Waste Management Bulletin 3 (2025) 100264

Contents lists available at ScienceDirect

Waste Management Bulletin

journal homepage: www.elsevier.com/locate/wmb

# Assessment of spent mushroom substrate recycling via soil biosolarization

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ARTICLE INFO

*Keywords:*
Biosolarization
Spent Mushroom Substrate
Cover Crops
Phytotoxicity
Fumigation Alternative
Agriculture

ABSTRACT

Biosolarization is an alternative to pesticides for soil pest management that can utilize food industry organic matter byproducts as soil amendments to create conditions conducive to pest suppression. Spent mushroom substrate (SMS) is the main byproduct of mushroom cultivation. New uses are needed for this waste stream as its production continues to increase. This study evaluated two types of SMS as sole amendments and as co-amendments with cover crop biomass for biosolarization. SMS consisted of rice bran with hardwood sawdust (BS SMS) and soybean hulls with oak sawdust (SO SMS). Cover crop biomass was a mix of *Secale cereale* and *Trifolium incarnatum*. Gas evolution, pH, biocidal organic acid (BOA) production, phytotoxicity, and soil nitrogen were tracked during and/or after simulated biosolarization. SMS and cover crop treatments were compared to rice bran-amended soil and unamended, solarized soil. Results indicate that SO SMS treatments can produce BOA concentrations and phytotoxic conditions comparable to rice bran-amended soil, with BOA content correlating to radish seed germination indices with a coefficient of determination of 0.683. The pH of treatments including cover crop biomass, whether as a sole or co-amendment, significantly decreased during biosolarization, but were higher than pH of rice bran controls. SO SMS and cover crop biomass as sole amendments significantly increased total nitrogen content compared to solarized soil ($P < 0.05$), likely due to the addition of nitrogen-containing organic matter, but not mineral nitrogen. These findings suggest that certain SMS compositions may be valuable amendments for agricultural pest control via biosolarization.

## Introduction

Conventional agriculture has become increasingly reliant on the use of synthetic chemicals for pest management (FAO, 2023). However, agrochemicals pose human-health and environmental risks. For humans, exposure to them can have impacts including neurotoxic effects, behavioral impairment, decreased hormone levels, and altered gut microbiomes, among others (Rendon-von Osten and Dzul-Caamal, 2017; Pereira et al., 2018; Ruuskanen et al., 2020; Kaikai et al., 2023; Kaikai et al., 2023). A variety of negative impacts have also been reported for mammals, birds, amphibians, fish, and insects (Yadav et al., 2024; Woodley et al., 2024; Fritsch et al., 2025; Gandara et al., 2024; Quandahor et al., 2024; Khoshnood, 2024).

Soil biosolarization can replace preplant pesticide applications by utilizing passive solar heating and fermentation by the soil microbiota. Incorporation of organic matter residue streams reclaimed from food systems can drive the fermentation and inactivate a variety of agricultural pests, from weeds to microbial pathogens, via thermal, low oxygen, and biocidal organic acid (BOA) stresses (Fernández-Bayo et al., 2018; Shea et al., 2022). These conditions are attained by amending organic matter into soil, covering with a clear plastic tarp, and irrigating (Ros et al., 2008). Accumulation of organic acids and other biochemicals produced through fermentation or introduced via the soil amendments can be phytotoxic (i.e., toxic to plants) thereby requiring a remediation period following biosolarization to allow for leaching, dissipation, or aerobic microbial degradation of these compounds before planting crops (Shea et al., 2021). Biosolarization can provide an avenue for waste valorization within the food industry. Byproduct streams, such as rice bran, tomato pomace, and almond hulls, have been used as amendments for biosolarization (Khadka et al., 2020; Haber et al., 2022; Pastrana et al., 2022).

Spent mushroom substrate (SMS) is the main waste product from mushroom production, which has been steadily increasing over the last 20 years (FAO, 2023). Specifically, there has been a recent surge in consumption due to the use of mushrooms as meat replacements and the increase in knowledge about their medicinal properties (Wang and

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https://doi.org/10.1016/j.wmb.2025.100264

Available online 13 November 2025

Zhao, 2023; Usman et al., 2021; Ajibola et al., 2024; Ahmad et al., 2023; Lang, 2020). Mushroom cultivation is an indoor practice that relies on the use of a substrate generally composed of fibrous byproducts from other industries, such as sawdust, straw, wheat bran, rice bran, soybean hulls or coffee grounds (Diamantopoulou et al., 2023; Melanouri et al., 2022; Jozífek et al., 2025; Karataş, 2022). The composition of the substrate can vary based on the nutritional needs of the mushroom species or strain being cultivated (Krupodorova et al., 2021; Aditya et al., 2024; Suwannarach et al., 2022). Currently, different reuse strategies are being investigated to divert SMS from landfills, including its use as a livestock feed supplement or soil amendment (Barua et al., 2024; Pintarič et al., 2024; Feng et al., 2025; Karageorgou et al., 2024). Recent advances include reports of SMS as a soil amendment for increased blueberry yield, an alternative solid fuel, and for production of biochar for use as a soil amendment or in wastewater treatment (Ravlikovsky et al., 2024; Aiduang et al., 2025). As mushroom consumption grows, the amount of waste produced increases dramatically, since approximately 5 kg of mushroom substrate are used for each kilogram of mushroom produced (Jordan et al., 2008).

Cover crops are grown by farmers for the variety of benefits they provide, including soil cover, organic matter inputs to the field, crop diversity, weed suppression, and nutrient scavenging (Osipitan et al., 2019; Haruna and Nkongolo, 2020; Hirsh et al., 2021; Mitchell et al., 2012). Research is also investigating the use of cover crop biomass as an alternative biosolarization amendment grown *in situ* (Morrissey et al., 2025). However, for the purpose of controlling weeds, cover crops can outcompete weeds during growth but will not inactivate propagules or prevent future weed growth unless the cover crop produces allelopathic chemicals, which is the case for cereal rye through its production of benzoxazinoids that can persist in the soil for several days after termination (Rice et al., 2022).

Cover crops are typically mowed and disked into the soil about 4 weeks before transplanting (Mitchell et al., 2012), giving an optimal window of time for biosolarization. Winter cover crops in California are typically grain crops such as triticale, barley, and rye (Mitchell et al., 2012). A rye cover crop would be left to decompose for several weeks before seeding or transplanting the cash crop to avoid nitrogen immobilization (Outreach, 2012).

Rye is an important cover crop in the California Central Valley for fighting weeds, pests and erosion (Outreach, 2012). Crimson clover has been used in combination with rye as a cover crop mixture for better nutrient management because of clover's symbiotic relationship with nitrogen fixing bacteria (Vann et al., 2018; Plumhoff et al., 2022). Cereal rye residues can be phytotoxic to other plants for up to 30 days after addition to the soil (Boselli et al., 2021; Teasdale et al., 2012). An improved method of incorporation is needed for this cover crop to avoid negative impacts on the following crop. Capitalizing on cover crop degradation as part of biosolarization could deliver pest control benefits by generating and trapping pesticidal biochemicals in the soil while accelerating biodegradation of the cover crop biomass to reduce its lingering phytotoxicity and reduce the plant-back time. In this way, coupling cover crop termination with biosolarization could allow cover crop biomass to act as a complement to other organic matter soil amendments.

In addition to pest suppression, biosolarization provides a range of secondary benefits linked to the increase in soil organic matter associated with the process, which improves water retention in the root zone, nutrient retention, and soil structure (Lal, 2020; Šimanský et al., 2019). This is true for SMS, which can be used as a soil amendment for different purposes, including improvement of physical characteristics and fertilization (Aiduang et al., 2025; Jordan et al., 2008; Othman et al., 2020; Medina et al., 2012). Increased soil porosity, water infiltration, microbial respiration and crop yield have been found after addition of SMS to soil (Medina et al., 2012; Nakatsuka et al., 2016; Stewart et al., 1998; Collela et al., 2019). SMS also contains residual chitin from the mycelial mass of the harvested mushrooms that grew in the substrate (López-Mondéjar et al., 2012). Chitin is a polymer of *N*-acetylglucosamine found in fungal cell walls and has been used as a soil amendment for a variety of benefits it can provide to soil and crops, including improving pest suppression and promoting crop growth (Andreo-Jimenez et al., 2021; Sharp, 2013; Kaku et al., 2006; Li et al., (2022); Sun et al., 2022; Gooday, 1990; Martínez et al., 2001).

SMS has not yet been evaluated as an amendment for biosolarization. Its use as a co-amendment may contribute to the degradation of cereal rye residues, which can contain 5–14 % lignin, 13–34 % hemicellulose, and 16–31 % cellulose on a dry matter basis (Shao et al., 2015; Brockmueller et al., 2023; Baker et al., 2024). SMS may provide a variety of lignocellulolytic enzymes from the fungal growth during mushroom cultivation that can help break down these components (Rajavat et al., 2020; Pérez-Chávez et al., 2022; da Luz et al., 2012; Del Cerro et al., 2021; Chai et al., 2022).

