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Assessment of the influence of using green tea waste and fish waste as soil amendments for biosolarization on the growth of lettuce (*Lactuca sativa* L. var. *ramosa* Hort.)

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Introduction: Safe and efficient treatment of organic waste is crucial to developing a sustainable food system around the world. Soil biosolarization (SBS) is a soil treatment technique that can use organic solid wastes to treat the soil in a way that is alternative to the use of chemical fumigants to improve soil fertility in agriculture.

Methods: In this study, two types of organic food wastes, green tea waste (GTW) and fish waste (FW), were evaluated for the feasibility of being applied as soil amendments within simulations of high-temperature cycle SBS. The evaluation was conducted by execution of three groups of measurements: gas and organic volatile emission profile, residual soil phytotoxicity and weed suppression, and cultivar growth (*Lactuca sativa L. var. ramosa Hort.*).

Results and Discussion: Green tea waste contributed to elevated levels of soil respiration and the evolution of signature volatile organic compounds during the simulated SBS. In the soil amended with green tea waste and then undergoing SBS the phyto compatibility was restored after residual phytotoxicity dissipation and a complete weed suppression was achieved. By using an application rate of 2.5% (w/w, mass fraction of green tea waste in total soil-waste mixture) green tea waste cultivar growth comparable to that of the non-treated soil (NTS) group was attained, with a more efficient nitrogen utilization and higher residual soil nitrogen content enabling the improvement of the continuous cropping system. FW at 1% (w/w, mass fraction of FW in total soil-waste mixture) promoted cultivar growth despite the significant reduction of the nitrogen (*p* value=0.02) and phosphorus (*p* value=0.03) contents in the cultivar leaves. A significant increase of the sodium content together with an increase of iron and chromium, which exceeded the permissible limit, were observed. These results provide new information about amendment selection for the SBS process.

KEYWORDS

organic food waste, biosolarization, fish waste, green tea waste, soil amendment, food sustainability

1. Introduction

Food waste is an enduring global concern. Many factors are involved in giving rise to this phenomenon, including poor storage and transportation facilities, quality defects, leftovers from meal preparation, and wasteful consumer behaviors (Gustavsson et al., 2011; Alexander et al., 2017; Spang et al., 2019; Barrera and Hertel, 2021). Reducing food waste can have positive social, environmental, and economic impacts. For example, it can lessen methane gas emissions (Fiore et al., 2017; Achmon et al., 2018; Chew et al., 2021), reduce purchase costs and waste disposal costs (Papargyropoulou et al., 2014) and redirect food to people from low-income and foodinsufficient regions (Walia and Sanders, 2019). Therefore, food waste reduction is crucial to the healthy functioning of the whole society. Food waste conversion into compost has been a measure to solve the food waste challenge with a positive impact on agriculture (Sullivan et al., 2002; Chew et al., 2018; Lin et al., 2021). Soil biosolarization (SBS) is a new agricultural practice that combines waste amendment and solarization (Achmon et al., 2016, 2018; Dar et al., 2022).

In SBS, chemical fumigation replacement is adopted to treat a broad spectrum of soil-borne pathogens. A proper organic amendment is added and mixed with the soil, which is subsequently covered by a transparent plastic sheet to achieve a soil pesticidal effect. Compared to solarization, SBS uses organic amendment to accelerate heat accumulation and transform the soil microbiome activity (Domínguez et al., 2014; Hestmark et al., 2019). Recent studies have shown several promising amendments used in SBS. For example, almond processing residues has been proposed to inactivate Pratylenchus vulnus, a plant pathogenic nematode, in biosolarized soil (Fernandez-Bayo et al., 2020); tomato plant debris applied in SBS could achieve yield and quality of tomato crops which is comparable to those achieved after inorganic fertilization (García-Raya et al., 2019; Haber et al., 2022). The selection of a proper amendment is vital for the feasibility of SBS on a larger scale and could be evaluated by indicators such as availability of amendment source, residual soil phytotoxicity (Achmon et al., 2016), soil pest mortality (Achmon et al., 2017; Shea et al., 2022), and transformation in salt immobilization (Sánchez-Navarro et al., 2022), soil composition (Fernández-Bayo et al., 2017; Sánchez-Navarro et al., 2022) and cultivar yield and quality (García-Raya et al., 2019; Haber et al., 2022), etc. The type of the soil amendment and optimal utilization conditions are crucial to achieve a high-performance SBS process.

Green tea is a widely popular drink all around the world. During the last decade, green tea consumption increased due to its suggested health benefits and reached about 30% of total tea production worldwide (WFP, WHO, and UNICEF, 2022). It is expected to reach 3.6 million tons by 2027 with an annual increment of 7.5% (Debnath et al., 2021). The current amount of green tea waste (TW) in China alone is around 2.5 million tons per year and most of it going to landfills (Li et al., 2022). In 2020, global fisheries and aquacultural production attained 214 million tons. The world capture of fisheries is projected to increase by 6 percent from 2020 to reach 96 million tons in 2030 (Wake and Geleto, 2019). The high production volume of both fish products and green tea also leads to a large amount of waste. However, the high contents of nitrogen, potassium and calcium in FW (Illera-Vives et al., 2015) and of organic matter and nitrogen (Kondo et al., 2004a) in GTW make them promising soil fertilizers and their use as such can reduce the waste disposal burden they cause. The conversion of waste into value is vital to achieving a sustainable food system.

Previous research has focused on soil status characterization during the SBS process. This study also examined the visible results, including residual soil phytotoxicity demonstrated by the growth of seedling of the consecutive crop, weed suppression and cultivar growth on SBS soil. The goal of this study is to investigate the feasibility of using GTW collected from beverage shops and FW collected from school canteens as amendments applied to field soils undergoing simulations in the lab of the high-temperature-cycle SBS (simulating solarized soil temperature field conditions). The following aspects of these simulations were evaluated: emission of gases and volatile organic compounds (VOC) during the simulated SBS process, residual soil phytotoxicity, weed suppression and cultivar growth. Cultivar growth of lettuce was primarily evaluated by the yield, followed by the profiling of macronutrient and micronutrient contents in the harvested cultivar and the post-harvest soil. These results could provide a vision for predicting the safety and efficacy of using FW and GTW in actual use of SBS in the field, thus enabling the development of a better food waste management system.

