

## **Compost induces the accumulation of biopesticidal organic acids during soil biosolarization**

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## **Abstract**

Current agricultural soil pathogen control methods that rely on fumigation with toxic synthetic chemicals are not sustainable. Combining soil organic matter amendment with soil hydrothermal treatment via solarization is a biological pest control alternative to chemical fumigation. The application and bioconversion of readily-available organic amendment resources, such as green wastes (GW), have not been considered. The impact of compost inoculum on the bioconversion of GW to pesticidal volatile fatty acids (VFAs) in soil during solarization was investigated. Laboratory experiments showed a significant positive effect of compost inoculum at a rate  $\geq 1\%$  on aerobic and anaerobic decomposition of GW, but only when soils were heated. Field trials showed that GW induced conditions in soil that supported fermentation and resulted in accumulation of VFAs. When soil was inoculated with compost, VFA accumulation was enhanced at increasing soil depth. The results indicate that green waste bioconversion can be used to improve pest control conditions in soil during solarization. The findings have environmental implications on green waste management and use of toxic synthetic chemical fumigants.

**Keywords:** Green wastes, compost, volatile fatty acids, soil biosolarization, soil fumigation

## **1. Introduction**

Soil fumigation with synthetic chemicals is a common and effective agricultural practice for economic control of soilborne pathogens and weeds, however, the toxicity of conventional fumigants presents public health and environmental concerns, especially where agriculture interfaces with densely populated areas (Hammond et al., 2010). This concern has resulted in the phase out of essential fumigants such as methyl bromide highlighting the need to find more sustainable alternatives. Soil solarization is a pre-plant pest control alternative that involves covering moist soil with a transparent film. The technique leverages elevated soil temperature that occurs via the accumulation of heat from incident solar short-wave radiation and the reduction in convection and evaporative cooling afforded by the tarp (Marshall et al., 2013; Stapleton and DeVay, 1986). The increase in temperature in the upper layers of soil has been shown to be lethal to agricultural pests such as weeds, fungi and nematodes (Dahlquist et al., 2007; Tuell-Todd et al., 2009). Currently, solarization is hampered by the requisite 4-6 week period of sun exposure, which often coincides with the growing season for many crops (Stapleton et al., 1985). Decreasing the treatment time by adding additional stresses in the soil and developing a better understanding of the underlying biochemical processes involved in pest inactivation could make solarization a more competitive technology.

Recent studies have shown that the addition of an organic carbon source to support microbial activity along with compost, as a microbial inoculum, can effectively decrease the time needed for solarization by increasing the temperature and biopesticidal volatile fatty acid (VFA) levels in the soil (Achmon et al., 2017; Simmons et al., 2016). Fermentation products that accumulate in soil, such as propionic, acetic and butyric acids, have been shown to enhance thermal inactivation of pests (Achmon et al., 2017; Huang et al., 2015). The combination of soil

organic amendment and solarization treatments is termed soil biosolarization (SBS) (Stapleton, 2000). SBS has proven to be effective for the control of a variety of pests; complete inactivation of the weed seeds *Solanum nigrum* and *Brassica nigra* was achieved after 8 days of treatment when tomato pomace was amended into soil prior to SBS (Achmon et al., 2017). The application of chicken manure or dried olive pomace (with mineral nitrogen) into soil prior to SBS achieved >90% inactivation for *Macrophomina phaseolina* (Chamorro et al., 2015; Dominguez et al., 2016). Chicken manure and olive pomace amendments also yielded good inactivation (>90%) of the nematode *Melodogyne* sp. (Dominguez et al., 2016).

The SBS process requires an amendment that is inexpensive, readily available at the time of treatment, and does not compete with markets for animal feed. Green waste (GW) is comprised of branches, lawn clippings, leaf litter and other organic waste from yard trimmings. This type of waste is currently generated on a large scale and municipalities have invested in infrastructure for its collection, making GW a potential amendment for SBS. However, experiments must be performed to ensure green waste is compatible with the technology.

While there is evidence that decomposition of soil organic matter improves pest inactivation during solarization (Achmon et al., 2017; Basallote-Ureba et al., 2016; Fernandez-Bayo et al., 2018; Gilardi et al., 2014), it is unclear to what extent the addition of compost inoculum further improves the process. Microbes that colonize compost are adapted to high temperatures and decomposition of organic matter under microaerobic conditions, so they have an inherent advantage for survival and growth under solarization conditions. In particular, mature compost from chestnut residues had a high relative abundance of microorganisms from hemicellulolytic, cellulolytic and ligninolytic groups such as fungal groups belonging to Ascomycota and bacterial groups belonging to *Bacilli* (Parillo, 2017; Ventorino et al., 2016).

Compost has also been shown to contain lignocellulolytic enzyme activities such as peroxidases, acetylerase and arabinofuranosidase (xylan-debranching enzymes), and endoglucanase, cellobiohydase and  $\beta$ -glucosidase (cellulose-degrading enzymes) (Ball and Jackson, 1995). Amending soil with a robust thermophilic, lignocellulolytic community could improve SBS performance compared to endemic soil microbes. For instance, a soil amended with a mix of 2% wheat bran along with 8% compost inoculum reached higher maximum temperatures at both 5.1 cm and 12.7 cm compared to the non-amended soil during solarization (Simmons et al., 2013). Another study involving wheat bran as a soil amendment showed that treatments containing compost inoculum had 50% higher metabolic activity in the first two days of treatment compared to treatments without compost (Simmons et al., 2016). Increased microbial activity may also increase the accumulation of VFAs to biotoxic levels. Tomato pomace and wheat bran amended in soil with compost inoculum achieved significant accumulation of VFAs such as acetic, butyric and iso-butyric acids (Achmon et al., 2017; Simmons et al., 2016). Since compost addition would be an added expense to SBS treatment, understanding the amount of compost inoculum required to achieve enhanced decomposition is needed.

This study aimed to determine the potential for green waste bioconversion to produce pest-controlling VFAs during soil solarization. An additional goal was to elucidate the impact of oxygen level and compost inoculation rates on microbial activity and VFA accumulation. Overall, the research will have environmental implications for green waste management and alternatives to fumigation using toxic synthetic chemicals.

## **2. Materials and Methods**

### **2.1. Green waste, compost and soil preparation**

A model GW was prepared by mixing 11.4 wt% (g/g) grass clippings, 7.2 wt% woody material, 41 wt% leaves and 40.4 wt% prunings, on a dry mass basis (Yu et al., 2017). The GW components were ground and sieved to a particle size < 2 mm. The prepared GW had a C/N ratio of 34 and a lignin, hemicellulose and cellulose composition of 15.0 wt%, 17.9 wt% and 28.9 wt% (dry basis), respectively. The compost inoculum had a C/N ratio of 19 and was collected from the Northern Recycling Compost Facility in Zamora (California, USA). Feedstocks treated by this facility included GW and food waste from the surrounding areas that were composted using the turned windrow method.

