

RESEARCH ARTICLE

# EFFECT OF SOLARIZATION TO KILL *BRADYSIA CELLARUM* ON CHINESE CHIVE GROWTH AND SOIL MICROBIAL DIVERSITY

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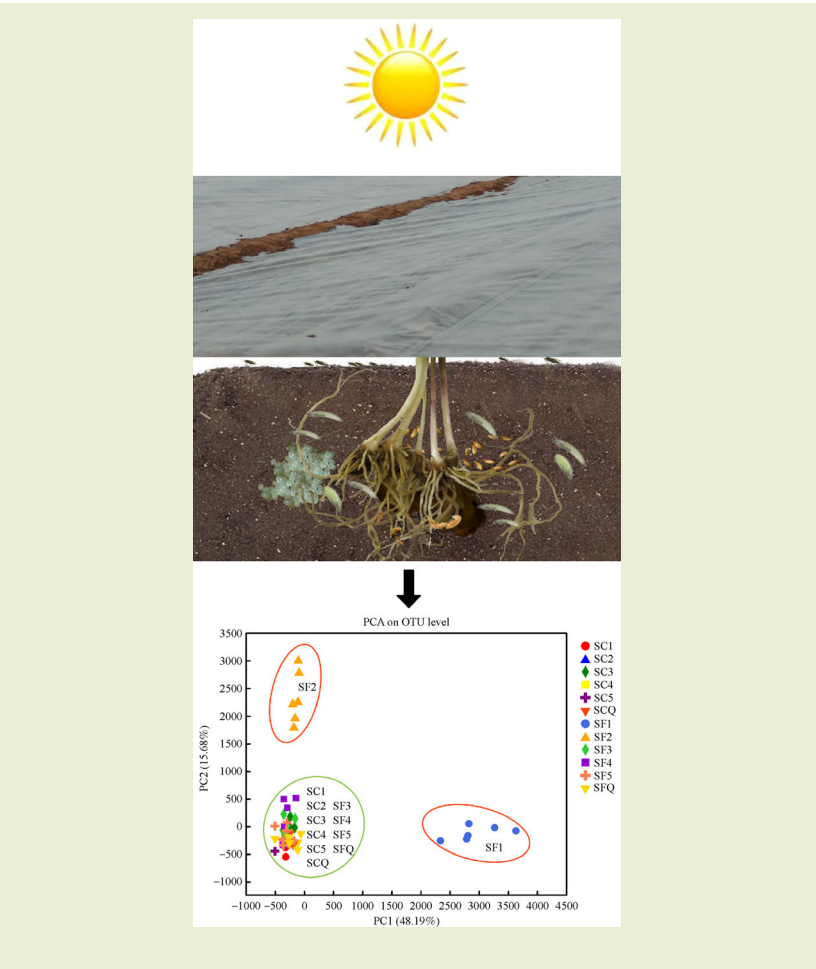
### KEYWORDS

*Bradysia cellarum*, Chinese chive, control, soil microbes, soil solarization

### HIGHLIGHTS

- Soil solarization achieved 100% control of *Bradysia cellarum*.
- The initial growth of Chinese chive was lower in solarized than control plots, but day 20 after treatment plants in the solarized had recovered and leaf height and yield were equivalent among the treatments.
- Soil microbial community diversity in the treatment group first decreased and then recovered gradually, and abundance of beneficial microorganisms increased significantly.

### GRAPHICAL ABSTRACT



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## ABSTRACT

*Bradysia cellarum* Frey (Diptera: Sciaridae) is an important subterranean pest and is especially damaging to Chinese chive. An effective and more environmentally safe method than pesticides is needed for its control. The efficacy of *B. cellarum* control, growth of Chinese chive and soil microbial diversity were investigated after use of soil solarization to exterminate this insect pest. The results show that on the first day after soil solarization 100% control of *B. cellarum* was achieved. Growth of Chinese chive was lower in solarized plots than in control plots over the first 10 days after treatment. Chive growth in solarized plots increased subsequently to match that in the control plots. Moreover, the soil microbial community diversity in the treatment group decreased initially before gradually recovering. In addition, the abundance of beneficial microorganisms in the genus *Bacillus* and the phyla Proteobacteria, Chloroflexi and Firmicutes increased significantly. Soil solarization is therefore practical and worthy of promotion in Chinese chive-growing regions.