2. Materials and methods

2.1. Raw material preparation and characterization

Fish waste (FW) residues were collected from the canteen of the Guangdong Technion Israel Institute of Technology (GTIIT, Shantou, China) in the winter semester, 2019. The FW was uniformly grinded by hand using a mortar into small particles (diameter smaller than 1 mm), dried under 60°C (Bluepard Instrument DZF-6000 Vacuum Drier) until the mass remained constant. The dried FW was well sealed in 4°C until usage. GTW was collected from the local beverage store (Yihetang, Dongmen Street, Jinping District, Shantou, China). The GTW was dried under 60°C (Bluepard Instrument DZF-6000 Vacuum Drier) for 24h and then uniformly ground into powder, sealed and stored in 4°C until usage. The soil was collected from fields in Qianxi Village, Chenghai District, Shantou, sieved through 4 mm sifters, and dried under 45°C until the mass remained constant at two separate times.

The physicochemical parameters of the nutrient element content in the waste and the original field soil were characterized by Nanjing Convinced-test Technology Co., Ltd. The volatile solid (*VS*) content was calculated as the ratio of the ash content over total dry solid content, where the ash content was presented as the remaining solid content in the sample undergoing 6-h incineration at 550°C (SAFTherm Furnace, STM-12-12). The pH value of the sample was determined on the supernatant of the sedimented mixture (waste (g)-water (ml) ratio at 1 to 5) after 30-min shaking at 550 rpm on an orbital/linear shaker (FOUR E's SCIENTIFIC). The properties of the sieved soil utilized in this study are presented in Table 1. The chemical composition profiles of the FW amendment and the GTW amendment are presented in Tables 2, 3.

The assessment of reusing waste as soil amendment was performed in four consecutive studies investigating the effects on the emission of gas and organic volatiles, residual soil phytotoxicity, weed suppression, and cultivar growth. As similar tests using FW have been performed on the gas and organic volatile emission, phytotoxicity and weed suppression (Liang et al., 2022), only the "cultivar growth" test has been performed in the current study. For the GTW group, all four

	Total N	Total P	Total K	Total C	C/N
	g/kg d.m.	g/kg d.m.	g/kg d.m.	g/kg d.m.	g/g
FW	N.A.	N.A.	23.480 ± 0.442	N.A.	N.A.
GTW	4.229 ± 0.155	0.726 ± 0.018	27.041 ± 0.290	47.50 ± 1.46	11.23 ± 0.17
	Са	Na	Mg	Fe	Mn
	g/kg d.m.	g/kg d.m.	g/kg d.m.	g/kg d.m.	g/kg d.m.
FW	0.329 ± 0.007	1.382 ± 0.077	1.414 ± 0.652	88.551 ± 3.180	0.194 ± 0.009
GTW	1.201 ± 0.027	1.683 ± 0.080	1.792 ± 0.073	30.057 ± 0.519	0.175 ± 0.005
	Zn	Ni	Cu	Мо	Se
	mg/kg d.m.	mg/kg d.m.	mg/kg d.m.	mg/kg d.m.	mg/kg
FW	59.718 ± 0.072	15.653 ± 0.483	18.909 ± 0.219	1.955 ± 0.064	N.A.
GTW	92.859 ± 4.387	4.852 ± 0.216	20.732 ± 0.926	0.444 ± 0.020	0.689 ± 0.020
	Density	WHC		рН	VS
	g/cm3	g water per g dry soil	Texture	Dilution fold of 5	g/100g dry soil
FW	1.3046 ± 0.0286	38%	N.A.	5.401 ± 0.033	2.339 ± 0.029
GTW	1.8889 ± 0.0031	68%	Between sand and loamy sand	5.216 ± 0.131	8.859 ± 5.845

TABLE 1 Soil properties including element nutrient content, soil density, water holding capacity (WHC), soil texture, pH and volatile solid content (VS).

FW, fish waste group; GTW, green tea waste group; d.m., dry matter; N.A., data not available. Mean values (±standard deviation) are shown in the table.

tests were performed. In all these four tests, waste (soil amendment), soil and distilled water were blended and incubated (Lab Companion, Low Temp. Incubator, Multi Chamber, 1L-11-4C) under aerobic conditions that simulate a SBS in a laboratory level (detailed in each corresponding section). For both the FW group and the GTW group, the incubator temperature was set at $30^{\circ}C-40^{\circ}C-45^{\circ}C-50^{\circ}C-55^{\circ}C-50^{\circ}C-45^{\circ}C-40^{\circ}C$ (4h-4h-3h-2h-2h-2h-3h-4h respectively) to simulate the temperature conditions in the upper level of the soil during a SBS [similar to what was previously done (Liang et al., 2022)]. The amendment load for the FW group was 1% (mass fraction of the FW in total soil-waste mixture). The amendment loads for GTW group were 0, 0.5, 1.5 and 2.5% (mass fraction of GTW in total soil-waste mixture). The waste mixture. Additional non-treated soil (NTS) was used as a control group in all experiments.

2.2. Gases and organic volatiles emission measurement

The bioreactor systems were established to represent possible situations that may occur during a SBS. The FW group used 250 mL (glass or plastic) bottles connected with gas collectors with either two-way valve or one-way valve to create either aerobic or anaerobic conditions. The temperature cycle was slightly different from the aforementioned incubator temperature (refer to (Liang et al., 2022) for detailed information). The GTW group used 250 mL (glass or plastic) bottles connected with gas collectors with a two-way valve to create the aerobic conditions. Gas collectors for each reactor were connected

to a MicroOxymax respirometry system (Columbus Instruments International) equipped with a Non-Dispersive Infrared CO₂ sensor with a 0–3% detection range (Columbus Instruments International Serial 200,135–4). Auto-sampling and auto-measurement of the accumulation and composition of gases were made using a system sampling pump (Columbus Instruments International Serial 200,135–1). The time intervals of the bioreactors depended on the number of bioreactors used in the system and ranged between 2 and 4h.

The proton transfer reaction-time of a flight-mass spectrometer (PTR-ToF-MS 1000, Ionicon Analytik Ges.m.b.H, Innsbruck, Austria) was used in this study to detect the VOCs emitted by samples. The conditions in the PTR drift tube where the actual ionization process of the VOCs took place were set as follows: drift pressure 2.30 mbar, drift temperature 80°C, drift voltage 630 V and thus the ratio of electric field strength to number density (E/N) was 142Td (1Td = 10–17 V cm2). H3O+ was set as a reagent ion. The mass-scale was calibrated using the signal at 21.022 m/z and 59.049 m/z (H3O+- isotope and protonated acetone). A sequencer model was then applied which permitted automatic switching between each channel that transfers VOCs in the sample. The extracting time for each channel was 30s with one spectrum per second. Four hour intervals were used after all channels finished the sampling process.

