of pests, including weeds.[12] Moreover, the ability to control weeds via soil amendment with various forms of organic matter, such as cultivated grasses and residues,[25] wheat bran, rice bran and molasses[44] and *Allium* spp. crop residues,[30] has been documented. As biosolarization is a newer technique, fewer studies have described weed seed inactivation when solar heating and soil amendment are combined.

In addition to previous results with GWC,[32] the results of this field study indicated that soil amendment with newly tested TP can complement or compensate for passive solar heating to control certain weed seeds. Owing to the unusually cool weather during the July field trial, ambient temperatures were below those typically targeted for solarization, particularly during the first half the experiment. This lessened soil temperature elevation during treatment. The decreased soil heating was reflected in the weed seed inactivation data in the non-amended soil treatment. For both *B. nigra* and *S. nigrum*, there was no significant difference in seed inactivation between non-amended soil that either underwent solarization or was incubated at room temperature. By contrast, seed inactivation was close to 100% for all biosolarized soil treatments. Additional field studies spanning multiple seasons are needed for a better understanding of the effect of climate variability on biosolarization efficacy.

Seed mortality results were more variable for amended soils incubated at room temperature. In the absence of solar heating, certain amendment treatments yielded seed mortality figures similar to those observed in biosolarized soil, while others produced more attenuated results. Based on the data showing that VFAs present in the freshly amended soil persisted or increased during room temperature incubation, it is possible that VFAs and other biochemicals heavily contributed to weed seed inactivation at lower soil temperatures. As a result, differences in seed mortality between non-solarized amendment treatments were likely related to the activity of different microorganism taxa present in each treatment. While previous simulated biosolarization laboratory studies using *Allium* spp. residue amendments suggested that amendment only enhanced weed seed inactivation under elevated temperatures,[30] the data presented here indicate a more complex interaction between the type and level of soil amendment, soil temperature and weed species targeted. Additional

research is needed to elucidate how differences in soil moisture, pH influence, nitrogen loading and other factors related to tomato pomace amendment affect soil microbial community structure under various soil temperature regimens.

A significant correlation between soil VFA levels and seed inactivation was detected. VFAs are known to play a role in inactivation of various pests.[29,39] Many of the VFA levels observed in this study corresponded to near complete weed seed inactivation, particularly under solar heated conditions. Possible explanations for this are that the weed species tested were sensitive to the minimum level of VFAs observed across most of the amendment treatments, that there were additional inhibitory compounds produced from the soil amendments or that other effects of microbial activity such as direct degradation of weed seed tissue by microorganisms were also present. These data motivate future studies to determine how soil temperature, compost inoculation and organic matter loading affect production of biotoxicants from tomato pomace, as well as to identify the full range of inhibitory compounds produced by soil microorganisms during biosolarization.

Overall, these results showed that amendment-driven biological activity in soil can positively interact with solar heating to enhance inactivation of certain weed seeds during biosolarization. In certain cases, such activity may entirely supplant the need for thermal inactivation during the biosolarization process. Moreover, the time required for weed seed inactivation, which the data suggested may be as few as 5 days, is much shorter than that required for soil solarization or ASD.[12,53] Together, these results are promising for the translation of biosolarization to commercial agriculture, as a decreased reliance on weather, climate, optimal calendar times, etc., will provide more flexibility in scheduling treatment in comparison with conventional soil solarization. Further research is needed to determine whether these results extend to other weed species and other soilborne pests.

5 CONCLUSIONS

The efficacy of biosolarization depends on the soil amendments used and on the extent of soil heating accumulated during treatment. Both the presence of GWC inoculum and the level of TP organic matter amendment can affect the accumulation of volatile fatty acids in the soil, which are known to inactivate soil pests such as fungal pathogens and nematodes. Soil temperature and depth can also influence volatile fatty acid production. For soil closer to the surface, where less VFA accumulation occurs and soil pH values remain close to neutral, the higher temperatures experienced at this depth during biosolarization may play a greater role in pest inactivation. Conversely, at lower depths that exhibit greater pH depression and VFA accumulation, chemical factors may be more responsible for pest inactivation. For seeds of the common weeds *B. nigra* and *S. nigrum*, biosolarization using tomato pomace as an organic matter amendment can effectively inactivate seeds in the soil in as little as 5 days of treatment, a much shorter time than the multiple weeks usually required for conventional solarization. These results show that biosolarization can outperform traditional soil solarization with regard to weed seed inactivation. However, in the absence of passive solar heating, soil amendment alone yields variable weed seed inactivation results. This variability cannot be explained solely through differences in final soil VFA levels, highlighting the need to gain a better understanding of interactions between soil heating and amendments as they relate to microbial production of weed-seed-inactivating compounds during biosolarization. Moreover, additional large-scale biosolarization field trials are needed to confirm that the VFA accumulation and weed seed inactivation data obtained in the soil mesocosms are reproducible at the scale of commercial agriculture.

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