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## 1 INTRODUCTION

Chinese chive (*Allium tuberosum* Rottler ex Sprengel) is a perennial herbaceous vegetable with medicinal qualities. It is generally grown in Malaysia, the Philippines, Vietnam, and other countries including China<sup>[1,2]</sup>. Unfortunately, Chinese chive crops, particularly those grown in northern or north-west China (such as in Hebei, Henan, Shanxi, and Shandong Provinces), are severely damaged by the soil insect *Bradysia cellarum* Frey<sup>[3]</sup>. *B. cellarum* (Diptera: Sciaridae) has a broad host range within seven families and 30 plant species including garlic, Welsh onion, radish, and melon<sup>[4,5]</sup>. Populations of *B. cellarum* are mainly found in humid and mild temperate soils, and in particular the surface soil to a depth of 5 cm<sup>[6]</sup>. This pest can reduce the yield of Chinese chive by about 50% in the absence of effective control<sup>[7]</sup>.

There are many methods used to control *B. cellarum* such as modifying crop cultivation patterns to stagger the peaks of pest populations<sup>[8]</sup>, deploying sticky black-colored traps to catch adults<sup>[9]</sup>, using sweet and sour liquids to lure adults<sup>[10]</sup>, and releasing entomopathogenic nematodes to attack larvae<sup>[11]</sup>. Unfortunately, each method has limitations including low efficacy, high cost, and environmental risks and this is why *B. cellarum* can still cause serious damage to Chinese chive in China. At present, application of pesticides to control *B. cellarum* remains the most common method used in Chinese chive production<sup>[12]</sup>. However, *B. cellarum* populations can become resistant to pesticides after long-term use of the same type of pesticide. Moreover, with a lack of better alternatives to control *B. cellarum*, farmers will increase pesticide application rates or apply a more toxic pesticide that is banned for use on

vegetable crops, thus contaminating crops and hampering the sustainable development of the Chinese chive industry<sup>[13]</sup>. Resolving this serious problem requires more research to find effective and safe methods to control *B. cellarum*.

Soil solarization has been used to control microbial, weed, and insect pests and Shi et al.<sup>[14]</sup> specifically demonstrated the effective control of *B. cellarum* by solarization. To thoroughly kill *B. cellarum* populations the method must be applied where there is sufficient intensity of sunlight (e.g., between late April and mid-September in Beijing, China). Also, Shi et al.<sup>[14]</sup> determined that light blue anti-dropping film (LBADF) of 0.10 or 0.12 mm thickness was more effective than other types of film. However, it is not known whether soil solarization affects soil microbial diversity. If soil solarization can kill *B. cellarum* and also avoid affecting Chinese chive growth and the soil microbial ecological balance, it will be an environmentally friendly control technology. Here, *B. cellarum* was controlled by soil solarization and the influence of soil solarization on Chinese chive growth and soil microbial diversity in the chive crop soil investigated. This study provides further understanding of the effects of soil solarization on soil microbial ecology and scientific data to support management strategies of crop pest control.

## 2 MATERIALS AND METHODS

### 2.1 Experimental site

The experiment was conducted on Yang Town farm in Shunyi (40°1'N, 116°6'E), Beijing, China. Chinese chive cv. Pingjiu No. 1 was grown in 0.2-m rows at a density of about 5 million plants

ha<sup>-1</sup>. The soil type was silt loam with a ratio of 40:40:18:2 sand, silt, clay and organic matter. The estimated densities of *B. cellarum* larvae inhabiting the soil ranged from 400 to 2000 individuals m<sup>-2</sup>. No insecticides were used in the experimental field in the previous year.