The fiber saturation point (FSP) of the GW and compost inoculum was estimated by wetting the material via capillary action for 20 hours until no change in mass was observed. Samples were allowed to drain onto a dry soil bed until no further change in mass was observed. The moisture content was measured by weighing both the saturated and oven-dry (105°C) samples. The FSP for GW and compost were 238% and 53% (dry basis), respectively.

Soil for experiments was collected from the UC Davis Plant Pathology Research Fields (Davis, CA 38.521028N, -121.760755W, elevation 18.5 m a.s.l). The texture of the soil was sandy clay loam (47 wt%, 27 wt% and 26 wt% of sand, silt and clay, respectively). The soil pH was 7.89. The total nitrogen and organic carbon (OC) content was 0.09 wt% and 0.79 wt%, respectively. The water retention at 0.33 atm of pressure (field capacity, FC) was 21.90% (wet basis). The soil was sieved using a 3.18 mm mesh screen in order to remove rocks, soil clumps and large pieces of organic matter.

## **2.2. Aerobic incubation experiments**

Prior to incubation, each component of the mixture was individually wetted to 80% of the FC for soil and 80% of the FSP for GW and compost. Materials were then mixed to the target amendment rate, homogenized and equilibrated in a 4°C refrigerator for one hour. Respiration rate was estimated using aerobic bioreactors, with three replicates per treatment, as described elsewhere (Reddy et al., 2009). One hundred grams (dry weight) of mixtures containing soil amended with 2 wt% GW (dry basis) and compost inoculum at 0 wt% (control), 0.01 wt%, 0.1 wt%, 1 wt% and 2 wt% (dry basis) were loaded into 250 mL reactors. Samples were incubated at 50 °C with an airflow of 20 mL/min for a 48-hour period (after the peak respiration occurred). The incubator system was equipped with both a CO<sub>2</sub> sensor (Vaisala, Suffolk, UK) and mass flow rate meter (Aalborg, Orangeburg, NY) that monitored influent and effluent air from the reactors. Readings for CO<sub>2</sub> and mass flow rate for each reactor were taken every 2.67 hours. Values were used to calculate CO<sub>2</sub> evolution rate (CER) and cumulative CO<sub>2</sub> evolved (cCER) as described elsewhere (Reddy et al., 2009).

## **2.3. Anaerobic Incubation experiment**

Soil mixtures with GW at 0.5 wt%, 1 wt%, 2 wt%, 3 wt% and 4 wt% (dry weight), and with either 0 wt% or 1 wt% compost (dry weight), as well as a soil-only control were incubated at 30°C and 50°C for 72 hours. The same wetting protocol as described for the aerobic incubations was used. For this experiment, the GW and compost were mixed together prior to wetting and a 3 g/L NH<sub>4</sub>Cl solution was used to achieve 80% of the combined FSP and a C/N ratio ideal for decomposition GW (Yu et al., 2017).

Forty grams, dry weight, of each sample was added to a 50-mL screw cap centrifuge tube to minimize the headspace. Each sample was prepared in triplicate. The tubes were capped and covered with parafilm to prevent oxygen contamination. Tubes were randomly arranged in the incubators at either 30°C or 50°C. After 72 hours, samples were removed from tubes for moisture content measurement and for preparation of water extracts for VFA and pH analysis.

#### **2.4. Volatile fatty acid and pH analyses**

An adapted version of a published VFA extraction protocol was used (Simmons et al., 2016). Samples were extracted using a 2:1 (w:w) water to dry mass weight ratio in 15mL screw cap plastic centrifuge tubes. The mixtures were shaken for 30 minutes at 450 rpm (Barnstead MaxQ 2000 Platform Shaker, San Diego, CA). The pH was measured while the soil-amendment mixture was fully suspended.

After pH analysis, the tubes were centrifuged for 10 minutes at 15,600xg and 25°C. An aliquot of 0.5 mL of the resulting supernatant was filtered through a 0.2µm PTFE syringe filter (Titan-3, 17 mm filter blue 0.2 µm PTFE membrane, Thermo Fisher Scientific Inc. San Diego, CA, USA) into 1 mL glass HPLC tubes.

An HPLC (Shimadzu, Columbia, MD) equipped with an ion exchange column (Bio-Rad Aminex HPX-87H, 300 mm x 7.8 mm) and a UV detector (SPD-20A Prominence, Shimadzu, Columbia, MD) was employed to determine the quantity of acid species present. The samples were acidified at a 1:1 (m:m) ratio with 10 mM sulfuric acid solution in Milli-Q water to ensure that all acid species were in the protonated form. 5 mM sulfuric acid in Milli-Q water was used as the mobile phase with a flow rate of 0.6 mL per minute. The column oven was set to 60°C and the UV detector wavelength was set to 210 nm. Standards were made for seven species: formic,

acetic, propionic, isobutyric, butyric, isovaleric and valeric acids. The standards were prepared in concentrations of 2000, 1000, 500, 250, 125, 63 and 31  $\mu\text{g/mL}$ .

## **2.5. Field experiments**

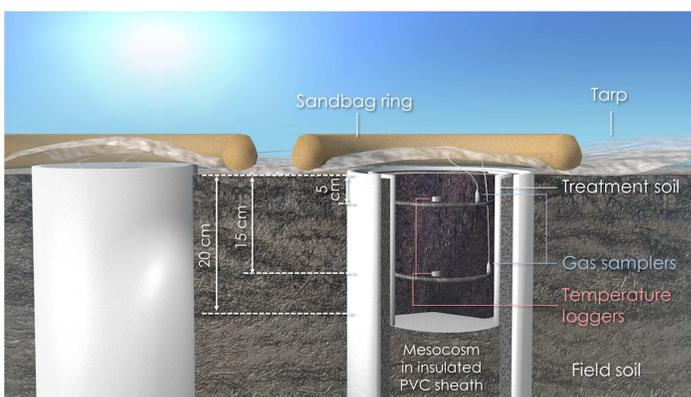
### *2.5.1. Mesocosm description*

Field solarization was completed using mesocosms that were isolated from the surrounding non-amended field soil through mesocosm insertion into larger PVC pipes embedded in the soil (Figure 1). This facilitated mesocosm isolation and simulation of a completely amended field. Two sizes of mesocosms were used. Large mesocosms (15 cm outer diameter x 20 cm height, Figure 1A) were used to monitor oxygen concentration and temperature at 5 cm and 15 cm from the soil surface (Table 1). In parallel, smaller mesocosms (5 cm outer diameter x 20 cm height) were used to monitor VFA accumulation over time at 5 cm and 15 cm from the soil surface (Table 1). Smaller mesocosms allowed direct soil sampling during solarization treatment without significantly disturbing the experimental plot. The depths were chosen because the 5 cm range falls directly within the primary seed germination range for many weed seeds while the 15 cm depth is within the rooting range for the crops planted post-solarization (Marshall et al., 2013). Both mesocosms had perforated stainless-steel plate attached to the bottom of each mesocosm and covered with weed barrier fabric to retain soil while still permitting drainage of water. Large and small mesocosms were wrapped with 1.9 cm and 0.95 cm thick pieces of foam rubber insulation (Buna-N/PVC Foam Insulation Sheet, McMaster-Carr, LA, California), respectively.