The carbon to nitrogen (C:N) ratio of amendments is important in determining how readily organic material will decompose in soil and how much nitrogen will be available for plant uptake (Nguyen and Marschner, 2016). C:N ratios greater than 25, like that of many cereal crops, will result in slow decomposition and lead to N immobilization (Weil et al., 2017). The C:N ratio of cereal rye has been reported between 27:1 to 46:1 (Finney et al., 2016; Balkcom et al., 2023; Kuo et al., 1997). Therefore, addition of a more nitrogen rich substrate, such as clover, may help balance the nutrient ratio and provide a more suitable combination of amendments to incorporate into the soil for microbial degradation. The C:N ratio of crimson clover can be between 12:1 and 24:1 (Yang et al., 2020; Parr et al., 2011; Ranells and Wagger, 1992; Kramberger et al., 2013).

The C:N ratio of the SMS used here is 18:1, so co-amending cover crop biomass with SMS can serve both purposes of contributing to a more balanced C:N ratio for microbial metabolism and serving as an inoculum of lignocellulolytic enzymes that can contribute to faster degradation of the lignocellulosic cover crop biomass, as mentioned above. Prior work has demonstrated that co-amending plant material with a stable material that can contribute lignocellulolytic enzymes enhances the degradation of the materials in the soil, producing a greater amount of BOAs (Achmon et al., 2017; Hestmark et al., 2019; Achmon et al., 2020). The combination of cover crop biomass with SMS allows for use of a sustainable soil management technique while also creating a new use case for an increasingly large food industry waste stream.

More research is needed to identify food system residue streams that can be incorporated into soil for biosolarization. The focus of this study was to gauge the efficacy of biosolarization using SMS, either as the sole amendment or as a co-amendment with cover crop biomass, in a microcosm format that simulated field biosolarization. Soil acidification, accumulation of BOAs, nitrogen content, and phytotoxicity were measured as indicators of soil pest inactivation potential and possible risk to subsequent crops. The data can inform future research and field work to recycle SMS as an agricultural soil amendment.

## Materials and methods

### *Soil and amendments*

The soil used was a Yolo series loam. Soil was collected from the Armstrong Plant Pathology Research Field in Davis, CA (collection coordinates 38°31′07.9″N 121°46′07.8″W). Soil was sieved through a 4 mm sieve before use. Spent mushroom substrate (SMS) was sourced from Goal Zen Mushroom in Dixon, CA and Solano Mushroom Farm in Esparto, CA. The SMS from Solano Mushroom Farm was composed of 20 % rice bran and 80 % hardwood sawdust by dry weight (BS SMS). The SMS from Goal Zen Mushroom was composed of 50 % soybean hulls and 50 % oak sawdust by dry weight (SO SMS). Dried rice bran (RB) was sourced from Frontier Ag in Davis, CA. The cover crop biomass used was *Secale cereale* (rye) and *Trifolium incarnatum* (clover) seeded at rates of 100 lb/

acre and 25 lb/acre, respectively, and grown in the Armstrong Plant Pathology field for 4 months before termination and collection. Both types of SMS were stored at −20 °C upon reception until use. Rice bran was stored at room temperature. Cover crop biomass was stored at −20 °C after collection until use.

### Gas evolution measurement

Simulated biosolarization was carried out in anaerobic bioreactors connected to a gas measurement system (Micro-Oxymax, Columbus Instruments International) to track gas evolution in soil amended with SMS as a proxy for microbial activity. For the bioreactors, 250-mL glass bottles were loaded and connected to gas collectors through a check valve to prevent oxygen intrusion during soil incubation and fermentation (Achmon et al., 2016). Each SMS type was amended into soil at a rate of 1.5 % by dry weight to determine the viability of using these materials as sole amendments for biosolarization, and an unamended (solarized) treatment was included as a negative control. Bioreactors were filled with 330 g of amended or unamended soil and enough water to reach field capacity (see *Moisture content and field capacity*) of the mixture. Four bioreactors were prepared for each treatment and incubated for fifteen days to capture any potential gas production that may occur outside the typical eight-day biosolarization timeframe. Incubation temperatures cycled between 35 °C and 55 °C every 12 h to simulate field soil temperature cycles during biosolarization.

The gas measurement system measured carbon dioxide, methane, and hydrogen production throughout the duration of the fifteen-day trial. The instrument was equipped with infrared detectors for $CO_2$ and $CH_4$ quantification and an electrochemical detector for $H_2$ quantification.

### Microcosms

A microcosm bioreactor setup was used to simulate biosolarization conditions in the field. The bioreactors were 8-ounce wide-mouth glass jars with tin-plated steel lids (Kerr). Each type of SMS was amended into soil as the sole amendment at a rate of 1.5 % by dry weight or as a co-amendment with cover crop biomass at rates of 0.5 % and 1.0 %, respectively (Table 1). The cover crop biomass was also amended into soil as the sole amendment at a rate of 1.5 % by dry weight. Bioreactors of soil amended with rice bran at 1.5 % by dry weight served as a positive biosolarization control and solarized soil (lacking any amendments) served as a negative control (Khadka et al., 2020; Shennan et al., 2017). Water was added to the amended and unamended soil to bring each treatment to field capacity (see *Moisture content and field capacity*) before filling the bioreactors that were then incubated for 8 days in a diurnal incubator cycling temperatures in the same way as described above (see *Gas evolution measurement*). All treatments had 3 replicates.

**Table 1**
Soil treatments for simulated biosolarization.

| Treatment abbreviation | Amendment | Amendment rate (% by dry weight) |
|---|---|---|
| CC | Rye and clover cover crop biomass | 1.5 % |
| BS SMS | Rice bran and hardwood sawdust spent mushroom substrate | 1.5 % |
| BS SMS + CC | Rice bran and hardwood sawdust spent mushroom substrate<br>Cover crop biomass | 0.5 %<br>1.0 % |
| SO SMS | Soybean hull and oak sawdust spent mushroom substrate | 1.5 % |
| SO SMS + CC | Soybean hull and oak sawdust spent mushroom substrate<br>Cover crop biomass | 0.5 %<br>1.0 % |
| RB | Rice bran | 1.5 % |
| Solarized | No amendment | 0.0 % |

### Moisture content and field capacity

Water content was determined gravimetrically by drying the soil and amendments to constant weight in a drying oven at 105 °C and comparing against the initial fresh weight. Moisture content was calculated using equation (1).

$$MC(\%) = \frac{water\ content(g)}{air\text{–}dry\ sample(g)} \times 100 \quad (1)$$

Field capacity (FC) was determined by saturating a known amount of dry soil or amendment with water and allowing it to drain freely for 24 h. The weight of the soil after the water content stabilized (fresh weight) and the weight of the oven-dried soil were used to calculate the field capacity of the sample using equation (2). Field capacity of the amended soil ($FC_{AS}$) was calculated using equation (3).

$$FC(\%) = \frac{fresh\ weight - dry\ weight(g)}{dry\ weight(g)} \times 100 \quad (2)$$

$$FC_{AS} = FC_{amendment} \times amendment\ rate + FC_{soil} \times (1 - amendment\ rate) \quad (3)$$

### pH and biocidal organic acid analysis

Soil from each bioreactor was sampled on Day 0, Day 3 and Day 8 of biosolarization as well as 1-week, 2-week, and 4-week remediation time points. Two 5-gram samples were taken from each bioreactor for pH and biocidal organic acid (BOA) analysis.

For pH determination, aqueous extracts of each sample were made by combining water and soil in a 1:1 ratio by mass, followed by vortexing (Fisher Scientific Analog Vortex Mixer) at 3,200 rpm for 30 s and centrifugation (Eppendorf 5810 R) at 13,600xg for 10 min. Soil pH was measured for each sample in the supernatant after centrifugation. For BOA analysis, the supernatant was filtered into 2-mL glass HPLC vials using a disposable syringe and a 0.22 μm PTFE filter. BOAs were identified and quantified in the filtrate via HPLC (Agilent 1260 Infinity II) using a sulfonated styrene–divinylbenzene column (Agilent Hi-Plex H) and a multiple wavelength detector (Agilent 1260 Infinity II MWD). The mobile phase was 5 mM sulfuric acid (Honeywell Research Chemicals) at a flow rate of 0.6 mL/min. For each sample, 20 μL were injected into the column (column temperature was 60 °C) with a run time of 30 min per injection. Citric, malic, formic (Sigma Aldrich), acetic, lactic, propionic, butyric and isobutyric acids (Fisher Chemical) were detected at 210 nm and succinic acid (MP Biomedicals) was detected at 195 nm (Fernandez-Bayo et al., 2020; Ball et al., 2011). Seven-point standard curves were produced with dilutions of each acid from 31.25 to 2,000 μg/mL in water.