## 2.2 Treatment application

The experiment was conducted from May to July 2018. Three treatments were applied, soil solarization and untreated control. Soil solarization was applied on a day with full sunlight. Plant leaves were cut prior to application of LBADF (0.1 mm thick) to cover the soil surface for 10 h from 8:00 to 18:00. Each plot was 6.5 m × 20 m. The mean numbers of *B. cellarum* larvae from five arbitrarily-selected soil samples (20 cm × 20 cm × 10 cm) per plot were recorded just before applying the treatments (day 0) and on days 1, 5, 10, 15 and 20 after treatment. In addition, automatic temperature meter probes (ZigWSN-C-A, Beijing, China) were inserted 5 cm into the soil in each plot but were covered with the film only in test one. Soil temperatures were recorded every 15 min during the treatment period. There were three replicate plots of each treatment.

## 2.3 Growth measurements

Leaf height of 60 arbitrarily-selected chive plants were measured and used to calculate mean leaf height per plant per plot. Leaf-height sampling was conducted on days 5, 10, 15 and 20 after treatment. The mean yield of Chinese chives was determined by taking the average plant yield from five arbitrarily-selected subplots (0.6 m × 0.2 m) within each plot on day 20 after treatment.

## 2.4 Sampling and determination of soil microbial diversity

### 2.4.1 Soil sampling and preparation

Soil cores (5 cm × 5 cm) were collected from each of the nine plots with a 5-cm diameter spiral sampler on the same days that larvae were sampled. Six soil cores were initially sampled from each of the plots. Instead of homogenizing all six samples for each plot we separately took one arbitrarily-selected soil core from the three replicate plots of each treatment and homogenized the samples as a new replicate in order to reduce the variation in the microbial community among replicate plots of the same treatment. We systematically repeated this process to give six replicate samples of each treatment. These soil samples were sieved to obtain only soil particles < 2 mm, cleared of any root material, and stored at -20 °C.

### 2.4.2 DNA extraction, amplification and sequencing

DNA extraction, amplification and sequencing were conducted by Allwegene, Beijing, China. We took a 1-g subsample from each soil sample to extract total DNA using a Fast DNA Spin Kit for Soil (MP Biomedicals, Solon, USA). To measure the concentration of total DNA we used a NanoDrop spectrophotometer 2000 (Thermo Scientific, Waltham, USA). Bacterial 16S rRNA genes were PCR-amplified (GeneAmp 9700, Applied Biosystems, Foster City, USA) in a 25 µL reaction composed of 12.5 µL KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, USA), 2.5 µL DNA template (5 ng · µL<sup>-1</sup>), 1 µL of each primer (F338: 5'-ACTCCTACGGGAGG CAGCAG-3' and R806: 5'-GGACTACHVGGG TWTCTAAT-3')<sup>[15]</sup> and 8 µL RNase-free water. The PCR amplification procedure consisted of: initial denaturation at 95 °C for 3 min, 27 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and final elongation at 72 °C for 5 min. A PCR reaction without DNA template was conducted as a negative control. The amplification products were run on 1.0% agarose gels and purified. The purified products were diluted to 20 nmol · L<sup>-1</sup>, mixed in equimolar proportions, and paired-end sequenced on the Illumina MiSeq platform (Illumina, San Diego, USA).

## 2.5 Data and bioinformatics analysis

Pest control efficiency (*CE*) was calculated based on the formula described by Shi et al.<sup>[14]</sup>.

$$CE = \left(1 - \frac{Nbc \times Nat}{Nac \times Nbt}\right) \times 100$$

where *Nbc* is the number of larvae before treatment in the control area, *Nat* is the number of larvae after treatment in the treated area, *Nac* is the number of larvae after treatment in the control area, and *Nbt* is the number of larvae before treatment in the treated area.

SPSS version 17.0 for Windows (IBM, Armonk, NY) was used for statistical analysis. We used Tukey's test to analyze the data described in Section 2.3. A significance level of *P* < 0.05 was used. Values are expressed as mean ± SD.