Table 1. Mesocosm sizes and associated experimental variables and measurements

Sample	Mesocosm size	Monitored variables*	Soil content (% d.w.)	Compost content (% d.w.)	Green Waste content (% d.w.)
Control soil (S)	Large	T, O <sub>2</sub>	100	0	0
Soil and compost (C)	Large	T, O <sub>2</sub>	99	1	0
Soil and Green Waste (GW)	Large	T, O <sub>2</sub>	98	0	2
Soil, compost and Green Waste (GW)	Large	T, O <sub>2</sub>	97	1	2
Soil and Green Waste (GW)	Small	pH, VFA	98	0	2
Soil, compost and Green Waste (GW)	Small	pH, VFA	97	1	2

\* T = Temperature; VFA = Volatile Fatty Acids; O<sub>2</sub> = Oxygen concentration



A



B

Figure 1. (A) Illustration of large mesocosm inside the PVC pipe embedded in the soil. Open section shows distribution of the temperature logger and gas sampling tubes in mesocosm. (B) Mesocosm placement in the field experimental plot including field prior to film placement (left), top view of a mesocosm embedded in soil (middle) and after tarp placement (right).

The large mesocosms were loaded with the four mixtures listed in Table 1. All percentages were by dry weight. The large mesocosms included oxygen sampling systems (Figure 1) and temperature loggers (Thermochron iButtons model 1922L, Embedded Data Systems, Lawrenceburg, KY) at 5 cm and 15 cm depths. Temperature was logged every 10 minutes. The oxygen sampling system is described elsewhere and consisted of a circular porous hosing tube connected to PE-50 plastic tubing that reached the surface (Achmon et al., 2018). A valve on the end allowed for oxygen sampling while preventing gas leakage.

The small mesocosms were loaded with the GW and GWC mixtures (Table 1). One of each sample type, GW and GWC, was removed from each of the five plots on days 1, 3, 5, and 8.

### *2.5.2. Mesocosm preparation*

To prepare the contents for each mesocosm, the appropriate amounts of air-dried GW, compost and soil were mixed (Table 1) thoroughly and then poured into PVC mesocosms. Five replicates per treatment were prepared. For initial time measurements, five replicates of 100 grams of each mix sample were transferred to 250 polyethylene containers with a draining opening in the base to allow water transfer. The night before inserting the mesocosms in the field, mesocosms and polyethylene containers were set in a bath of water (one for each treatment to avoid cross contamination) filled to a depth of 15 cm to allow direct contact between the base of the mesocosms and water layer to allowed soil wetting by capillary action. The baths were

placed in a 4°C refrigerator and left to equilibrate overnight. Prior to inserting the mesocosms in the PVC pipes in the field, mesocosms were placed on dry soil for one hour to drain excess water. Time

zero samples were immediately frozen at -80°C for later analysis.

### *2.5.3. Field preparation*

The field site was located at the UC Davis Plant Pathology Research Fields where lettuce had been planted annually with the exception of winter 2015. Prior to solarization, the field was rototilled and an orchard float was used to flatten the plots. The field was irrigated for 7 days with drip irrigation and then left to dry for 2 days.

PVC columns that were 60 cm in length were buried in the soil to accommodate mesocosms and isolate treatments in the horizontal direction for heat and oxygen transfer (Figure 1). Two sets of columns were used; 22 cm diameter and 7.6 cm diameter for large and small mesocosms, respectively. The top 20 cm of soil in the columns was removed to provide void space for the mesocosms.

The field site contained five replicate plots (2 m x 5.5 m), with a 2-m buffer zone between plots. The plots were oriented lengthwise from west to east. Each plot had four large mesocosms (S, SC, GW and GWC), and eight small mesocosms (GW and GWC). Mesocosms were spaced at 0.6 m increments within a plot.

### *2.5.4. Mesocosm placement in the field*

Mesocosms were inserted into the embedded columns in the field (Figure 1B). Each mesocosm was topped off with field soil to minimize the air gap between the soil and solarization film. The plots were irrigated for one hour before being covered with 0.7 mil

transparent plastic (Husky Film Sheeting; Poly-America, Inc., Grand Prairie, TX). In order to ensure each mesocosm's headspace was isolated from that of other mesocosms and the surrounding non-amended soil, nylon mesh bags filled with wetted gravel and sand were placed around the circumference at the surface of each PVC pipe (Figure 1).

#### *2.5.5. Small mesocosm removal from the field during kinetic studies of VFAs*

To remove small mesocosms from the field during the experiment, small holes were cut in the tarp on days 1, 3, 5 and 8. Sand filled barriers were placed in between those samples remaining and those being harvested in order to prevent air contamination in the remaining mesocosms in the field. The soil in each mesocosm was separated into top 0-10 cm to represent the 5-cm depth measurements, and bottom 10-20 cm samples to represent the 15-cm depth measurements. The soil from each depth was well mixed for VFA, pH and moisture content analysis.

## **2.6. Gas sampling and oxygen analysis**

Gas samples were collected from large mesocosms during the experiment. Prior to collecting each sample, gas in each tube was purged using a 3-mL syringe. Then, 1mL syringes with three-way stopcocks to prevent air leaks, were used to collect gas samples from the two depths. Then 100 $\mu$ L of air in the syringe was taken with a glass, gas tight syringe and injected into an Agilent 6890N Gas Chromatograph equipped with a 12.2 m (40 ft) HayeSep, packed stainless steel, 3.18 mm (1/8 inch) OD column. ChemStation software was used for collecting data and integration. Oxygen samples were collected at 0, 4, 8, 12.5, 24, 36, 48, 81.5, 105.5, 129.5, 153.5 and 194 hours after mesocosm placement in the field.