### Phytotoxicity assays

Phytotoxicity of the treated soil was determined on day 8 of biosolarization as well as 2-week and 4-week remediation time points to determine the appropriate time needed for the soil to remediate and be able to support plant growth. Phytotoxicity was determined via a radish seed germination and seedling growth assay (Shea et al., 2021; Fernandez-Bayo et al., 2020). Forty-five grams of soil from each bioreactor was spread evenly across the surface of a 100 mm-diameter petri plate with 10 radish seeds and incubated for 3 days at 25 °C (Shea et al., 2021). Two plates were made from each bioreactor. After incubation, the number of germinated seeds (N) and their average radicle length ($L_{avg}$) was measured to determine germination rate and germination index of each plate (Pastrana et al., 2022). The number of germinated seeds ($N_0$) and their radicle lengths ($L_0$) in unamended soil were used to calculate the germination index (GI) using equation (4). Soils with germination indices below 80 % were considered phytotoxic (Tiquia et al., 1996).

$$GI = \frac{L_{avg}N}{L_0N_0} \times 100\% \quad (4)$$

*Soil nitrogen analysis*

Soil was sampled four weeks after biosolarization to evaluate the impacts of amendment composition and biosolarization on soil nitrogen dynamics. Total nitrogen, nitrate nitrogen ($NO_3^-$-N) and ammonium nitrogen ($NH_4^+$-N) were analyzed in the SO SMS and cover crop-amended samples as these were the experimental amendments that showed significant BOA production and phytotoxicity, and therefore promise as amendments for biosolarization. The solarized samples were included as a control to compare amended treatments to. The original SO SMS amendment was analyzed as well to provide further insight into potential changes in nitrogen content of amended soils.

Total nitrogen, $NO_3^-$-N and $NH_4^+$-N were quantified for the subset of samples by the UC Davis Analytical Laboratory (AnLab, 480 Sprocket Bikeway, Davis, CA). Total N was determined via the combustion method as outlined by the AOAC (AOAC, 1997). Briefly, samples were oxidized via combustion, converting all organic and inorganic nitrogen into combustion gases that were then detected with thermal conductivity and infrared detectors. $NH_4^+$-N and $NO_3^-$-N were determined colorimetrically in potassium chloride extracts (Knepel, 2003; Hofer, 2003). For $NH_4^+$-N determination, extracts were heated with salicylate and hypochlorite in an alkaline phosphate buffer to produce a green color measurable at 660 nm. For $NO_3^-$-N analysis, extracts were combined with nitrate reductase, sulfanilamide in hydrochloric acid, and naphthyl ethylenediamine dihydrochloride for formation of a magenta azo dye measurable at 520 nm.

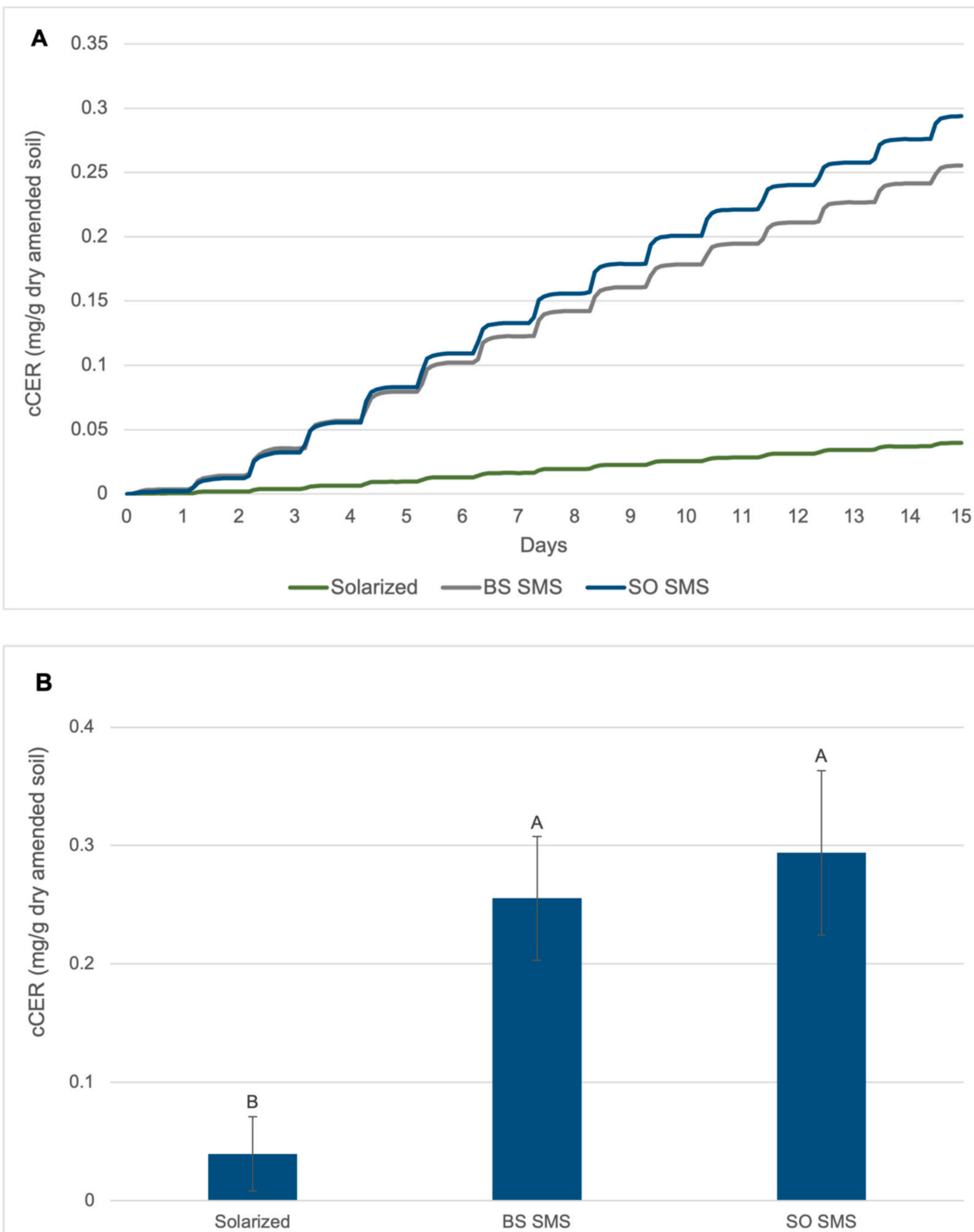


**Fig. 1.** Cumulative carbon dioxide evolution rate (cCER) produced in anaerobic bioreactors throughout (A), and at the end of (B), 15 days of simulated biosolarization. Soils were amended with 1.5 % by dry weight of spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), 1.5 % spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), or no amendment (solarized). Error bars represent ± 1 standard deviation. Values that do not share a letter are significantly different ($P \leq 0.05$).

### Statistical methods

For gas evolution measurements, pH, BOA content, phytotoxicity responses, and nitrogen analyses, comparison of treatment means was conducted via one-way ANOVA. Where significant differences ($P \leq 0.05$) were found, Tukey's Honest Significant Difference post-hoc test was used to compare all treatments to each other and the controls. Main and interaction effects of biosolarization process variables were evaluated using multiway ANCOVA for BOA content, pH and germination indices. When values of zero were reported for BOA or nitrogen content and the data did not follow a normal distribution, comparison of treatment means was also conducted via Kruskal-Wallis and Dunn's post-hoc test. When mineral nitrogen values were below the limit of detection (0.10 ppm), they were analyzed as zero to allow for determination of mean and variance values. Linear regression was used to correlate total BOA content with germination indices. Statistical analyses were performed using JMP 18 software (SAS Institute Inc.).

## Results

### Gas evolution measurement

Carbon dioxide, methane and hydrogen gases were measured as indicators of microbial degradation and fermentation of soil amendments, as well as potential greenhouse gas production. At the end of the simulated biosolarization, cumulative carbon dioxide evolution rate (cCER) in both amended treatments was significantly different from the solarized control (Fig. 1). Production of $H_2$ and $CH_4$ was not detected in any of the bioreactors.

### Simulated biosolarization

#### Soil pH during biosolarization

Treatment and time showed significant main effects on soil pH ($P < 0.0001$), and there was a significant interaction effect between the two factors ($P = 0.0317$, Table 2). Specifically, the effect of treatment on soil pH became greater over time. Rice bran-amended control soils (RB) reached the lowest pH of all treatments (6.13 on Day 3), while BS SMS-amended soil (BS SMS) and the solarized control had the highest pH on Day 3 of biosolarization (7.40 and 7.37, respectively). The pH of all three treatments that included cover crop biomass decreased by Day 3, but only the treatment of cover crop as the sole amendment (CC) maintained a pH lower than its initial pH until the last day of biosolarization (Fig. 2A).