2.3. Residual phytotoxicity and weed suppression

The residual phytotoxicity was determined as the germination rate of lettuce seeds growing on the waste-amended soil [similar to a

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IABLE 2	Chemical	composition	characterization	of green	tea waste	(GTW) (II	n dry m	lass).

Total N	Tot	al P		К	Tota	lC	C/	/N	Ca	
g/kg	g/	kg		g/kg	g/k	g	g/	/g	g/kg	
43.381±0.111	3.396	±0.077	5.7	02 ± 0.020	400.789±	7.577	9.239 ±	±0.198	3.082±0.0)10
Na	Mc	Mg		Fe		Mn		In	Ni	
g/kg	g/k	g	m	g/kg	mg/l	kg	mg	J/kg	mg/k	g
0.092 ± 0.007	1.563±0	0.003	227.20	54±5.424	523.965±	25.316	42.065±0.019		3.532±0.0	046
Cu		٨l	Мо		B		Se		Ti	
mg/kg	mc	/kq	n	ng/kg	 mg/l	q	mq/	′kq	mg/kc	1
18.063±0.029	0.759	±0.026	0.0	74±0.002	15.483±0).153	1.517±0.063		17.125±0.3	321
Cu				۸ -	C.r.				Ç.,	
Cr	Co		As		Sr		Ca		Sn	
mg/kg	mg/	kg	m	g/kg	mg/kg	μg/kg		9	μg/kg	
3.994 ± 0.218	$0.349 \pm$	0.007	0.128	±0.003	24.074±1.8	63	84.037±2.355		128.278±41.0)33
Sb		Ва			Pb		V		Τι	
μg/kg		mg/kg	ı mg		g/kg		μg/kg		μg/kg	
20.485 ± 3.855		33.364±1.2	91	2.613	3±0.299	26	55.439±3.449		74.822±2.568	
Crude protein			Cellulose		Total sugar		Crude fat			
g/100g			mg/g			mg/g	mg/g		%	
25.061 ± 1.507	25.061±1.507		147.778 ± 10	.439	24	8.986±18.028	3		1.694 ± 0.031	
Lianin		Coarse_fil	ber			Die	etarv fiber			
%		%		Soluble d g/1	ietary fiber 00g	Insolub	le dietary fi g/100g	iber [.]	Total dietary fiber g/100g	
10.104±1.097		0.156 ± 0.00	12	5.872	± 0.340	46	.711 ± 2.280		52.582 ± 1.940	1

Mean values (±standard deviation) are shown in the table.

TABLE 3 Chemical composition characterization of fish waste (FW) (in dry mass).

К	Ca	Na Mg		Fe		Mn	
g/kg	g/kg	g/kg	g/kg	mg/kg		mg/kg	
5.078 ± 0.274	28.279 ± 4.172	20.227 ± 1.645	20.227 ± 1.645 1.056 ± 0.062 45.469 ± 1.394		6.421 ± 0.355		
-	NĽ					0	
Zn	NI	Cu	Al		-r	Co	
mg/kg	μg/kg	mg/kg	μg/kg	mg	/kg	μg/kg	
46.391 ± 2.201	106.274 ± 5.753	1.561 ± 0.219	50.649 ± 6.602	1.996	±0.053	21.527 ± 3.578	
Мо		Cd Pb		b		S	
μg/kg		μg/kg	μg/kg	μg/kg g/kg		g/kg	
298.231 ± 13.273		53.764 ± 4.180	157.237±24.32	9		8.28±0.55	

Mean values (±standard deviation) are shown in the table.

previous studies (Achmon et al., 2016; Liang et al., 2022)]. For each GTW amendment load level, 150 g soil-waste mixture was placed into one 250 mL media bottle. Distilled water was added up to 60% water holding capacity (WHC) and this was maintained on a daily basis. The cap was perforated to allow oxygen exchange with the environment. After 14-day incubation under the pre-set temperature condition, the

soil mixture was transferred to a greenhouse and around 45 g of the soil mixture was transferred into each deep well ($6 \text{ cm}^*6 \text{ cm}^*5.3 \text{ cm}$). The control groups were soil mixtures amended with 0, 0.5% (w/w), 1.5% (w/w) and 2.5% (w/w) of GTW that did not undergo the simulating-SBS incubation and were prepared after 0 days, 3 days and 7 days following the transferring day. In these days, six lettuce seeds

(*Lactuca sativa* L. var. *ramosa* Hort.) [China Vegetable Seed Technology Co., Ltd. (Beijing)] were seeded equidistantly in each soil sample. The temperature during the seedling growth ranged between 15°C and 35°C. Distilled water was added twice a day at 9 am and 4 pm to maintain consistent moisture. The residual phytotoxicity was measured by the germination rate of the lettuce seedlings harvested 14 days after seeding.

A weed suppression test was performed by measuring the germination of Bidens (Bidens pilosa) incubated in the soil undergoing simulated SBS. Bidens seeds were collected from paths near the field where the soil was collected. For each GTW amendment load level, 30g soil-waste mixture was placed in one 50 mL Conical Centrifuge Tube (CorningTM FalconTM). Six Bidens seeds were covered with soil and positioned in the upper level of the tubes. Specifically, they were positioned at the 40 mL marker when the total soil reached 45 mL marker. Distilled water was added to 40% WHC and maintained on a daily basis. The tubes were slightly uncapped to allow oxygen exchange with the environment. The Bidens seeds were checked after 14-day incubation under the pre-set temperature condition. The weed suppression efficacy was measured by the germination condition of Bidens as seen by counting the evident weed seedlings if the radicle extended to more than 3 mm in each well through 20 days (Achmon et al., 2017).