## 2.7. Statistical analyses

Stepwise regression and Tukey's Honest Significant Difference (HSD) post-hoc tests were performed using JMP-IN software (version Pro 12, SAS, Cary, NC). The significance level was set at 0.05. The stepwise regression models were optimized using the minimum least square method considering the leverage effect using the following equation (eq. 1):

$$E\{Y\} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 \quad (\text{eq. 1})$$

Where  $E\{Y\}$  = expected value of the response variable,  $\beta_0$  = intercept,  $X_1$  = settings for variable 1,  $X_2$  = highest and lowest settings for variable 2,  $X_3$  = highest and lowest settings for variable 3,  $\beta_1$  = parameter estimate for variable 1,  $\beta_2$  = parameter estimate for variable 2,  $\beta_3$  = parameter estimate for variable 3,  $\beta_{12}$  = parameter estimate for the interaction between variable 1 and variable 2,  $\beta_{13}$  = parameter estimate for the interaction between variable 1 and variable 3,  $\beta_{23}$  = parameter estimate for the interaction between variable 2 and variable 3, and  $\beta_{11}$  = parameter estimate representing second-order effects of variable 1.

A Shapiro-Wilk test was performed to test for normality. It was necessary to perform Box-Cox transformations of the volatile fatty acid data to satisfy the requirement for normality. The value for lambda in the transformation ranged between 0.40 to 0.78.

## 3. Results and Discussion

### 3.1. Aerobic laboratory incubations for GW and compost amended soil

Aerobic incubations at 50°C were completed to measure decomposition rates during the initial aerobic phase of biosolarization and to determine the effect of compost inoculum level on green waste decomposition in the soil. Cumulative respiration increased with increasing compost

inoculum up to 1 wt% compost (Table 2). Respiration in samples amended with 0.1 wt% compost was not significantly different from that measured in non-inoculated samples, while samples inoculated with 1 wt% and 2 wt% compost had significantly higher respiration ( $p < 0.05$ ).

The results suggest that microbial communities in the compost inoculum beneficially impacted the initial rate of carbon breakdown under aerobic conditions. Accumulation of VFAs in soil by microbial activity requires rapid depletion of  $O_2$  in air-filled void spaces followed by anaerobic fermentation of soil organic carbon. However, there was a maximum compost inoculation rate above which there was no additional benefit for improving GW degradation for the GW amendment level tested. This beneficial impact was also observed in a study of soil amended with wheat bran and compost (Simmons et al., 2016), which similarly did not derive additional benefit from increasing the compost inoculum rate from 2 wt% to 5 wt% for a 2 wt% level of wheat bran amendment. The positive effect of compost addition at lower amendment levels may stem from elevated initial concentrations of compost-borne lignocellulolytic microorganisms in the soil (Ball and Jackson, 1995; Parillo, 2017; Ventorino et al., 2016) or from increased proximity of these microorganisms to biomass in the soil. The proximity effect may become irrelevant at concentrations above 2% or there may be other limiting factors, such as nutrient limitations in the soil, that mitigate any benefits from greater compost concentrations.

Table 2. Cumulative respiration (cCER) values after 15 hours of incubation for samples with varying levels of compost inoculum

Mixture	Mean cCER (mg $CO_2$ /g dry weight soil)*
0% compost	0.82 a
0.01% compost	0.99
0.1% compost	0.80 a
1% compost	1.14 b
2% compost	1.17 b

\* Three replicates for all treatments except 0.01 wt% compost which had a single replicate. Means followed by the same letter within columns are not statistically different at  $\alpha = 0.05$  based on Tukey-Kramer HSD test. Mean

respiration for 0.01 wt% compost could not be calculated due to two out of the three bioreactors receiving insufficient aeration.

### **3.2. Anaerobic incubations of GW and compost amended soil**

The impact of GW amendment rate and compost inoculum on soil microbial activity under anaerobic conditions was determined by measuring the accumulation of VFAs over a 72h incubation period at 30°C and 50°C. Of the analyzed VFAs, only formic, acetic, propionic and butyric acids were detected. Formic acid concentration measured at 72 h increased with increasing GW addition (Figure 2A). The greatest increase was observed in the samples containing compost inoculum that were incubated at 50°C.

Acetic acid concentration was higher than formic acid concentration and increased with increasing GW incorporation (Figure 2B). However, acetic acid levels peaked at 3% GW for compost inoculated samples. The lower acid concentration observed at 4% GW compared to 3% GW may have been due to inhibition of microbial activity as acid accumulated. Samples without compost inoculum and incubated at 30°C produced more than twice the amount of acetic acid compared to similar samples incubated at 50°C. This difference suggests that the native mesophilic microbial community in soil alone was effective at fermenting organic carbon in GW to acids under moderate temperatures. For instance, it has been argued that mesophilic bacteria involved in the degradation of lignocellulosic material can compete with *Bacilli* for carbon sources and nutrients (Ventorino et al., 2016). *Bacilli* has been suggested to be one of the dominant species in compost material because their high thermo-tolerance and ability to secrete polymer-degrading enzymes critical for lignocellulose decomposition (Li et al., 2013).