Primers, adapters, low quality reads and redundant tags were trimmed or deleted from microbial DNA sequences to obtain unique tags. The unique tags were aligned against the 16S rRNA V3-V4 database using the BLASTN algorithm. Operational taxonomic units (OTUs) with a 97% cutoff in similarity were clustered using UPARSE ver. 7.1<sup>[16]</sup> and chimeric sequences were identified and removed using UCHIME ver. 3.0.617<sup>[17]</sup>. Rarefaction analysis (Mothur v. 1.30.1) was conducted to investigate microbial diversity using species richness (Chao),

evenness (ACE) and diversity (Shannon) indices<sup>[18,19]</sup>. Differentially abundant OTUs were found between solarization and the controls identified using linear discriminant analysis effect size (LDA-LEFSE) analysis. The LDA-LEFSE analysis was coupled with the microbiota community structure to search for statistically different biomarkers between the solarization and control groups using the Kruskal–Wallis test ( $P < 0.05$ ) and an LDA score threshold of  $> 4.0$ <sup>[20]</sup>. The larger the LDA score the greater the differences in taxonomic abundances of the groups compared.

### 3 RESULTS

#### 3.1 Pest control efficiency by soil solarization

The control efficiency of *B. cellarum* by solarization was 100% on the first day after treatment (DAT). The maximum soil temperature was 48.7 °C on the test day. The time for which the soil temperature was  $> 40$  °C was 8.5 h on the test day.

#### 3.2 Effect of soil solarization on the growth of Chinese chive

The growth of Chinese chive was lower in solarized plots than in control plots for the first 10 DAT but subsequently was faster in

the solarized plots (Table 1). At day 5 after treatment, the mean leaf heights in the controls were 9.8 cm, significantly higher than with solarization (5.3 cm). By day 10 after treatment, the leaf heights in the controls were 16.6 cm, significantly higher than with solarization (10.6 cm). However, by days 15 and 20 after treatment, there were no significant differences in leaf heights between the two treatments ( $P > 0.05$ , Table 1).

#### 3.3 Effect of soil solarization on soil microbial diversity

##### 3.3.1 Microbial alpha diversity

A total of 4,759,435 reads with a mean length of 440 bp were obtained in the solarization and control treatments through the MiSeq sequencing analysis. The alpha diversity with solarization, based on Chao, ACE, and Shannon indices, initially decreased and then gradually increased compared with the controls. The lowest diversity was observed in the soil one day after solarization (Table 2).

##### 3.3.2 Microbial beta diversity

Principal component analysis shows that the first and second components (PC1 and PC2) accounted for 48.2% and 15.7% of

**Table 1** Effect of soil solarization on height and yield of Chinese chive on days 5, 10, 15 and 20 after treatment

Treatment	Height of Chinese chive after treatment (cm)				Yield at day 20 (g)
	Day 5	Day 10	Day 15	Day 20	
Solarization	5.3±0.8 b	10.6±1.7 b	27.4±2.2 a	35.3±2.8 a	375.8±39.4 a
Control	9.8±1.3 a	16.6±1.6 a	29.2±1.4 a	33.2±3.7 a	347.1±36.5 a

Note: Values are (means±SD) of three replicate plots. Mean leaf height was determined by measuring the leaves of 60 arbitrarily-selected Chinese chive plants for each plot. Mean yield was determined by averaging yield measured from five arbitrarily-selected subplots (0.6 m × 0.2 m) within each plot. Means followed by the same letter within a column are not significantly different according to Tukey's test ( $P < 0.05$ ).

**Table 2** Analysis of microbial alpha diversity indices

Sample	Shannon		Ace		Chao	
	C	F	C	F	C	F
SQ	7.31	7.31	8190.43	8180.50	8204.23	8226.22
S1	7.27	6.90	8113.53	7874.91	8197.81	7985.25
S2	7.33	7.15	8060.24	7841.15	8105.82	7886.50
S3	7.24	7.22	7785.04	7559.13	7808.12	7604.25
S4	7.34	7.33	8184.54	8125.65	8214.36	8158.71
S5	7.27	7.22	8120.60	8088.59	8212.83	8144.34

Note: C and F indicate control and solarization treatments, and SQ, S1 to S5 indicate sampling days: SQ sampling on day 0 (just before treatment application) and S1 to S5 sampling on days 1, 5, 10, 15 and 20 after treatment.

the total variance in the bacterial communities of the 72 soil samples (Fig. 1). All bacterial communities overlapped each other except for two distinct bacterial communities obtained from the soil samples one and five days after solarization (Fig. 1).