2.4. Cultivar growth

Cultivar growth was analyzed based on three parameters: leaf analysis, nutrient content of lettuce (*Lactuca sativa* L. var. *ramosa* Hort.) [China Vegetable Seed Technology Co., Ltd. (Beijing)], and nutrient content of post-harvesting soil. For each amendment (GTW and FW) load level, 1,200g soil-waste mixture was placed in one 1,000 mL media bottle and this was repeated in triplicate. The prepared soil was positioned in the incubator running at a pre-set high temperature cycle for 28 days. Distilled water was added to the initial mass value (at 60% WHC for TW group and 30% WHC for FW group) on a daily basis. Changes in pH and VS were monitored regularly (Supplementary Figures S1–S4).

To ensure the normal growth of the cultivar., seedlings were prepared 1 month prior to transplantation. For the FW group, 4–6 seeds were sown in each seedling block positioned outdoors for 30 days and watered at 7 am and 5 pm every day. For the GTW group, one seed was sown in each seedling block positioned in the greenhouse under 20°C for 18 days. Seeds were watered after 4 pm to maintain the soil moisture every day. The watering amount depended on the soil condition and watering was continued until the whole seedling block became moist.

After 28-days incubation under the pre-set temperature condition, soil mixture from each media bottle was transferred to one pot (upper radius*lower radius*height: 8 cm*5.75 cm*16 cm). The control group consisting of 1,200 g of soil (with water addition to 60% WHC for GTW and 30% WHC for FW) was freshly prepared. The thickest seedlings with uniform growth were selected and gently transplanted into the soil samples.

During the incubation, the average daily temperature monitored by the temperature logger (Jiankongbao, ZL-TH10TP) during the greenhouse incubation was 25°C, ranging between 22°C and 33°C. Distilled water was added daily to keep the soil well moist every day. The pots were exposed to sunshine for $11 h (6:00 \sim 17:00)$ with no shade intentionally imposed. The pot position was changed clockwise for one step and the individual pot was reversed for 180° (Supplementary Figure S5) for uniform sunlight. The lettuce plants were carefully examined for any abnormality throughout the greenhouse incubation period and special traits were recorded. The leaf number and leaf length were recorded regularly. Beginning at the transplantation, the cultivar growth of the GTW group lasted for 66 days and that of the FW group lasted for 61 days. The cultivars were gently harvested for biomass measurements including leaf length, leaf area and leaf weight (fresh mass). After being peeled and washed, every leaf was wiped as dry as possible to avoid measurement error. For each individual cultivar., only leaves with lengths longer than 2 cm were weighed and numbered. Each selected leaf was then orderly placed on a white paper with a ruler presenting a standard of length, and a photo was taken for area and length measurements.

The leaves were dried under 105°C for 20 min and 80°C until the mass remained constant. The nutrient element contents in leaves and soil were characterized by Convinced, ltd. Elements and include N, P, K, Ca, Na, Mg, Fe, Mn, Zn, Ni, Cu, Al, B, Se, Ti, Cr, Co, As, Sr., Mo, Cd, Sn, Sb, Ba, Pb, V, Tl and S. The content of total N, total P and total K in lettuce leaves was measured by the Kjeldahl method, the spectrophotometric method and the atomic absorption flame photometric method, respectively, (NY/T 2017–2011) after leaf digestion with sulfuric acid-hydrogen peroxide mixture. Content of the other elements and the K content of the FW-group were measured by ICP-MS or ICP-OES with appropriate dilution folds following nitric acid digestion (GB 5009.268–2016).

The nutrient contents in the soil after harvesting the cultivar growth were determined on the soil taken near the lettuce root system. Soil samples were air dried until the mass remained constant. The tested elements included N, P, K, C, Ca, Na, Mg, Fe, Mn, Zn, Ni, Cu, Se and Mo. Total N content was measured by the Kjeldahl method following sulfuric acid-catalyst digestion (LY/T 1228-2015 Chinese standard). Total P content was measured by alkali fusion with NaOH and the Mo-Sb anti spectrophotometric method (NY/T 88-1988 Chinese standard). Total K content was measured by alkali fusion by NaOH and the flame photometric method (NY/T 87-1988 Chinese standard). Total C content was measured directly by an elemental analyzer (HJ 695-2014). Measurement of Ca, Na and Mg contents followed the protocol of NY/T 296-1995 Chinese standard. Measurements of Fe, Mn, Zn, Ni, Cu, Mo contents were performed by an ICP-OES after four-acid digestion. Measurement of Total Se content was done by atomic fluorescence spectroscopy after aqua regia digestion. The nutrient/element measurements of soil and leaf samples were performed by Convinced, Ltd. The samples were sealed and stored under room temperature until analyzed.

ImageJ (Version 1.53, National Institutes of Health, Bethesda, MD) was utilized for measurements of leaf length, leaf area, seedling root length and seedling shoot length. Leaf length was determined as the longest length from the petiole to the tip along the main vein. Seedling's root length was determined as the longest root length. When using the software, the scale was set according to the measure of the ruler in the photo and then used the segment line to measure the leaf length, seedling root length and seedling shoot length. To measure the leaf area, the photo was changed into a grayscale 8-bit menu item, and the threshold was adjusted until the whole leaf was covered in red. After applying the threshold, the wand tool was used



to choose the measurement area. The final step was to set "Analyze Particles," changing "Show" to "Masks" and selecting "include holes." All data were shown on a popup window.

2.5. Data analysis

The statistical test was performed with R (Version 1.0.153 – $^{\circ}2009-2017$ RStudio), using a *t*-test with unequal variance for two groups and one-way ANOVA followed by post-hoc analysis with Tukey's test for comparisons between three or more groups.

3. Results and discussion

3.1. Gases and VOCs emission of tea waste

One of the main purposes of SBS is to improve soil quality by transforming the soil microbial community by adding soil amendments. Soil respiration is often positively correlated with microbial biomass and activity (Allen et al., 2011). To study soil respiration status during the simulating SBS process with GTW as the amendment, CO_2 evolution profile during the initial 30 days was characterized. The highest CO_2 accumulation was observed in the system amended with 2.5% (w/w) green GTW with an evolution of 16 mg CO_2/g soil at the end (Figure 1). It may be related to the enhanced microbial activity in the soil. The slowing trend of CO_2 accumulation started around the 8th day and may indicate the stabilization of the microbial community in the system. The CO_2 evolution rate of the GTW group with the amendment rate at 2.5% was the highest in the initial 10 days, reaching a peak of CO_2 evolution rate of 0.99 µL/ming soil between the third and the fourth day (Supplementary Figure S6). The GTW group with the amendment rate at 0.5% (w/w) showed a similar CO_2 evolution profile to the control group (Supplementary Figure S6).