On Day 3 of biosolarization, the pH of RB was significantly lower than all other treatments ($P \leq 0.05$, Fig. 2B). Both treatments of SMS alone had pH values that were not significantly different from the solarized control, indicating that SMS as a sole amendment does not produce significant decreases in soil pH during biosolarization. The pH values of both SMS/cover crop combinations and the cover crop alone were significantly lower than that of the solarized control, but higher than that of the soil amended with rice bran ($P \leq 0.05$). By Day 8 of biosolarization, the pH of the CC and SO SMS treatments were not statistically different from the pH of RB.

#### Biocidal organic acid production during biosolarization

To characterize the biopesticidal acids produced, HPLC was performed on soil extracts. Biocidal organic acid (BOA) content was identified and quantified in all sampled time points before, during and after biosolarization.

BOA content of all treatments peaked on either Day 3 or Day 8 of biosolarization, with the maximum concentration reaching 1,232.5 μg BOA/g dry soil in the CC treatment on Day 8 of biosolarization. BOAs in RB peaked again at the 2-week-remediation time point (Fig. 3A). At this time, succinic acid was the main acid produced in the soil, which differs from the acids present in biosolarization time points (Fig. 4).

On Day 3 of biosolarization, there were no significant differences between the total BOAs of the four treatments with the highest concentrations: RB, CC, SO SMS + CC and BS SMS + CC (Fig. 3A). SO SMS and BS SMS were significantly different from the rest of the treatments ($P \leq 0.05$), but not from each other. BOA concentrations in co-amended soils were significantly higher than each of the treatments of SMS alone ($P \leq 0.05$). The presence of cover crop biomass led to consistent production of BOAs by Day 3 of biosolarization, regardless of its use as a sole amendment or in combination with either SMS. However, by Day 8, cover crop alone had significantly greater ($P \leq 0.05$) BOA concentration than either of the co-amendment treatments.

On Day 8 of biosolarization, CC and RB had the greatest total amount of BOA production followed by both SO SMS treatments, which were not significantly different from RB. The BS SMS and solarized treatments had zero or near-zero totals. Between 96 and 100 % of total BOA content on Day 8 was composed of acetic, propionic and butyric acids. All three follow the same general trend across treatments (Fig. 4 A-C).

Cumulative BOA concentrations for the 8-day treatment were greatest in CC and RB, followed by SO SMS + CC, but RB and SO SMS + CC were not significantly different ($P = 0.505$, Fig. 3B). The two co-amended treatments were also not significantly different from each other ($P = 0.115$), but they were each different from the treatments with their SMS as the sole amendment ($P < 0.05$). There was no detectable production of BOAs in the solarized soil.

The above results are based on Tukey's HSD as an ANOVA post-hoc test performed on treatments that had non-zero values of BOA content. For analysis of BOA concentrations on Day 8 of all treatments, including the zero values, Kruskal-Wallis indicated significant differences among the treatments ($P = 0.008$). The solarized, BS SMS, and BS SMS + CC treatments exhibited means at or near zero, in contrast with the other treatments (Fig. 4). However, Dunn's post-hoc test was not sufficiently sensitive to identify significant differences between individual treatments.

#### Phytotoxicity of biosolarized and remediated soil

Phytotoxicity assays were carried out every 2 weeks for 4 weeks after biosolarization to determine the time needed for the biosolarized soil to remediate. Remediation is necessary prior to seeding or transplanting a crop of interest in the treated soil. Treatment and remediation time both showed significant main effects on soil phytotoxicity ($P < 0.0001$) and the two variables had a significant interaction effect ($P = 0.0094$, Table 3), as the effect of treatment on phytotoxicity became more pronounced with longer remediation times. Soils with SMS were either not phytotoxic at any time or only phytotoxic on the last day of biosolarization, or 0 weeks of remediation (Fig. 5). CC was phytotoxic for the first two timepoints (0 and 2 weeks of remediation) and RB showed phytotoxicity at all sampled times, as demonstrated by germination indices below 80 %.

On Day 8 of biosolarization, soil from CC, SO SMS, SO SMS + CC, and RB had a phytotoxic effect on radish seeds. Both SO SMS treatments resulted in phytotoxic soil that inhibited germination at rates comparable to RB and CC after biosolarization. However, the difference between the germination index of SO SMS and the solarized control was also not statistically significant (Fig. 5).

After 2 weeks of remediation, both SO SMS treatments were no longer phytotoxic, as demonstrated by germination indices above 100 %. This is also the case for BS SMS treatments, but these were not

**Table 2**
ANCOVA effect tests of soil treatment and time point on soil pH during and after biosolarization. Asterisks indicate statistically significant values ($P \leq 0.05$).

| Source | Degrees of freedom | F Ratio | Prob > F |
|---|---|---|---|
| Treatment | 6 | 10.3892 | <0.0001* |
| pH time point | 1 | 47.4367 | <0.0001* |
| Treatment*pH time point | 6 | 2.4062 | 0.0317* |

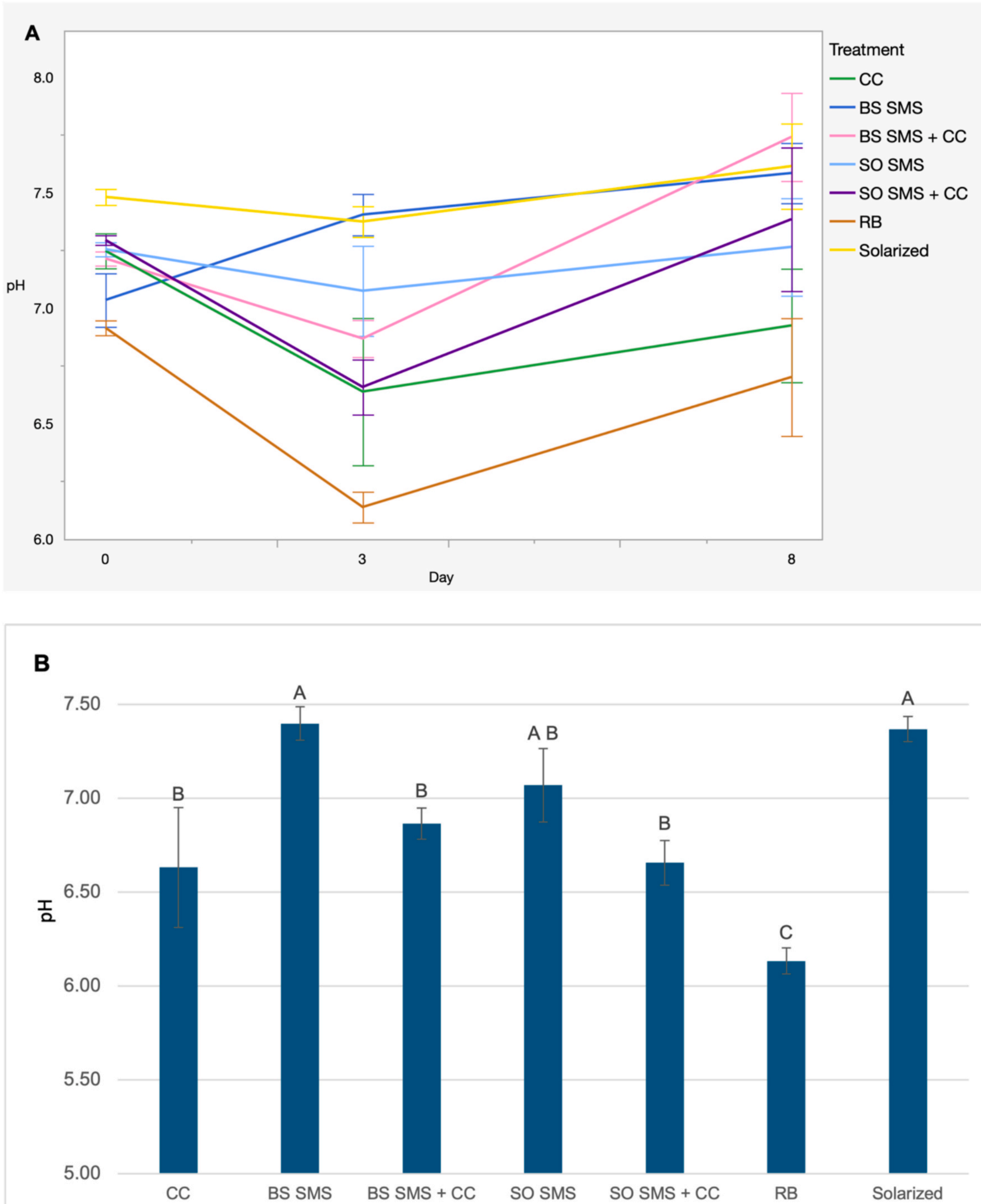


**Fig. 2.** Soil pH throughout (A) and on Day 3 (B) of biosolarization of soil amended with spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), rice bran (RB) or no amendment (solarized). Soils were sampled before (Day 0), during (Day 3), and after (Day 8) biosolarization. Error bars represent ± 1 standard deviation. Values that do not share a letter are significantly different ($P \leq 0.05$).

phytotoxic on any of the time points, including Day 8 of biosolarization. After 4 weeks of remediation, the cover crop treatment was no longer phytotoxic, but rice bran still presented lingering phytotoxicity, with germination indices of 90.1 and 74.2 %, respectively (Fig. 5).