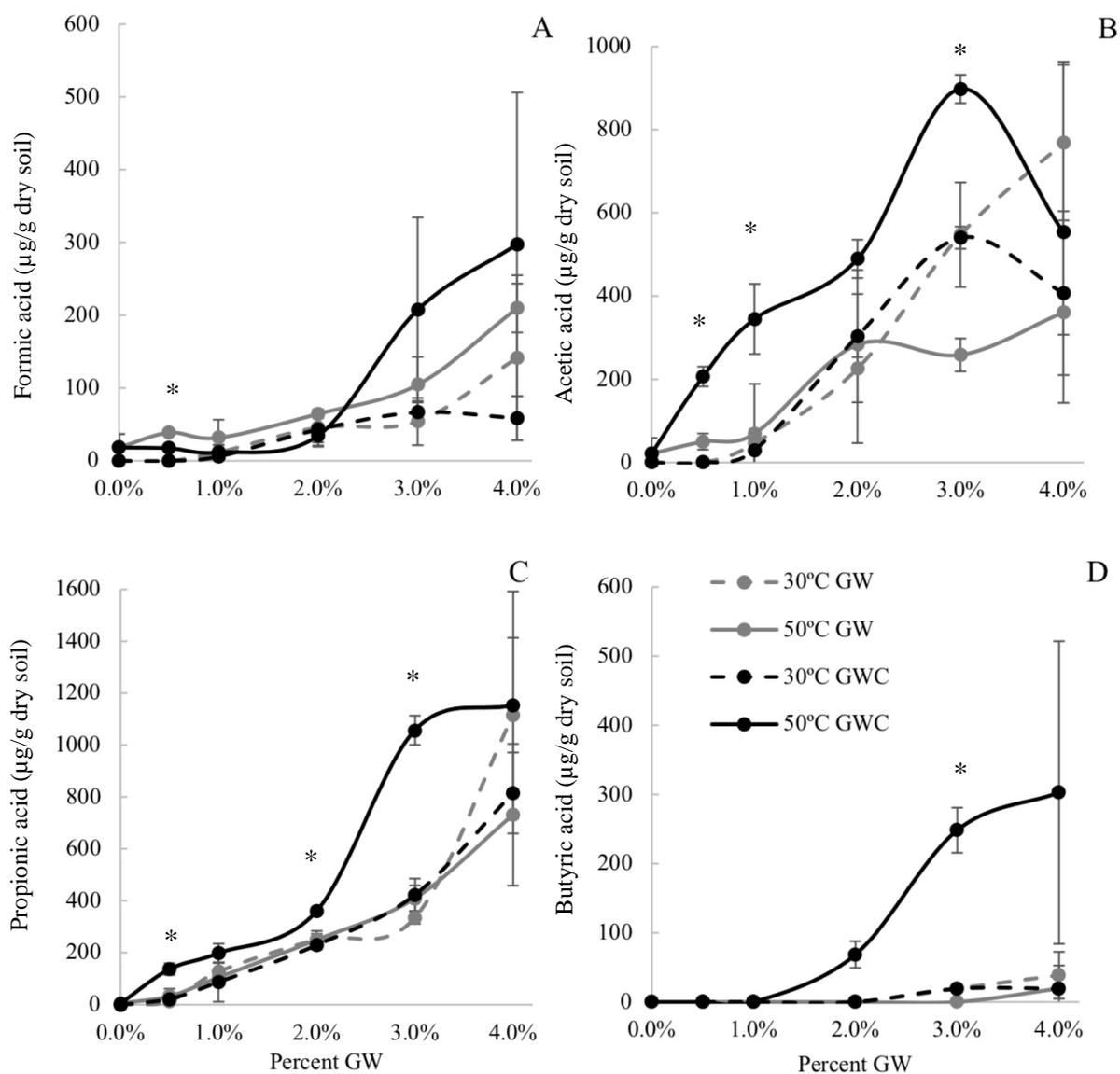


Figure 2. Levels of formic (A), acetic (B), propionic (C) and butyric (D) acids in samples incubated under anaerobic conditions with (GWC) and without (GW) 1 wt% compost at 30 °C and 50°C at different levels of green waste (GW) amendment. Lines included to guide the eye. Bars represent standard deviation (n=3). Stars denote significant differences between incubation regimes at the same GW level ( $P < 0.05$ )

The results also indicate that moderate temperatures are more favorable for acetic acid accumulation compared to thermophilic temperatures in the absence of compost inoculum. When

samples were incubated at 50°C, samples with compost inoculum produced twice as much acetic acid compared to samples without compost.

Propionic acid was observed at the highest concentration of all measured acids during laboratory incubations (Figure 2C). The compost-inoculated samples incubated at 50°C had more than twice the level of propionic acid than all other treatments at 3% GW.

Finally, butyric acid was only detected in samples inoculated with compost and incubated at 50°C. As the GW amendment level increased, so did the butyric acid concentration (Figure 2D).

A stepwise regression analysis was performed to test whether compost inoculum, temperature, and GW amendment level had significant effects on acid levels in experimental soil. Increasing GW amendment level had a significant positive effect on formic acid in incubated soil samples ( $p < 0.05$ , Table S1). Formic acid also was significantly greater in soil samples incubated at 50°C compared to 30°C ( $p < 0.05$ ). There was no significant effect of compost inoculum on formic acid accumulation in soil.

Acetic acid significantly increased with increasing GW amendment level, compost inoculum and incubation temperature ( $p < 0.05$ , Table S1). The interaction between compost inoculum and incubation temperature was highly significant ( $p < 0.0001$ ); in incubations at 50°C compost had a greater positive effect on acetic acid accumulation compared to 30°C incubations. The second order estimate for GW was also significant suggesting an optimum amendment level exists between 0 and 4 wt% for the production of acetic acid under the conditions tested.

Propionic acid levels significantly increased with GW amendment, compost amendment and incubation temperature ( $p < 0.05$ ). Like acetic acid, the interaction between compost inoculum and temperature was significant for propionic acid ( $p < 0.05$ ); under thermophilic

conditions compost had a greater positive effect on propionic acid accumulation compared to incubation under mesophilic conditions. In addition, the second order estimate for GW was significant indicating an optimum level exists within the range of GW amendment rates tested.

The increased respiration rates and VFA accumulation in GW-amended soil under thermophilic conditions during laboratory scale experiments indicated that the addition of compost inoculum could be beneficial for fermenting green waste during SBS. Many lignocellulose degrading thermophilic microorganisms have been identified from compost and GW degrading microbiota have been shown to secrete enzymes that hydrolyze cellulose and hemicellulose (Allgaier et al., 2010; Reddy et al., 2011; Yu et al., 2017). In particular, during thermophilic composting processes an enrichment of microorganisms known to degrade lignocellulosic material has been observed (Ball and Jackson, 1995; Li et al., 2013; Liu et al., 2018; McCarthy, 1987). Similar microorganism would play a significant role in the breakdown of the complex polysaccharides into fermentable sugars and VFAs under anaerobic conditions (Baumann and Westermann, 2016).

In addition, although composting is an aerobic process, in general, anaerobic microenvironments may develop allowing anaerobic bacteria to survive (Tuomela et al., 2000). These anaerobic bacteria can be highly cellulolytic, playing a significant role in the degradation of organic macromolecules (Tuomela et al., 2000). Moreover, thermophilic conditions can select a thermophilic microbiota able to decompose a high-lignin source of lignocellulose such as GW (Ceballos et al., 2017).

The laboratory experiments also demonstrated the potential for green waste to be converted to VFAs under simulated SBS conditions. There have been several recent studies demonstrating the use of agricultural residues as soil amendments to enhance the soil solarization

process. These have included livestock manures (Basallote-Ureba et al., 2016; Chamorro et al., 2015), food processing by-products (Achmon et al., 2017; Achmon et al., 2016; Dominguez et al., 2016), and composts and anaerobic digestates of said by-products (Basallote-Ureba et al., 2016; Fernandez-Bayo et al., 2017a; Fernandez-Bayo et al., 2017b; Fernandez-Bayo et al., 2018; Kanaan et al., 2017). While other lignocellulosic biomass sources have been shown to be effective for soil treatment, green waste is unique in that it is produced year-round and does not compete with animal feed markets or organic farming operations that rely on manure for fertilizer. Given the substantial concentrations of acid accumulated at the amendment rate of 2 wt% GW and considering that treatment application costs would increase with increasing amendment levels, 2 wt% GW was selected as the amendment level for field experiments.

### **3.3. Temperature profiles in large mesocosms over the 8-day field trial**

Temperature was measured at two depths in all large mesocosms. The average temperature at the 5 cm depth varied between 20°C and 45°C (Figure 3A) while temperatures at 15 cm varied between 25 and 35°C (Figure 3B). Recent SBS studies that used organic amendments originating from same source of GW mixed with food waste and partially treated either via composting or anaerobic digestion also showed >90% reduction for *Fusarium oxysporum* f. sp. *lactucae* at a similar range of temperatures after 8 days of treatment (Fernandez-Bayo et al., 2018).