The principal component analysis (PCA) diagram (Figure 2) showed a correlation in the VOCs content generated from the three simulating systems during the initial 16 days. The VOCs content in the GTW group with the amendment rate at 0.5% was strongly correlated with two other groups, and the control group and the GTW group



with an amendment rate at 2.5% possessed their own signature VOCs. Previous research showed that GTW was an effective silage additive due to its good fermentability and associative effects with other fermentable nutrients (Kondo et al., 2004b). The potential candidates for the major emitted VOCs may include ammonia (NH₃) due to the high nitrogen contents in GTW which is believed to degrade into ammonium (Kondo et al., 2004b). The analyses by PTR-ToF-MS suggested that the transformation of the emitted VOCs during the initial 16 days of SBS proceeded gradually with time as indicated by the changes in the indicator peaks (Supplementary Figure S7). Characterization of the signature VOCs will be conducted in the future.

3.2. Phytotoxicity and weed suppression

The SBS technique could serve the purpose of substituting conventional chemical fumigation. The compatibility with the originally-grown cultivar and the pesticidal efficacy against the soilborne pathogens would be desirable. To determine if SBS with the GTW addition could attain these purposes the residual phytotoxicity and the weed suppression rate were measured. The GTW group with the amendment rate of 0.5% (w/w) and 2.5% (w/w) demonstrated a significantly lower lettuce seed germination rate than the four control groups at seedling day 0 (Figure 3A). It indicates that a significant residual phytotoxicity effect existed immediately after the end of the SBS process with GTW, possibly due to remaining phytotoxic compounds. The difference in the lettuce seed germination rate

between the control groups and the experimental groups was gradually diminished in day-3 and day-7 groups, possibly because the phytotoxic compounds may be volatile and dissipated throughout the exposure period to open-air. In the day-7 group, the GTW1 group even attained a significantly higher germination rate than the FTW2 group and the C group. It probably indicates that the phytocompatibility of the soil undergoing SBS with a lower GTW concentration was restored more quickly. In future experiments it is possible to postpone the seeding time in order to measure if SBS with GTW could achieve an even higher germination rate after a longer-term phytotoxicity dissipation. Other growth indices, including biomass, root length, and shoot length of the seedlings, can also be measured to better characterize the phytotoxicity effect (Achmon et al., 2016; Radziemska et al., 2019; Liang et al., 2022; Wang et al., 2023).

The weed suppression rate by the SBS technique was determined on Bidens, which are common weeds in the field the soil samples were taken from. The germination rates of Bidens seeds were zero across all the groups undergoing high temperature treatment, demonstrating a significantly lower value than the control groups (Figure 3B). It showed that the high temperature treatment had an obvious weed suppression efficacy. However, whether GTW exerted the weed suppression effect cannot be determined in this study because both the solarization group and the SBS groups demonstrated zero weed germination rate. The likelihood that the weed seed was not fully inactivated cannot be excluded because a low germination rate of Bidens seeds long after the seeding was observed in the previous research (Liang et al., 2022). A large field trial should also



be implemented to determine the weed suppression efficacy in environmental and edaphic conditions where a larger quantity of weeds and other soil-borne pathogens are encountered.

3.3. Cultivar growth

3.3.1. GTW amendment group

The SBS technique aims to improve agricultural efficiency by achieving a higher cultivar yield. Important cultivar growth parameters include leaf number, edible mass, leaf area and leaf length. In this part, leaf analysis was performed on the lettuce growing on soil undergoing SBS with GTW as the amendment. The amendment load was GTW1: 0.5% (w/w), GTW2: 1.5% (w/w) and GTW3: 2.5% (w/w). The results showed that as the GTW loading rate increased, the values of all these four growth indicators increased (Table 4). The highest values in leaf number, edible mass and leaf area were observed in the GTW3 group, and the leaf length was not significantly different between NTS and GTW3 (two highest). These results met the expectation that leaf growth increases with the loading increment of the GTW, which is a rich source of nitrogen (Kondo et al., 2004a). Previous studies have also shown that these four indicators of plant growth (leaf number, edible mass, leaf area and leaf length) would increase in value as the applied N rate increases within a certain range (Boroujerdnia and Ansari, 2007). Compared to the solarized group (Control), lettuce in the GTW3 group showed significantly larger values of all four parameters (p value<0.05), and lettuce in the GTW2 group showed significantly larger values in leaf number (p value = 0.03) and leaf length (p value<0.001). It indicated that the GTW applied at 1.5% (w/w) and 2.5% (w/w) in the simulated SBS restored the leaf growth which was inhibited by the solarization process. Although a larger edible mass and a larger leaf area than non-treated soil (NTS) were observed in the GTW3 group, the difference was not significant. During the greenhouse incubation, lettuce in the Control group was observed to wilt earlier than in other groups. The NTS group was first observed to have large amounts of weeds which belonged to a different species (evaluated based on its appearance) from that of weeds growing in other groups (Supplementary Figure S8).

Analysis of nutrient element content in lettuce leaves showed varying NPK distributions among groups (Table 5). The highest N content was observed in GTW1 (51.554±7.631g/kg) which was significantly different from NTS (p value<0.01) and GTW3 (p value<0.02). Interestingly, by comparing the leaf growth (Table 4) and the leaf N content (Table 5) in GTW1, GTW2 and GTW3, the leaf growth enhancement was negatively correlated with the total leaf N content. Previous research has found that weeds might germinate after a long period at the post-SBS stage. Despite the high N content, the weed incidence in SBS groups with a low GTW concentration may cause decreased growth as was also observed in the other study (Iqbal Khan et al., 2007). Another factor for the negative correlation between the N content and the leaf growth performance could be the dilution of leaf nitrogen during the leaf expansion stage as recorded in a previous study (Mediavilla and Escudero, 2003). The application of tea compost at a higher rate within a certain limit could increase the chlorophyll content (Talei, 2018). Therefore, the soil amended with GTW (especially at 2.5% loading rate) and undergoing SBS treatment might enhance the photosynthesis and result in enhanced plant growth. In SBS, N was provided in the form of an organic fertilizer

TABLE 4 Leaf analysis of lettuce growing on the soil without simulated-solarization (NTS), with simulated-solarization (Control), with simulatedbiosolarization (SBS) at 0.5% (w/w) green tea waste (GTW1), with simulated-SBS at 1.5% (w/w) GTW (GTW2), and with simulated-SBS at 2.5% (w/w) GTW (GTW3).