Concentration of BOAs and remediation time both showed significant main effects on germination index ($P < 0.0001$) and the two variables had a significant interaction effect ($P = 0.0358$, Table 4). Generally, the greater the remediation time, the lower the BOA concentration (Fig. 3A), with the exception of the rice bran-amended soil, in which succinic acid peaked after 2 weeks of remediation. Additionally, the greater the BOA concentration the lower the germination index (Fig. 6), indicating greater phytotoxicity.

*Nitrogen analysis*

Biosolarized treatments that included cover crop biomass had the least amount of $NH_4^+$-N, with 4.1 and 6.1 ppm for CC and SO SMS + CC, respectively. SO SMS and solarized soil had 12.4 and 13.2 ppm, respectively, but were both significantly different only from CC ($P \leq 0.05$, Fig. 7B). The $NO_3^-$-N of the amended treatments was below the detection limit (0.10 ppm) and therefore reported as zero in Fig. 7B.

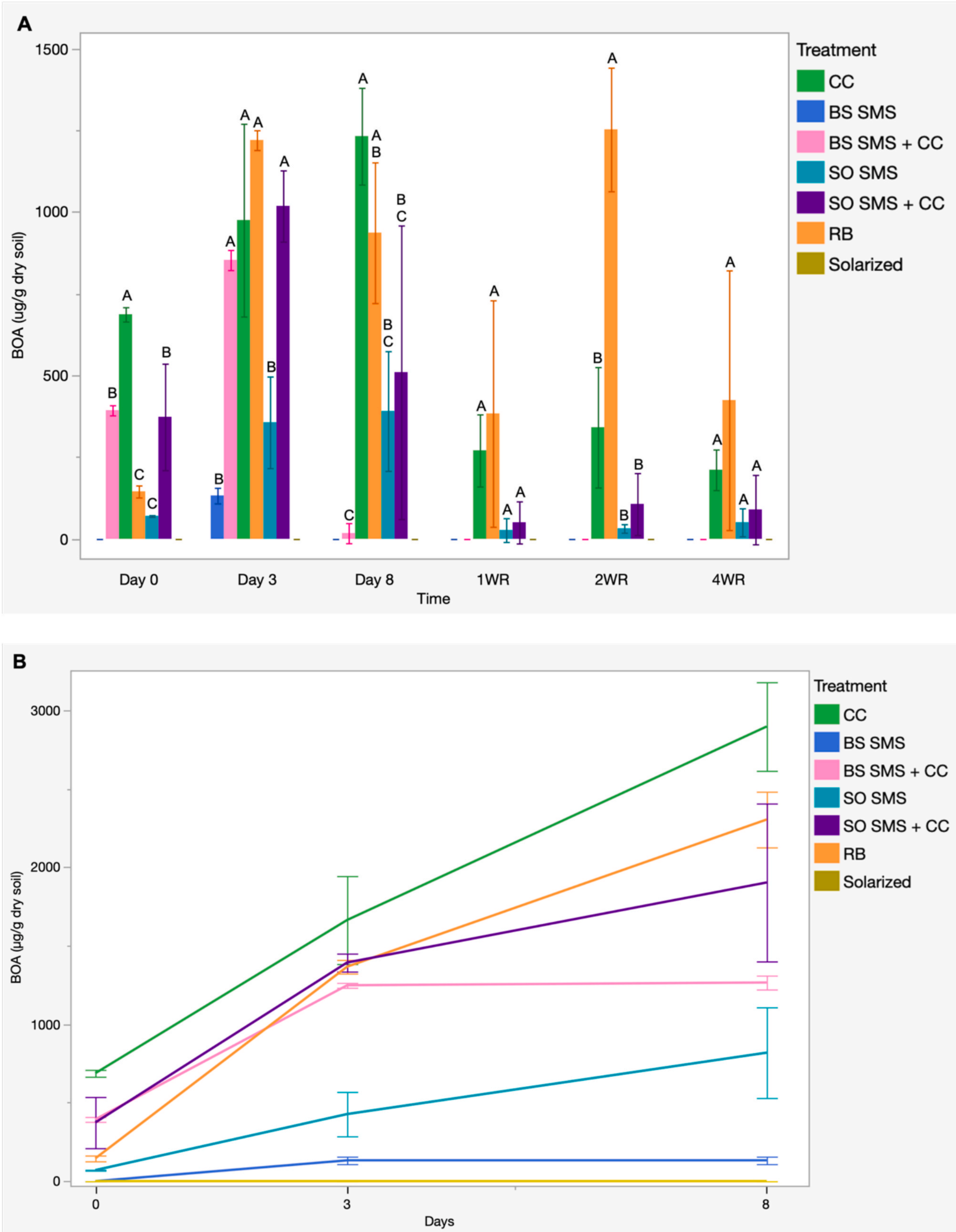


**Fig. 3.** Total biocidal organic acid (BOA) content before, during and after biosolarization (3A) and cumulative BOAs during biosolarization (3B). Soil was amended with spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), rice bran (RB) or no amendment (solarized). Soil was sampled on Day 0, 3 and 8 of biosolarization and after 1, 2 and 4 weeks of remediation (1WR, 2WR and 4WR, respectively). Values that do not share a letter within each time point are significantly different ($P \leq 0.05$). ANOVA and Tukey's post-hoc test were performed on treatments with non-zero values. Error bars represent $\pm$ 1 standard deviation.