### 3.4 Effect of solarization on soil bacterial species

#### 3.4.1 Differences in bacterial species within groups

The distribution of phyla of the OTUs shows that the clean sequence reads were classified in Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, Gemmatimonadetes, Nitrospirae, Planctomycetes, Saccharibacteria, Latescibacteria and Cyanobacteria. Members unassigned to a phylum (others) were likely not true bacteria (Fig. 2). The abundance of Firmicutes (23.8%) one day after solarization was significantly higher than the abundance of Firmicutes in other soil samples.

The distribution of classes of the OTUs shows that the clean sequence reads were classified in Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Gemmatimonadetes, Anaerolineae, Sphingobacteriia, Nitrospira, Bacilli, Thermomicrobia, Clostridia, Chloroflexi and Cytophagia. The abundance of Clostridia (14.9%) one day after solarization was significantly higher than that in other soil samples, and the abundance of Gammapro-

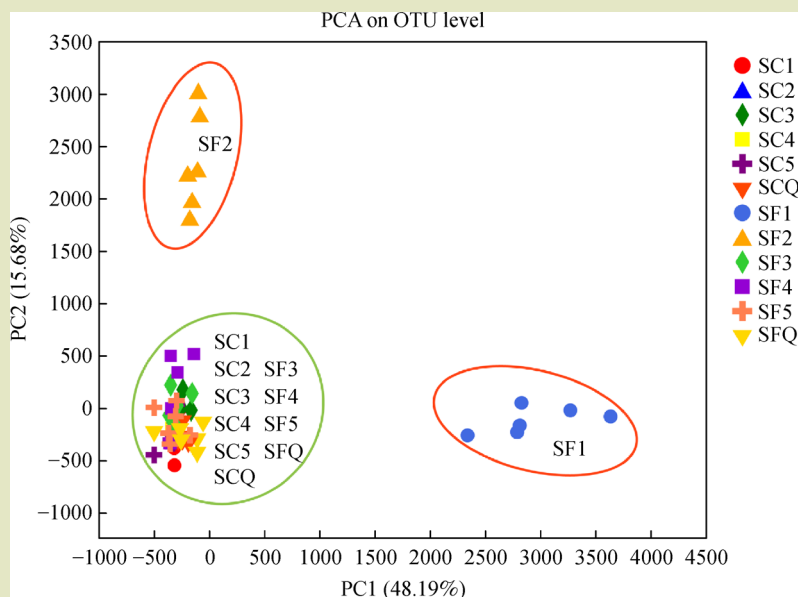
teobacteria (11.0%) five days after solarization was significantly higher than that in other soil samples (Fig. 3).

The distribution of orders of the OTUs shows that the clean sequence reads were classified in Rhizobiales, Nitrosomonadales, Anaerolineales and Sphingobacteriales. The abundances of Clostridiales (14.81%) and Bacillales (8.23%) one day after solarization were significantly higher than those in other soil samples, and the abundance of Pseudomonadales (5.14%) five days after solarization was significantly higher than that in other soil samples (Fig. 4).