	Leaf number	Edible mass	Leaf area	Leaf length
NTS	20 ± 3 a	21.77 ± 9.12 ab	29.18 ± 7.61 a	$8.16\pm0.87~\mathrm{a}$
Control	7 ± 1 b	$1.92 \pm 0.67 \text{ c}$	5.77 ± 2.22 b	$3.94\pm0.51~b$
GTW1	11 ± 6 ab	5.68 ± 4.34 c	12.86 ± 6.18 b	5.50 ± 0.69 bc
GTW2	18 ± 3 a	15.37 ± 4.38 bc	21.51 ± 2.07 ab	$6.84\pm0.37ac$
GTW3	20 ± 2 a	29.23 ± 1.48 a	32.33 ± 0.34 a	7.66 ± 0.01 a

Mean values (\pm standard deviation) are shown in the table. One-way ANOVA followed by Tukey's test (confidence level = 0.95) was performed to determine the significant differences among groups. Data within columns without common indices are significantly different (p < 0.05).

TABLE 5 Leaf nutrient element content analysis of lettuce growing on the soil without simulated-solarization (NTS), with simulated-biosolarization (SBS) at 0.5% (w/w) green tea waste (GTW1), with simulated-SBS at 1.5% (w/w) GTW (GTW2), and with simulated-SBS at 2.5% (w/w) gGTW (GTW3).

	N	P	K		Ca		Na	Mg	
	g/kg	g/kg	g/k	g	g/kg		g/kg	g/kg	
NTS	23.750 ± 0.745 a	1.616 ± 0.012 a	24.478 ± 8	8.313 a	6.823 ± 1.754 a	7	.725 ± 1.321 a	2.342 ± 0.457 a	
GTW1	51.554 ± 7.631 b	1.107 ± 0.175 b	23.121 ± 0	0.053 a	N.A.		N.A.	N.A.	
GTW2	41.906 ± 2.259 bc	0.977 ± 0.026 b	25.389 ± 0	0.719 a	N.A.		N.A.	N.A.	
GTW3	27.231 ± 1.304 ac	1.043 ± 0.042 b	30.308 ±	1.597 a	7.173 ± 2.088 a	8	.312 ± 1.217 a	2.565 ± 0.960 a	
	Fe	Mn	Zn		Ni		Cu	Al	
	g/kg	g/kg	g/kg		mg/kg		mg/kg	g/kg	
NTS	0.870 ± 0.615 a	0.462 ± 0.170 a	0.157 ± 0.024 a		6.927 ± 4.605 a	9.3	301 ± 1.049 a	1.435 ± 1.019 a	
GTW3	0.259 ± 0.281 a	1.343 ± 0.576 a	0.181 ± 0.074 a		4.995 ± 2.216 a	4.995 ± 2.216 a 10		0.548 ± 0.268 a	
	В	Se	Ti		Cr		Co	As	
	mg/kg	mg/kg	mg/kg		mg/kg		mg/kg	mg/kg	
NTS	42.548 ± 8.655 a	0.672 ± 0.455 a	55.482 ± 16.	.946 a	14.176 ± 8.868 a	0	.925 ± 0.168 a	0.290 ± 0.158 a	
GTW3	44.206 ± 6.908 a	0.601 ± 0.590 a	33.648 ± 21.	.595 a	11.607 ± 5.533 a	1	.752 ± 0.514 a	0.231 ± 0.064 a	
	Sr	Мо	Cd		Sn		Sb	Ва	
	mg/kg	mg/kg	mg/kg		μg/kg		µg/kg	mg/kg	
NTS	34.865 ± 11.150 a	0.078 ± 0.018 a	1.479 ± 0.128	3 a	175.658 ± 25.219 a	29	.382 ± 25.674 a	7.091 ± 0.756 a	
GTW3	30.039 ± 13.543 a	0.024 ± 0.025 a	1.775 ± 0.812	2 a	124.788 ± 106.706 a	13	8.621 ± 4.062 a	$2.814\pm0.804~b$	
		Dh			V			ΤI	
		FU		V					
		mg/kg		mg/kg			μg/kg		
NTS		1.451 ± 0.59	6 a		1.919 ± 1.480 a		48.29	4 ± 14.213 a	
GTW3		0.598 ± 0.30	0 a		0.842 ± 0.302 a		11.6	37 ± 1.150 a	

N.A.: data not available; *p* value of Ba content is 0.03195.

Mean values (\pm standard deviation) are presented in the table and the unit shown is element content per leaf dry mass. For NPK content, one-way ANOVA followed by Tukey's test (confidence level = 0.95) was performed to determine the significant differences among groups. For other nutrient element content, *t*-test with unequal variance (confidence level = 0.95) was performed to determine the significant differences between groups. Data within columns without common indices are significantly different (p < 0.05).

(i.e., GTW). The N availability is worth investigating in order to determine the most efficient amount of application of GTW in SBS to optimize the plant growth. The highest P content was observed in NTS ($1.616 \pm 0.012 \text{ g/kg}$) and was significantly higher than in the other three groups (*p* value<0.05). The similar cultivar growth in the NTS and the GTW3 (Table 4) indicated that the total P content alone does not exert a significant effect on the lettuce growth in terms of leaf

number, edible mass, leaf area and leaf length. Unlike N and P, the K content did not vary significantly among the four treatments after the GTW addition during the simulated SBS. The control group was excluded from this analysis due to the insufficient biomass after drying.

Characterization of other nutrient elements was performed on NTS and GTW3 that possess comparable high edible mass. Among all the characterized elements, the significant difference was

observed only in Ba (p value = 0.03), whose content was over 3 times higher in the NTS $(7.091 \pm 0.756 \text{ mg/kg})$ than in the GTW3 (2.814±0.804 mg/kg) (Table 5). A low Ba concentration is beneficial to plant growth as an elevated Ba level may inhibit plant growth and render plants more prone to oxidative damage (Sleimi et al., 2021). Some trace elements, including Fe, Al, Mo, Sn, Sb, Pb, V and Tl, were more than twice higher in leaves of the NTS group than in the GTW3 group, despite the insignificant difference due to the large deviation among replicates. The lower element contents of Sb, Pb, V, and Tl would be beneficial to the cultivar yield and to consumer health (Bassuk, 1986; Gil et al., 1995; Pillay and Jonnalagadda, 2007; Evans and Barabash, 2010), while the low content of Fe may reduce the cultivar yield (Keat et al., 1999). This study envisioned that, among other methods such as microbial immobilization (Han et al., 2020), using 2.5% GTW during SBS could be a potential method to reduce certain heavy metal contents in lettuce. However, the results were not consistent among replications and thus a significant difference was not observed in these elements. More intensive sampling is needed to quantify better the element content.