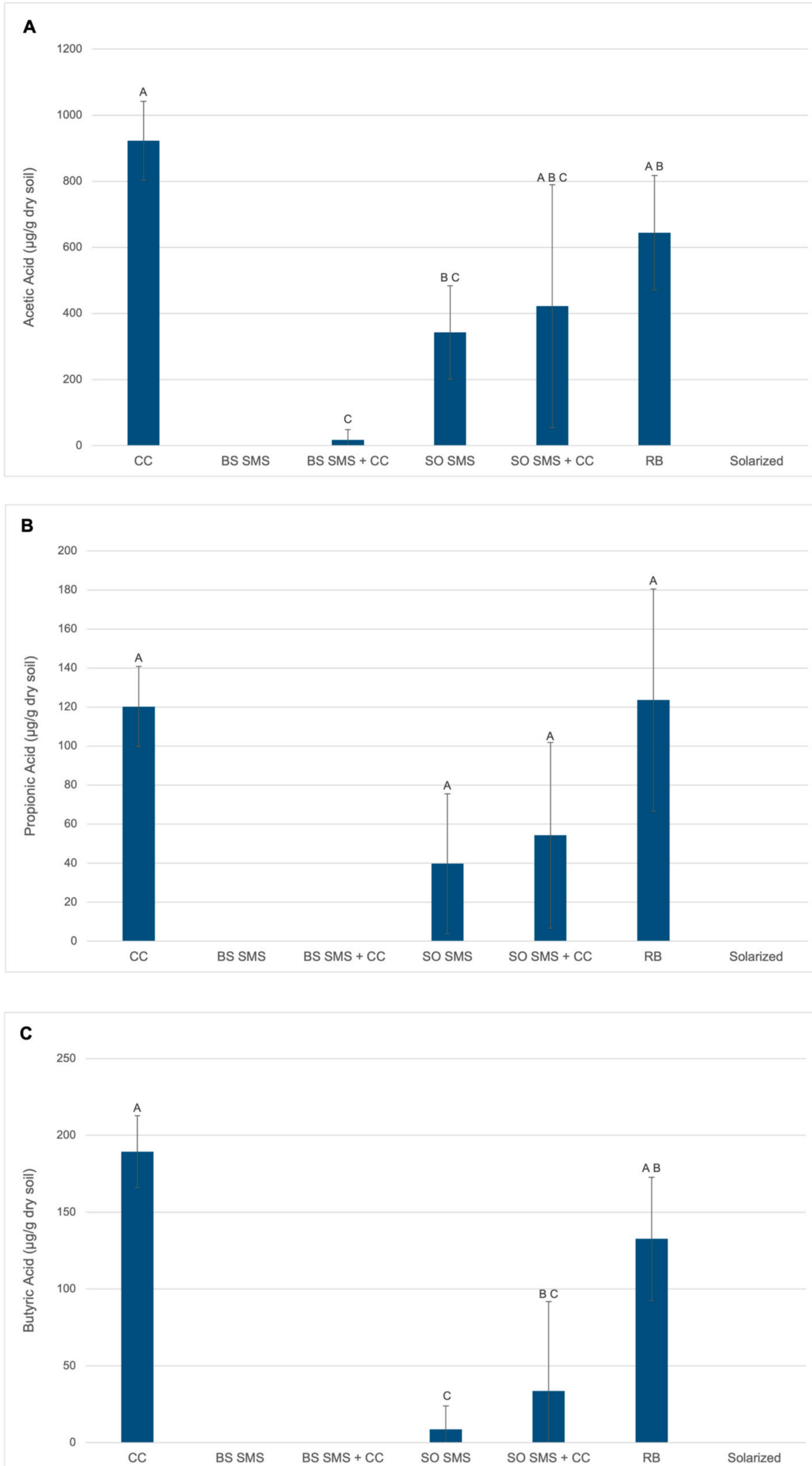


**Fig. 4.** Acetic acid (A), propionic acid (B) and butyric acid (C) content at the end of biosolarization (Day 8) of soils amended with spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), rice bran (RB) or no amendment (solarized). Treatments without a visible bar did not have detectable levels of BOAs. Error bars represent $\pm$ 1 standard deviation. Values that do not share a letter within graphs are significantly different ($P \leq 0.05$). ANOVA and Tukey's post-hoc test were performed on treatments with non-zero values.

**Table 3**
ANCOVA effect tests of soil treatment and remediation time on radish germination indices after biosolarization. Asterisks indicate statistically significant values ($P \leq 0.05$).

| Source | Degrees of freedom | F Ratio | Prob > F |
|---|---|---|---|
| Treatment | 6 | 13.7768 | <.0001* |
| Weeks of remediation | 2 | 28.9812 | <.0001* |
| Treatment*Weeks of remediation | 12 | 2.6656 | 0.0094* |

Average $NO_3^-$-N content of the solarized samples was 1.65 ppm. The nonparametric Kruskall-Wallis test reported a significant difference in the $NO_3^-$-N dataset ($P = 0.011$), but the post-hoc Dunn test was not sensitive enough to identify a difference between the solarized samples and the zero values of the amended treatments. The $NH_4^+$-N and $NO_3^-$-N content as a percentage of total nitrogen followed the same trends as those described for each mineral nitrogen type.

There was no significant difference between total nitrogen content of all the amended treatments, but the solarized soil had significantly less total N than CC and SO SMS ($P \leq 0.05$, Fig. 7A).

Nitrogen analysis was also done on the SO SMS amendment itself. The $NH_4^+$-N, $NO_3^-$-N and total N content of the amendment was 9.2, 3.2, and 8926.7 ppm, respectively. ANOVA and Tukey's post-hoc test confirmed that there was no significant difference between the $NH_4^+$-N content of the SO SMS and any of the analyzed treatments. However, the total nitrogen of the amendment was well above, and significantly different from ($P < 0.0001$), any of the treatments, with the amended treatments averaging between 576.7 and 638.3 ppm.

## Discussion

This study investigated the potential for SMS and cover crops, both alone and in combination, as amendment sources for biosolarization. Specifically, gas evolution, pH, BOA content, phytotoxicity, and soil nitrogen concentrations were examined in biosolarized soil amended with these materials. The amended treatments were compared to positive and negative controls to quantify characteristics which may lead to successful biosolarization treatments and increased soil health.

The gas evolution experiment was used to measure microbial activity induced in SMS-amended soil and characterize the kinetics of biodegradation. In a previous simulated biosolarization trial using 5 % tomato pomace combined with 2 % green waste compost as the amendment, cumulative $CO_2$ reached 1.2 mg/g dry amended soil in 12 days (Achmon et al., 2016). This was achieved with a total amendment rate of 7 %, while the SMS evaluated here were incorporated at 1.5 %, reaching 0.26 to 0.29 mg/g dry amended soil (Fig. 1). Due to the lack of methane production over the 15-day experiment, there was no evidence to suggest that methane emissions from SMS degradation in soil would be a

**Table 4**
ANCOVA effect tests of biocidal organic acid (BOA) concentration and remediation time on radish germination indices after biosolarization. Asterisks indicate statistically significant values ($P \leq 0.05$).

| Source | Degrees of freedom | F Ratio | Prob > F |
|---|---|---|---|
| BOA concentration | 1 | 26.3804 | <.0001* |
| Weeks of remediation | 2 | 25.1397 | <.0001* |
| Weeks of remediation*BOA concentration | 2 | 3.5329 | 0.0358* |

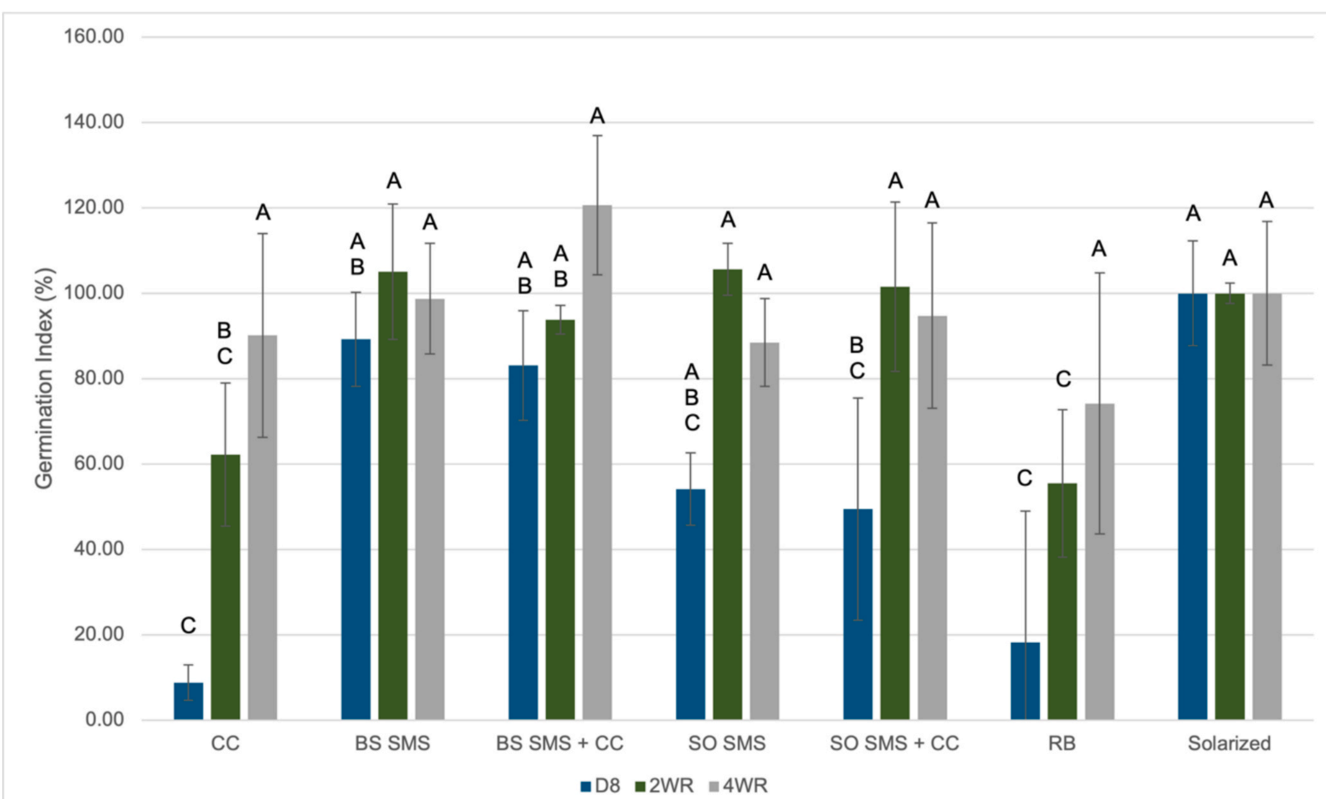


**Fig. 5.** Average germination index of radish seeds during remediation of soil amended with spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), rice bran (RB) or no amendment (solarized). Soil was sampled on the last day of biosolarization (D8), and after 2 and 4 weeks of remediation (2WR and 4WR, respectively). Values that do not share a letter within each time point are significantly different ($P \leq 0.05$). Error bars represent ± 1 standard deviation.