The difference in mean temperature between GW and soil-only samples and GWC and soil-only samples (Figure S1) showed that both amendment combinations resulted in increased daily maximum temperatures. The temperature difference between GW and GWC samples and the non-amended soil was between -0.5°C and 1.4°C at the 5-cm depth and between -1°C and 0.5°C at the 15 cm depth. In addition, at the 5-cm depth the mean maximal daily temperatures

were generally higher in the GW and GWC samples than in the non-amended or compost-amended samples. After 3 days of biosolarization, GW showed significantly higher maximum temperatures than the compost-amended soil ( $p < 0.05$ ). The greater positive temperature difference at 5 cm could be biologically relevant. Other studies have observed increases in soil temperature and soil heating when soil was solarized using wheat bran and compost as amendments (Simmons et al., 2013; Simmons et al., 2016). This increase in temperature may have been due to a boost in metabolic activity when an organic food source is introduced in soil. The lower temperature difference at 15 cm compared to 5 cm may have been due to lower overall temperatures at 15 cm, lower metabolic activity at these temperatures and slightly lower moisture content (Figure S2) which would have reduced the thermal capacity of the soil reducing its capacity to retain heat.

#### **3.4. Oxygen profiles in large mesocosms over the 8-day field trial**

Gas phase oxygen concentration was measured at two depths in large GW and GWC mesocosms. The initial oxygen concentration at both depths was lower than the oxygen concentration in ambient air (21%) (Figure 4), indicating oxygen was consumed while mesocosms were equilibrating overnight at 4°C. At the 5-cm depth the oxygen concentration dropped to below 2% for both amendment mixtures in the first 12.5 h. This indicates a rapid uptake of the available oxygen in the soil atmosphere and in the reservoir between the tarp and the soil surface. Over the remaining 8 days of treatment the average oxygen concentration for both amendment types remained under 5% until the final two samplings.

At the 15-cm depth, there was a rapid decrease in oxygen concentration in the first 12.5 h (Figure 4B). Concentrations remained below 5% oxygen for 36 hours. Concentrations increased to nearly 10% by the end of the 8-day period.

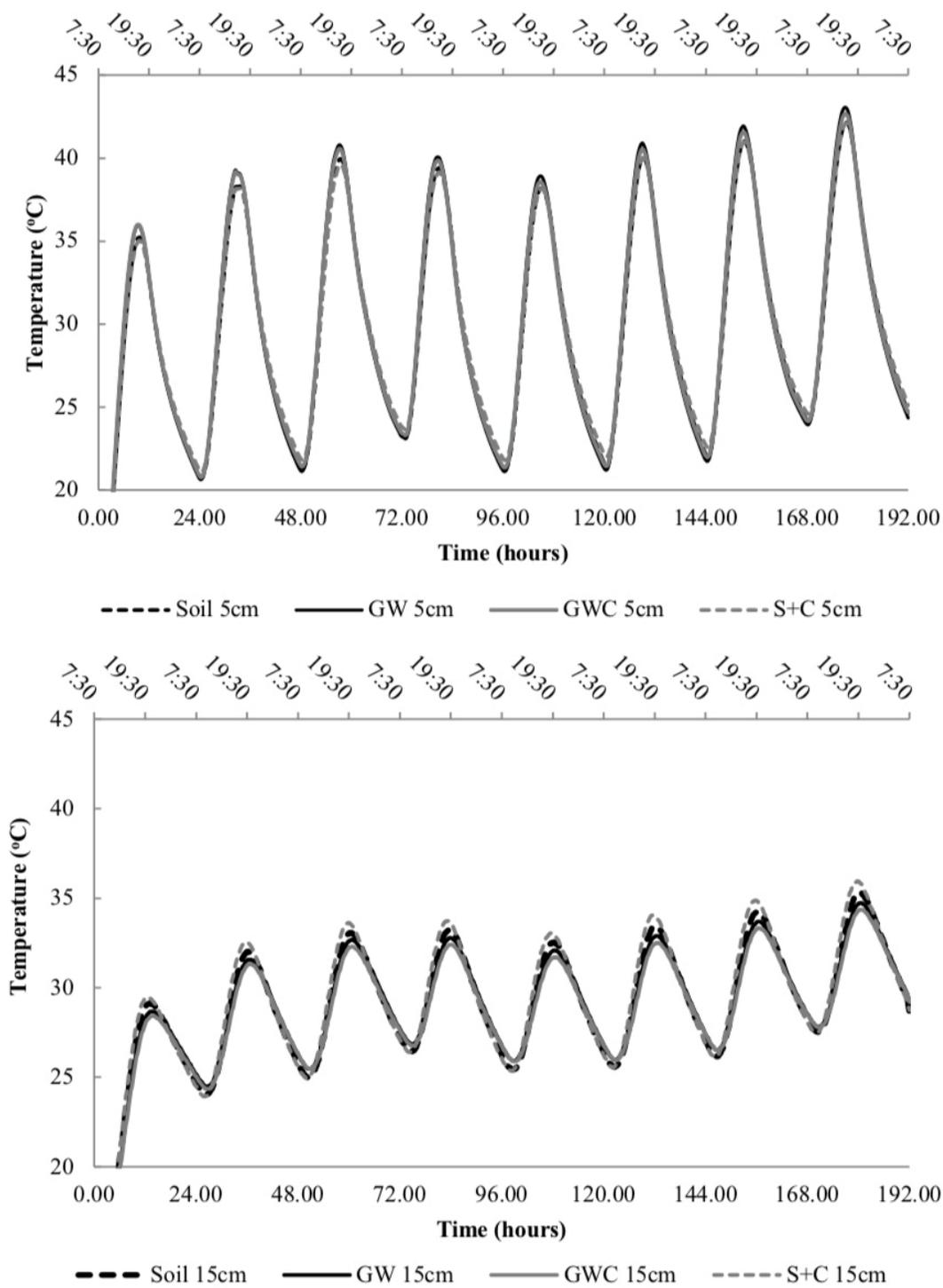


Figure 3. Average temperature measured at 5 cm (A) and 15 cm (B) depths in soil amended with green waste (GW) and co-amended with green waste plus compost (GWC). Lines included to guide the eye.

The observed levels of oxygen were not sufficient to classify the environment as completely anaerobic, however, they were low enough to foster respiration or fermentation by obligate and facultative anaerobic or microaerophilic organisms which resulted in the metabolism of VFAs (Krieg and Hoffman, 1986). At both depths, the difference between the oxygen concentrations in GW and GWC samples was not significant, suggesting that samples with and without compost consumed oxygen at similar rates. Regarding a depth effect, gas samples collected from GW treatments at 12.5, 24 and 36 hours of solarization showed significantly lower oxygen concentration at 5 cm compared to 15 cm ( $p < 0.05$ ). This difference may have been due to a combination of oxygen diffusing upward from the lower non-amended soil layer into the lower depths of mesocosms and greater microbial consumption of  $O_2$  at 5 cm. Although oxygen levels for GWC at 5 cm were generally lower than at 15 cm, these differences were not significant.