From the post-harvesting analysis of soil nutrient element content (Supplementary Table S1), the samples in all the groups undergoing SBS showed higher N and P contents compared to the NTS and Control groups. The highest N was observed in GTW3. This residual N can be used for consecutive cropping rounds and promote crop growth with reduced dependence on fertilizers, thus leading to a more sustainable, environmentally safer and economically more efficient agricultural food production system (Ma et al., 1999; Grant et al., 2002). The residual total P content in the soil after cultivar harvesting was relatively high in biosolarized groups but the highest P leaf content was observed in NTS. We thus postulate that the addition of GTW during high-temperature-cycle SBS might limit P uptake into the lettuce. To have a better confirmation, the total P content in the soil after a simulated SBS should be analyzed. Similar to the lettuce K content, the soil residual K content did not vary significantly among groups, which indicated that the GTW addition may not alter the K profile in either the lettuce leaves or in the soil undergoing hightemperature-cycle SBS. The total C content was the lowest in the NTS group and increased with the amendment loading rate. Elements Na, Fe, Mn and Cu showed a higher level in the NTS and the Control groups compared to the amendment-loaded groups (especially GTW2 and GTW3). Elements Ca, Ni and Mo showed a significantly higher level in the Control group (p value<0.05), while Mg was significantly higher in the NTS group (p value<0.05). The significant difference in Zn content was observed between GTW1 (highest) and GTW2 (lowest) (p value<0.05). The GTW may promote efficient micronutrient transfer to the lettuce via biosolarized soil because the contents of certain elements (Na, Mg, Mn and Cu) were significantly lower in GTW3 post-harvesting soil but higher (though not significantly) in lettuce leaves.

Additionally, samples from the Control group were observed to wilt while GTW1, GTW2 and GTW3 persisted throughout the greenhouse incubation period. This phenomenon provided basis for the assumption that addition of the GTW could elongate the leaf longevity by providing essential nutrients from the amended soil. Therefore, it is worth profiling the soil nutrients' content directly after SBS treatment in order to find if nutrient deficiency could be remedied by the addition of GTW in an SBS process.

3.3.2. FW amendment group

Leaf analysis of the lettuce growing on the simulated biosolarized soil amended with 1% FW demonstrated a significant increase of all four growth indicators (all *p* values <0.03, Table 6). The leaf number increased from 17 ± 1 leaves per lettuce to 20 ± 1 leaves per lettuce. The edible mass and the leaf area reached 29.99 ± 1.37 g and 40.73 ± 2.17 cm² respectively, so both increased by a factor of approximately 2 compared to the non-treated soil (NTS). The average leaf length of the FW group increased by 2.47 cm. It met the expectation because fish residues are a rich source of protein, which is correlated with a high N content. Similarly, cultivar growth improvement by the addition of FW compost blended into non-solarized soil was previously observed (Radziemska et al., 2019). Although the soil used in this study was treated with SBS, which is believed to better improve cultivar growth via soil heating compared to a compost-only treatment (Achmon et al., 2017), the actual cultivar growth enhancement might have been diminished in this case by the properties of the original soil which was barren with many weeds growing. During the greenhouse incubation, the FW group lettuce demonstrated a darker green color and a harder leaf texture compared to the NTS group (Supplementary Figure S9). Such changes may influence the consumer choice and the commercial value of the lettuce.

Post-harvesting analysis of nutrient element content in lettuce leaves showed that the N (p value = 0.02) and P (p value < 0.05) content decreased significantly in the FW group. Their negative correlation with the leaf growth (Table 6) indicated that the reduction of the NP content in the lettuce leaves alone could not imply reduced cultivar growth. Significant percentage increases (p value <0.05) were observed in certain elements' content (Table 7). These increases ranged from 1096.8% (Na) to 42.8% (Fe) and the percentage increment was in the following descending order: Na > Ni > Cr > S > Cu > Co > Mg > As > Tl > Ba > V > Sr. > Ti > Zn > Fe. Themost significant difference was in the sodium content (20.166 ± 0.943 g/ kg), which was uncommon compared to another study results (Kleiber, 2014) and was possibly enhanced by the extremely high sodium content in the FW used in this work (from a mixed source of fish) than in others (Radziemska et al., 2019; Kandyliari et al., 2020). In our study, there was no evidence that the high sodium content limited cultivar growth (Coria-Cayupán et al., 2009) since the FW lettuce demonstrated improved growth, with the edible mass being doubled. On the contrary, the high sodium content may improve the lettuce salinity tolerance, which is important to the cultivar by promoting molecular and physiological parameters such as growth

TABLE 6 Leaf analysis of lettuce growing on the soil without simulating-solarization (NTS), and with 1% (w/w) fish waste (FW).

	Leaf	Edible mass	Leaf area	Leaf length	
	number	g	cm^2	cm	
NTS	17 ± 1	14.36 ± 0.30	23.75 ± 0.50	6.99 ± 0.02	
FW	20 ± 1	29.99 ± 1.37	40.73 ± 2.17	9.46 ± 0.17	
p value	0.0269	0.0014	0.0033	0.0013	
(if <0.05)	*	**	**	**	

***p<0.001, **p<0.01, *p<0.05.

Mean values (±standard deviation) are shown in the table. T-test (confidence level = 0.95) was performed to determine the significant differences between groups. Only p values smaller than 0.05 are presented.

TABLE 7 Leaf nutrient element content analysis of lettuce growing on the soil without simulating-solarization [non-treated soil (NTS)], and with 1% (w/w) fish waste (FW).