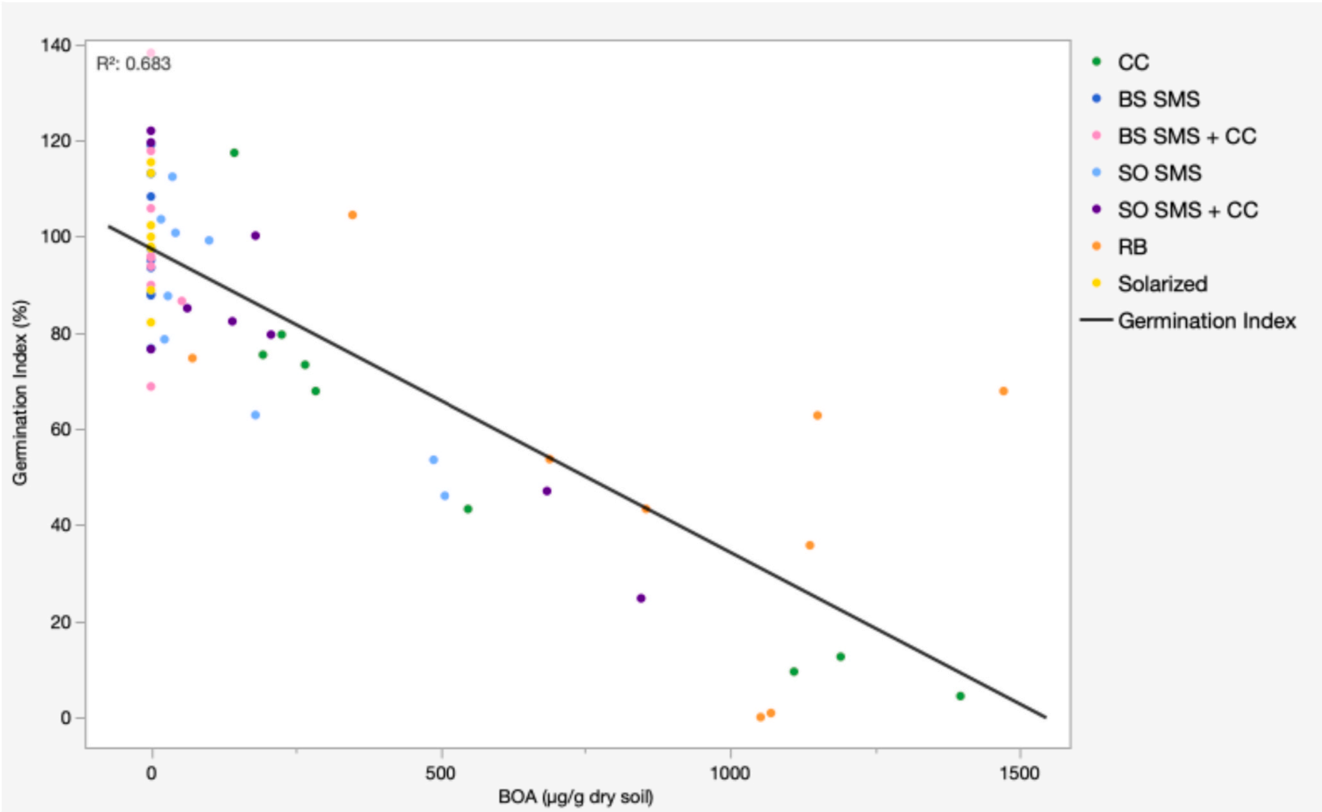


**Fig. 6.** Correlation between germination index and biocidal organic acid (BOA) concentration of all treatments and remediation timepoints. Soil was amended with spent mushroom substrate made of rice bran and hardwood sawdust (BS SMS), spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), rice bran (RB) or no amendment (solarized). Remediation timepoints were 0, 2 and 4 weeks after biosolarization. Each dot represents a mean of 2 germination index replicates and 2 BOA replicates. The solid line indicates the line of best fit.

concern within the biosolarization timeline.

The gas evolution data indicate that SMS induced microbial activity, which can potentially generate pesticidal conditions in soil, such as reduced oxygen, lower pH, and BOA accumulation as a result of amendment fermentation. However, the data showed that gas evolution is not a consistent predictor of soil acidification as indicated by pH. Day 3 of biosolarization was the time point at which pH was lowest for all treatments except BS SMS (Fig. 2). The fact that both treatments of SMS as sole amendments did not show significant pH drops by Day 3 when compared to the solarized control suggests that SMS as a sole amendment may not be a viable amendment for soil acidification during biosolarization. This is further supported by the low concentration of BOAs detected via HPLC for Day 3 samples of both SMS treatments (Fig. 3).

Phytotoxicity was tested not only as an indicator of weed inactivation potential, but also risk to subsequent crops. In practice, SO SMS may produce pest –specifically, weed– suppressive properties in the soil as demonstrated by the fact that the germination index on the last day of biosolarization was not significantly different from that of the rice bran control. The low germination index may be due to BOA production. The $R^2$ of 0.683 for the regression shown in Fig. 6, which captures the amount of variance in germination indices that can be explained due to BOA concentration, suggests BOA content is a predictor of phytotoxicity. Several BOAs are known to be phytotoxic, but they may also co-occur with other fermentative phytotoxic compounds, such as alcohols or ketones (Achmon et al., 2017; Osipitan et al., 2022). Nonetheless, the BOA content of soils containing SO SMS at 1.5 % at the end of biosolarization (391.5 μg BOA/g dry soil) is comparable to BOA concentrations of other biosolarized soils (80.8 to 546.9 μg BOA/g dry soil) in which there was significant mortality of the weed *Brassica nigra* along with the plant pathogen *Fusarium oxysporum* f.sp. *lactucae* (Fernandez-Bayo et al., 2017).

In another biosolarization trial in which > 95 % mortality of *Brassica nigra* and *Solanum nigrum* seeds was achieved, approximately 1,600 μg BOA/ g dry soil were generated in soil amended with 2.5 % tomato pomace (Achmon et al., 2017). The cumulative BOA concentrations achieved during the biosolarization in this study in soils amended with CC, RB, and SO SMS + CC exceeded this value (Fig. 3B), suggesting that these treatments may also lead to biopesticide production sufficient for inactivation of these weed seeds.

Biosolarization microcosm experiments have been validated at field scale (Simmons et al., 2016). Comparison of the BOAs observed in soil amended with SO SMS to previous studies mentioned above suggests that the treatments applied here may be feasible at a field scale for pest management. Future research applying the treatments from this study in a field trial can serve as validation that the BOA production observed in the microcosms can indeed be achieved and contribute to conditions needed to inactivate pests in an infested field within the 8-day treatment period.

The BOA totals on Day 3 were similar among all cover crop treatments, but decreased in the co-amendment treatments by Day 8 (Fig. 3), showing that acid production was not sustained throughout the 8-day period in the treatments with less cover crop biomass. The significantly lower total BOA concentrations on Day 8 in the co-amended treatments compared to the cover crop alone suggests that the cover crop biomass was the driving amendment for acid production in the co-amended soils. Additionally, each SMS treatment had significantly lower cumulative BOA concentrations than its co-amended counterpart, further demonstrating that SMS did not enhance acid production during biosolarization.

Remediation after biosolarization is necessary to avoid negative growth effects from the phytotoxins on seeds or seedlings. However, soil amended with rice bran or cover crop alone required at least 4 weeks to