### **3.5. Acid concentration profile over the 8-day field trial**

The predominant VFAs detected in the field trial samples were acetic acid and propionic acid (Figure 5). At the 5-cm depth, the GW and GWC samples yielded maximum levels of acetic acid in the first 24-72 hours (Figure 5A). Levels averaged 150  $\mu\text{g/g}$  for the remainder of the 8-day field trial and were similar for GW and GWC samples. At the 15-cm depth, acetic acid levels increased for the first 72 hours of treatment; in both mixtures the maximum acetic acid levels occurred on day three. In general, the GWC samples accumulated more acetic acid than the GW samples. The stepwise regression analysis revealed a significant positive effect of increasing depth and time on acetic acid concentration ( $p < 0.05$ , Table S2). There was also a significant positive interaction between depth and compost amendment ( $p < 0.05$ ); the presence of compost

amendment increased acetic acid accumulation as depth increased. As oxygen levels were similar in both layers and the plastic tarp reduced the rate of VFA volatilization from the soil surface, the difference in behavior between the GW and GWC samples at the two depths was likely due to differences in the microbial community responsible for metabolism of GW into VFAs.

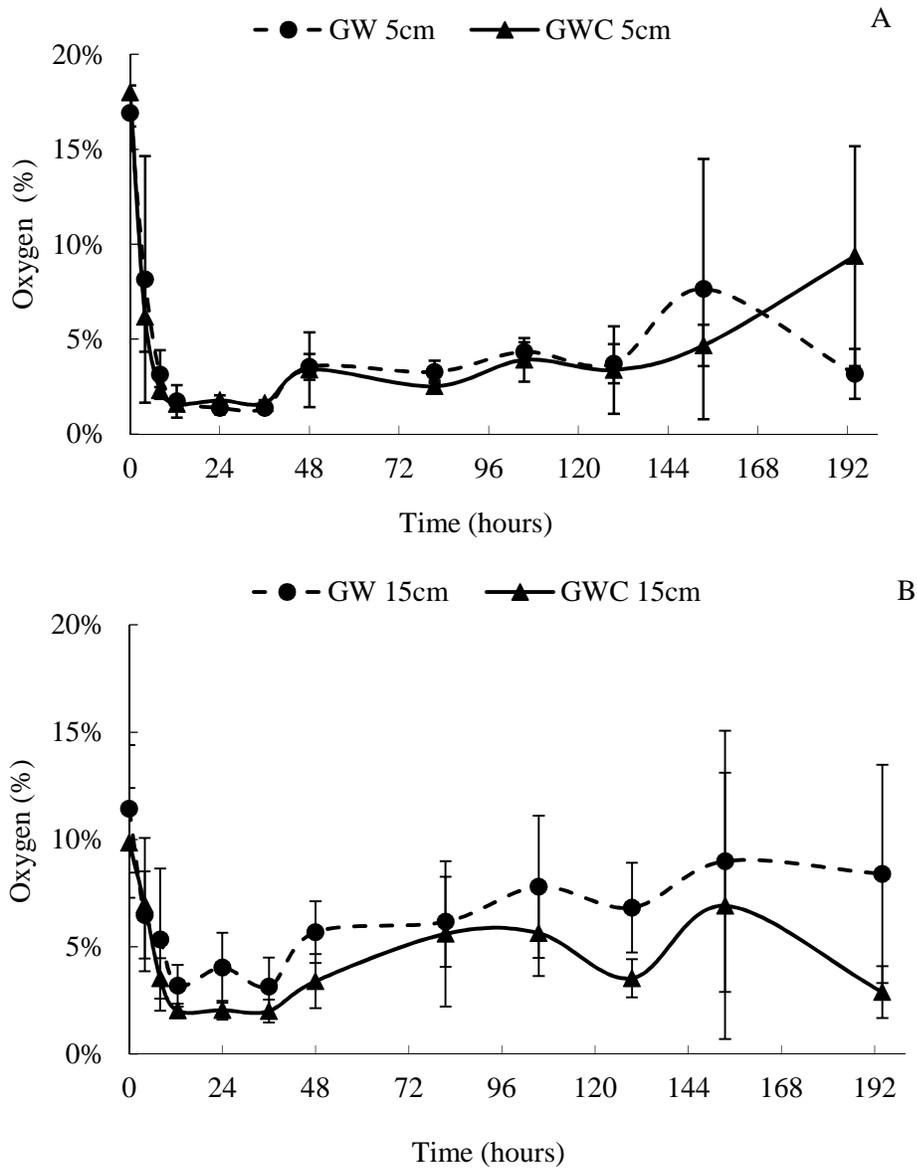


Figure 4. Average oxygen concentration measured at 5 cm (A) and 15 cm (B) depths in soil amended with green waste (GW) and co-amended with green waste plus compost (GWC). Lines included to guide the eye. Bars represent standard deviation.

There was a significant interaction between depth and time for acetic acid concentration ( $p < 0.05$ , Table S2). At the 15-cm depth, during the first 72 hours of treatment, acetic acid production rate was higher than consumption, promoting acid accumulation. However, after 72h, acetic acid levels decreased suggesting decomposition by microbial activity. Acetic acid is a confirmed biotoxic compound used for pathogen inactivation and weed control (Malkomes, 2005). It has also been reported to eliminate black root rot, brown root rot and bed rot in tobacco plants when applied at a rate of 1.0-1.2% by volume (Doran, 1928). Acetic acid has also been shown to kill *Verticillium dahliae* when applied at a 30 mM concentration (Tenuta et al., 2002), slightly above the elevated levels produced during this study (~24 mM in the liquid phase at the maximum). Acetic acid and high temperature stress have also shown enhanced pesticidal efficacy which would result in reduced treatment duration for SBS (Achmon et al., 2017; Fernandez-Bayo et al., 2018).

Propionic acid concentrations were highest at the beginning of the experiment and ranged between 300  $\mu\text{g/g}$  to 350  $\mu\text{g/g}$  for both treatments (Figure 5 C and D). Concentrations declined over the course of the field trial to less than 50  $\mu\text{g/g}$  for both treatments at both depths. The rate of decline was greater at 15 cm compared to 5 cm. The rapid consumption of propionic acid at 15 cm in the first 24 hours suggests a faster metabolism of propionic acid at lower temperatures.

The stepwise regression analysis revealed a significant negative effect of increasing time, compost and depth on propionic acid concentration ( $p < 0.05$ , Table S2). Furthermore, there was a significant interaction between compost amendment and depth; the decline in propionic acid

concentration was greater at 15 cm and in treatments containing compost. These findings indicate the compost inoculum may have enhanced the decomposition of propionic acid.