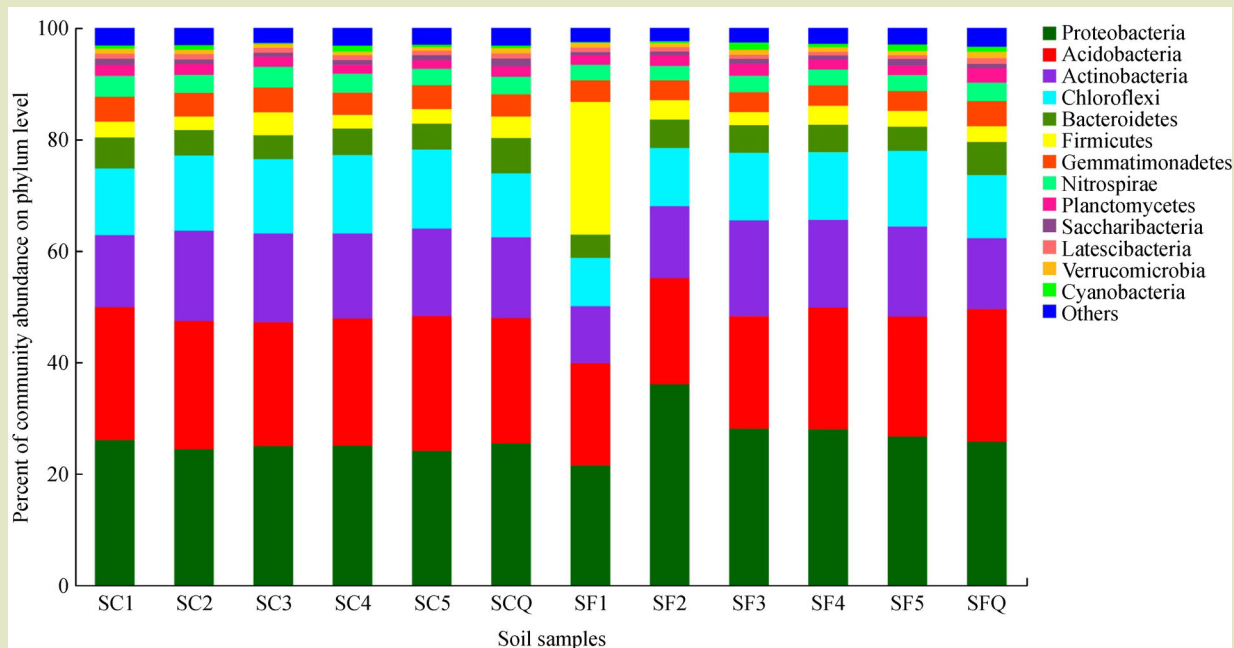
The distribution of families of the OTUs shows that the clean sequence reads were classified in Nitrosomonadaceae, Anaerolineaceae, Gemmatimonadaceae, Paenibacillaceae and Clostridiaceae. The abundance of Clostridiaceae (12.25%) in SF1 was significantly higher than that in other soil samples, and the abundance of Pseudomonadaceae (6.13%) five days after solarization was significantly higher than that in other soil samples (Fig. 5).

#### 3.4.2 Differences in bacterial species between groups

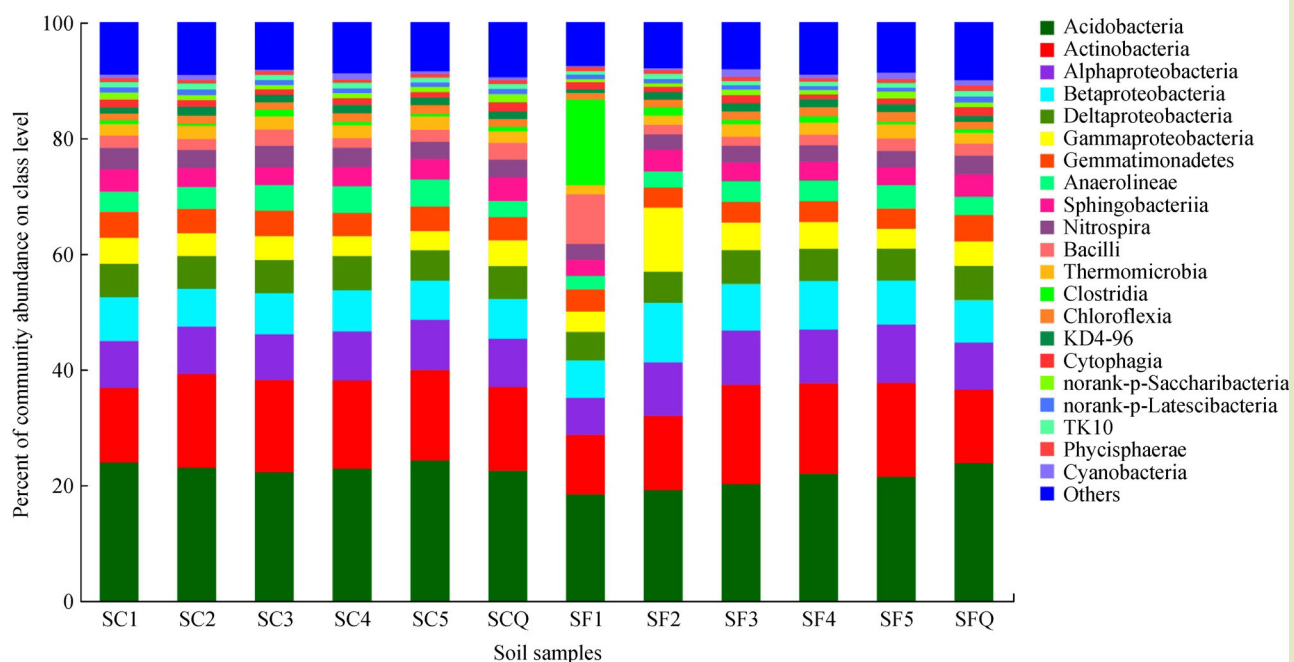
LDA-LEFSE analysis between solarization and the controls indicates that there were 1 enriched taxa prior to treatment application in the control, and 12, 9, 2, 1, 2 enriched taxa on days 1, 5, 10, 15, 20 after treatment with solarization, respectively,