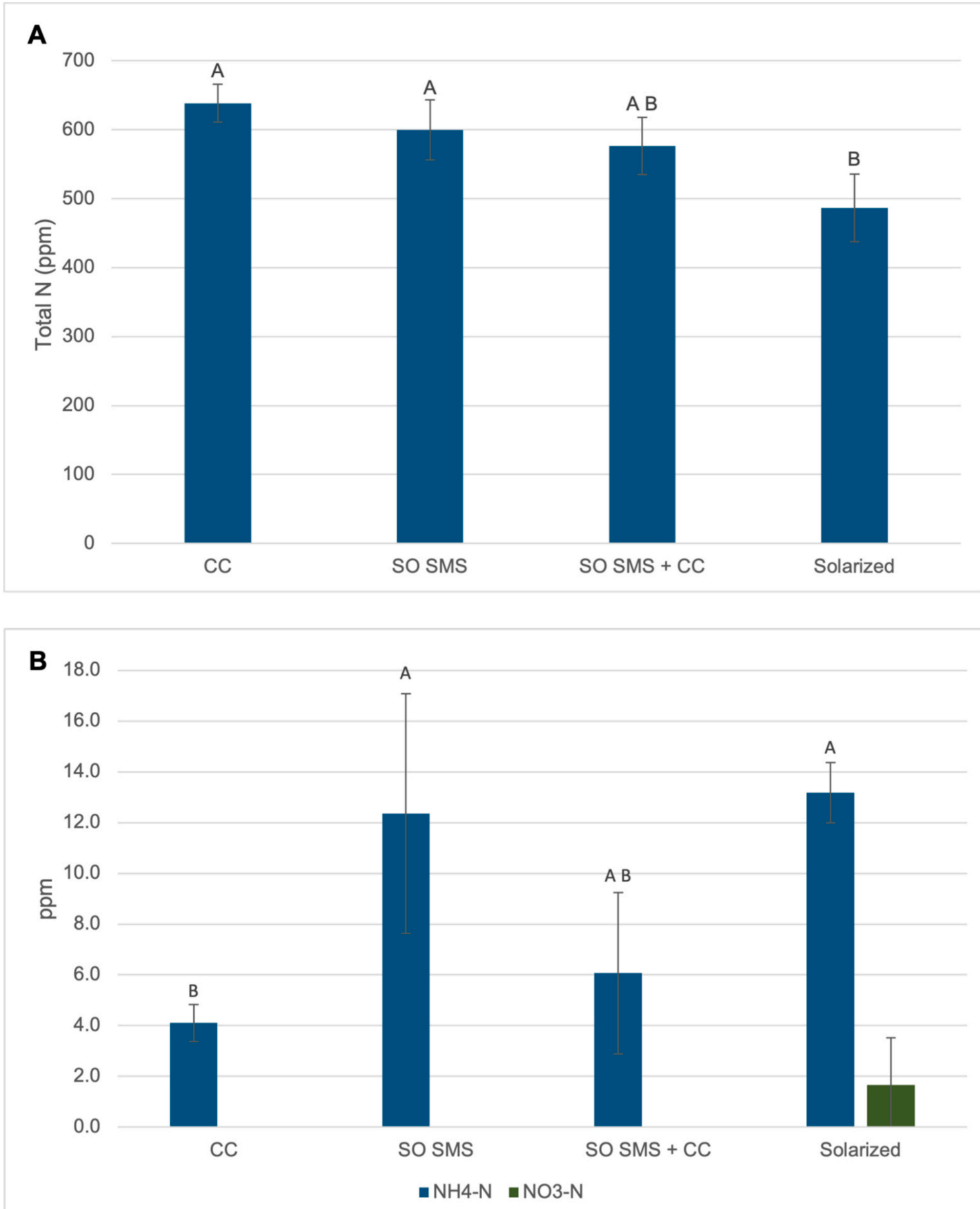


**Fig. 7.** Total nitrogen (A), ammonium nitrogen and nitrate nitrogen ($NH_4^+$-N and $NO_3^-$-N, B) of a subset of soil samples after biosolarization of soil amended with spent mushroom substrate made of soybean hulls and oak sawdust (SO SMS), rye and clover cover crop biomass (CC), a combination of both (SO SMS + CC) or no amendment (solarized). Treatments without a visible $NO_3^-$-N bar did not have detectable $NO_3^-$-N levels. Values that do not share a letter within the same nitrogen type are significantly different ($P \leq 0.05$). Error bars represent ± 1 standard deviation.

remediate and no longer be phytotoxic. This longer remediation time could present challenges for growers that need to plant crops in the treated soil. For all treatments with SMS, the results suggest 2 weeks of remediation is a sufficient amount of time for the soil to support subsequent plant growth (Fig. 5). However, neither of the treatments with BS SMS were phytotoxic at any time point, suggesting that BS SMS is not a suitable amendment for production of phytotoxic conditions, even when paired with cover crop biomass. On the other hand, as mentioned above, SO SMS may be a useful amendment alone or when combined with cover crop biomass. Since SO SMS + CC had phytotoxicity and BOA concentrations on Day 8 that were not significantly different from the rice bran control, this combination may provide a suitable strategy for biosolarization using both a food industry waste stream and an amendment grown *in situ* that protects soil health. While biosolarization with SO SMS + CC decreased BOA accumulation by 34 % compared to CC alone, there was a disproportionate decrease in phytotoxicity immediately after simulated biosolarization (461 % increase in germination index). This suggests that SO SMS co-amendment could be used to balance BOA accumulation during biosolarization with a reduction in post-treatment phytotoxicity. Additional research is warranted to refine such strategies.

Although the cover crop biomass used here as the sole amendment showed the greatest phytotoxicity, the proportion of biomass used here is not feasible to reach in typical production on a field (Finney et al., 2016; Balkcom et al., 2023). Also, the presence of cover crop biomass led to decreases in nitrogen mineralization (Fig. 7B). Given that all biosolarized treatments had comparable total nitrogen content (Fig. 7A), the decrease in $NH_4^+$-N seen in both of the treatments that contained cover crop residues suggest that the biomass may contain bioactive compounds that inhibit mineralization in the soil or has a high carbon to nitrogen ratio that causes immobilization by soil microbes (Weil et al., 2017). This decrease in mineral nitrogen may impact fertilization strategies and would therefore need to be considered when determining biosolarization amendments for soil in which crops will be planted.

The addition of organic matter amendments in the biosolarized treatments increased the total N of the samples compared to the solarized soil (Fig. 7A). However, mineral nitrogen did not follow the same trend, suggesting that there were differences in the nitrogen dynamics within the soil, likely due to differences in microbial composition or activity. The low amounts of $NO_3^-$-N and lack of significant differences between any treatments suggest that nitrification may have been inhibited, possibly by inactivation of nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* during biosolarization since these microbes are temperature and pH sensitive, with their activity decreasing above 40 °C and below pH 7 (Grunditz and Dalhammar, 2001). Both of these conditions occur during biosolarization, so microbiological studies would help shed light on the effects that these amendments and the biosolarization process can have on nitrogen cycling in soil by understanding the effects that exposure to these conditions for 8 days may have on these bacteria during and after treatment.

In terms of treatment duration –or time during which the field cannot be used for planting– the 8-day biosolarization period presents a more viable option for pest management than other fumigation alternatives. Previous findings report that three weeks is the most common anaerobic soil disinfestation (ASD) incubation period and incubation periods of greater than 10 weeks were most effective for weed suppression (Shrestha et al., (2016)). Recent studies using solarization as a pest management strategy have employed treatments lasting from 21 to 60 days (Rippa et al., 2025; Castello et al., 2024; Tziros et al., 2024; Wada et al., 2024). Therefore, biosolarization presents an alternative option for pest control with reduced treatment periods and disruptions to crop cycles. Since the treatment requires high temperatures, growers in regions that have a sufficiently warm climate may be able to integrate this alternative into cropping schedules before a fall planting date, or after a summer harvest.

The pest control opportunities discussed here may be complemented by secondary benefits resulting from incorporation of organic matter byproducts, such as improved soil physicochemical properties (Lal, 2020; Šimanský et al., 2019; Medina et al., 2012; Stewart et al., 1998). A variety of alternative effects, from heavy metal remediation to restoration of soil biological health after pesticide applications, have also been reported after biowaste incorporation (Rai et al., 2024; Virk et al., 2025). These additional benefits can be assessed in future biosolarization work to further understand the downstream impacts of adopting this process as an alternative pest management strategy.

## Conclusion

The findings of this study confirm that SO SMS and cover crop treatments produce phytotoxicity results and BOA concentrations comparable to those of biosolarization with rice bran amendment. Since rice bran has been successfully used for agricultural pest inactivation, further studies with SO SMS are justified (Khadka et al., 2020). There was no evidence that BS SMS produced phytotoxic effects, indicating that it likely does not have value as a biosolarization amendment for pest suppression. The main difference between BS and SO SMS is the proportion of sawdust they contain: 80 % and 50 %, respectively. Sawdust may reduce the fermentation potential of the amendment because it is a lignocellulosic, recalcitrant material (El Hage et al., 2023). Future work should focus on evaluating the efficacy of SMS with nutrient ratios similar to SO SMS in biosolarization as an applied pest management strategy by incorporating direct analyses of pest inactivation, such as weed propagule mortality, to determine if the lower microbial activity and BOA concentrations produced are sufficient to inhibit specific pests. Given the differences reported in mineral nitrogen, future research should also investigate the effects of the treatments on subsequent soil and crop health.

## Funding

This work was supported by a grant from the National Institute of Occupational Safety and Health (grant #5U54OH007550), the USDA NIFA Multistate Research Project S1075: The Science and Engineering for a Biobased Industry and Economy, and the Fellowship for Research Doctorates (Convocatoria de Doctorado de Investigación) of the Panamanian National Secretariat for Science, Technology and Innovation (SENACYT).

## CRediT authorship contribution statement

**Carolina R. Conte:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Abigail Nagano:** Visualization, Investigation, Formal analysis. **Maya C. Gentry:** Visualization, Investigation, Formal analysis. **Christopher W. Simmons:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data described here is available via Mendeley Data using the following information.

Conte, Carolina (2025), "Data for Assessment of Spent Mushroom Substrate Recycling via Soil Biosolarization", Mendeley Data, V1, https://doi.org/10.17632/zc67tbgxg8.1.

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