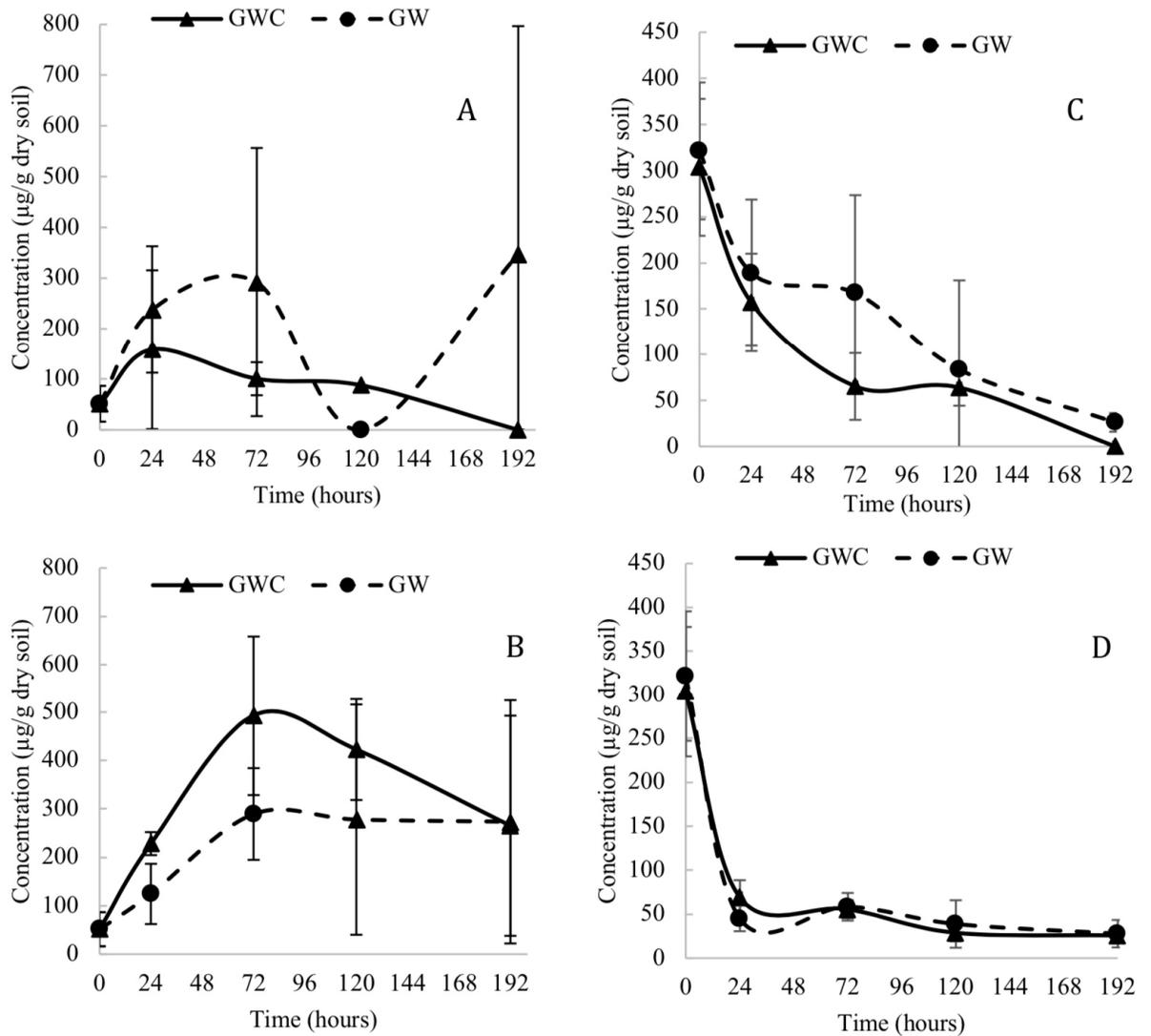


Figure 5. Kinetic accumulation of acetic acid at 5 cm (A) and 15 cm (B) depths and propionic acid at 5 cm (C) and 15 cm (D) depths in field mesocosms amended with green waste (GW) or co-amended with green waste plus compost (GWC). Lines included to guide the eye. Bars represent standard deviation.

Propionic acid has been reported to be toxic to *V. dahliae* at 30 mM concentration and to *Fusarium oxysporum* at 50 mM concentration (Huang et al., 2015; Tenuta et al., 2002). The

lower concentrations encountered during this study (10 mM in the liquid phase) and its rapid depletion suggest a reduced impact of this GW-derived VFA in pest inactivation during treatment, as compared to acetic acid.

The field trial results largely bolstered findings from the laboratory experiments. For example, acetic acid was the predominant VFA found in the highest concentration in both field and laboratory studies. Furthermore, the acetic acid concentrations from samples in the field mesocosms were comparable on day 3 to concentrations in the laboratory experiments containing 2% GW. On the other hand, concentrations of propionic acid in the field trial were lower than those observed in the lab and propionic acid consumption was lower in the laboratory experiments than in the field. This discrepancy was likely due to the completely anaerobic conditions achieved in the lab experiments, whereas oxygen was present during the first 24 hours of the field trial. Thus, the availability oxygen in the field mesocosms likely facilitated microbial consumption of propionic acid. Also, in the anaerobic laboratory scale experiments, the GWC samples incubated at 50°C yielded consistently higher concentrations of VFAs than the other three treatments. This result was not replicated in the field; acetic acid levels were greatest at the 15-cm depth, where temperature averaged 30°C over the 8-day trial, and when compost was amended.

#### **4. Conclusions**

The accumulation of biopesticidal VFAs, particularly acetic acid, in laboratory and field experiments demonstrated the potential of GW as an amendment to improve soil hydrothermal treatment using soil solarization. Compost inoculum increased the accumulation of acetic acid in the first 72 hours of field solarization, but only at the 15-cm depth. Given that pathogen/pest

inactivation would decrease with a decrease in soil temperature, the enhanced accumulation of pesticidal acids associated with GW and compost inoculum would be important at lower soil depths. This enhancement would need to be considered in the context of increased costs associated with soil compost amendment and would need to be tested with additional soil types and at an industrial scale. In addition, measuring the impact of the compost inoculum on spatial and temporal changes in the soil microbiome during solarization would be important to elucidate mechanisms of enhanced VFA accumulation; this is the subject of future work. It is also important to note that SBS is only effective in the region of soil amended with organic matter. Other approaches like deep application of amendments and irrigation practices that promote VFA transport and accumulation with increasing soil depth would need to be implemented to achieve treatment in deep soil. Overall, the research will have significant impacts on the direct use and management of green wastes in agricultural systems and on alternatives to soil fumigation using synthetic chemicals.

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