	Ν		Р	К	Ca		Na	Mg
	g/kg	g	/kg	g/kg	g/kg	n	ng/kg	g/kg
NTS	26.317 ± 1.096	3.989	0±0.189	17.624 ± 0.072	4.760 ± 0.005	1.6	85±0.232	1.340 ± 0.023
FW	11.386 ± 0.301	1.964	1 ± 0.041	20.662 ± 0.662	7.425 ± 0.467	20.1	66 ± 0.943	3.168 ± 0.176
<i>p</i> value (if <0.05)	0.0229	0.	0363				0.0163	0.0400
	*		*				*	*
	Fe		Mn	Zn	Ni		Cu	AL
	ma/ka		a/ka	ma/ka	ma/ka	r	na/ka	a/ka
NITS	575 802 + 20 224	0.1	97 Kg	1197 Kg	1 804+0 118	2.0	07+0.142	97 Kg
IN 15	5/5.892 ± 29.234	0.1	28 + 0.016	43.909 ± 2.240	0.378 + 0.504	3.5	170 + 0.400	0.848±0.031
FW (16 -0.05)	822.4/5±15.404	0.2	58±0.016	72.042±0.802	9.578±0.594	11.	0.0100	1.595±0.259
<i>p</i> value (if <0.05)	0.0261			0.0201	0.0296		0.0199	
	·**			*			~	
	В	S	e	Ti	Cr		Co	As
	μg/L	μς	ı/L	μg/L	mg/kg	n	ng/kg	mg/kg
NTS	7.696 ± 0.478	0.169	± 0.009	48.971 ± 2.199	5.519 ± 3.444	0.36	54 ± 0.027	0.171 ± 0.015
FW	7.915±0.018	0.254	4±0.069 81.098±3.090		22.686±2.338	0.87	70±0.018	0.404 ± 0.031
<i>p</i> value (if <0.05)				0.0092	0.0075	0.0038		0.0274
				**	**		**	*
	Sr	~	10	Cd	Sn		Sb	Ва
	mg/kg	μg	/kg	mg/kg	μg/kg	ŀ	ıg/kg	mg/kg
NTS	13.362 ± 1.361	124.13	0±3.819	0.758 ± 0.101	189.051 ± 26.455	24.9	952 ± 5.345	5.461 ± 0.576
FW	22.481 ± 0.669	126.14	7±1.055	0.997 ± 0.362	266.145 ± 26.246	32.8	376±5.107	10.886 ± 7.045
<i>p</i> value (if <0.05)	0.0324							0.0104
	*							*
	Pb			V	TI			S
	ma/ka			ma/ka	ua/ka	1		a/ka
NTS	1.132±0.067		1	.685±0.250	$\mu g / \kappa g$		0.	849±0.065
FW	1.961±0.229		3	0.082 ± 0.283	38.196±4.	172	2.	478 ± 0.118
<i>p</i> value (if <0.05)				0.0357	0.0349			0.0003
/				*	*		***	

***p<0.001, **p<0.01, *p<0.05.

Mean values (±standard deviation) are shown in the table and the unit is element content per leaf dry mass. *T*-test (confidence level = 0.95) was performed to determine the significant differences between groups. *p* values smaller than 0.05 are presented.

rate and nutrition uptake (Rajendran et al., 2009; Jouyban, 2012; Angelo et al., 2021). The high sodium content also explained the darker green color of FW group lettuce leaves observed in this study (Bartha et al., 2015). Additionally, the lettuce growing on soil amended with fish-waste and undergoing a high-temperature cycle might have a crisper texture and a saltier taste (Bartha et al., 2015) and its popularity among consumers might be evaluated in terms of organoleptic properties.

From the analysis of soil nutrient element content (Supplementary Table S2), the FW group assumed a significantly reduced content of Mg at 0.997 ± 0.030 g/kg (*p* value<0.05), which was

0.253 g/kg lower than that of the NTS group. The soil structure might be consequently influenced as magnesium carbonates were found to improve soil structure via the creation of carbonate coatings and soil particle binding (Huang et al., 2010). The Na content showed little difference between the groups. It indicated that Na from FW was transported to the lettuce without obvious accumulation in the soil (this is very important as high Na level can alter the soil characteristics and reduce its health toward plant growth). Soil treated by FW could be susceptible to heavy metal contamination, and a future study that will examine heavy metal content in post-harvesting soil should be conducted to investigate whether its application within high-temperature SBS may cause a significant accumulation of heavy metals in the soil.

In terms of heavy metals which are considered toxic when excessive intake occurs (Hokin et al., 2004; Ščančar et al., 2013), Cr and Fe content of the FW group exceeded the permissible threshold in plants while Zn, Ni and Co were still below the safety limit (Macnicol and Beckett, 1985; Mensah et al., 2009; Hu et al., 2021). Whether Cu content met the permissible value is arguable (Mensah et al., 2009; Osmani et al., 2015). High levels of heavy metal elements could be attributed to the heavy metal content remaining in the FW (gills, liver, scales, etc.) of the commercial fish products, which are more likely to be contaminated by heavy metals as indicated from previous studies (Darafsh et al., 2008; Lake et al., 2018; Huang et al., 2022).

4. Conclusion

This study evaluated the feasibility of using GTW and FW as amendments in simulated high-temperature SBS influence on the growth of lettuce (Lactuca sativa L. var. ramosa Hort.). The GTW waste amended soil undergoing SBS presented an elevated respiration profile and a distinct VOCs' evolution profile. Its phytocompatibility was restored after 7 days of residual phytotoxicity dissipation and a complete weed suppression was achieved. GTW applied at 0.5 and 1.5% (w/w) reduced the cultivar growth but the group with the 2.5% (w/w) loading rate reached a growth which was comparable to the NTS group, with a likely more efficient utilization of nitrogen and a continuous future cropping enabled by the higher amount of residual soil N content. Amending with 1% of FW (w/w) had a significant effect in promoting cultivar (Lactuca sativa L. var. ramosa Hort.) growth, despite the significant reduction in the N (p value = 0.02) and P (p value<0.05) contents in the cultivar leaves. Nutrient element analysis showed a high Na accumulation in the leaves. As a material which is prone to heavy metal contamination, FW elevated the content in leaves of Fe, Zn, Ni, Cu, Cr and Co while Fe and Cr even exceeded the permissible value.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

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Author contributions

YA, YZ, and AS conceived of the presented idea. YZ, BQ, WW, RG, FL, JX, and ZZ conducted the laboratory and greenhouse experiments. YA, YZ, BQ, and AS wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1174528/full#supplementary-material

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