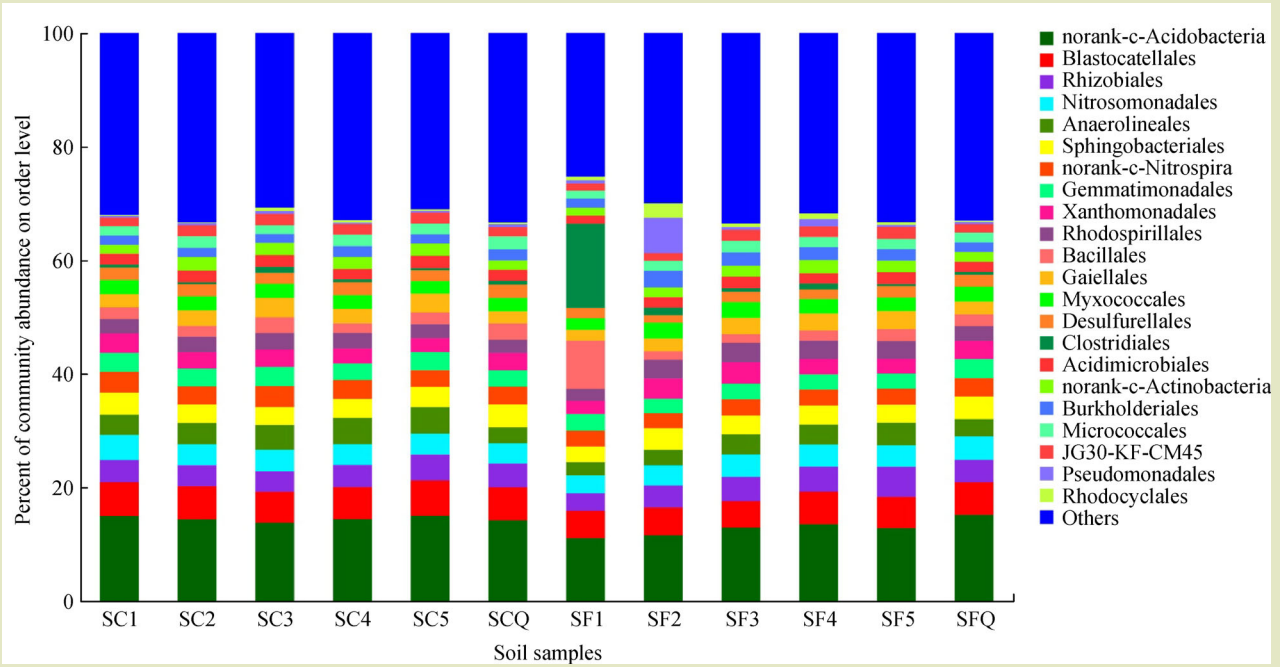
**Fig. 1** Principal component analysis (PC) of bacterial communities. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1–5 for sampling on days 1, 5, 10, 15 and 20 after treatment. The six replicates of each treatment group are indicated by hyphenated numerical suffixes.



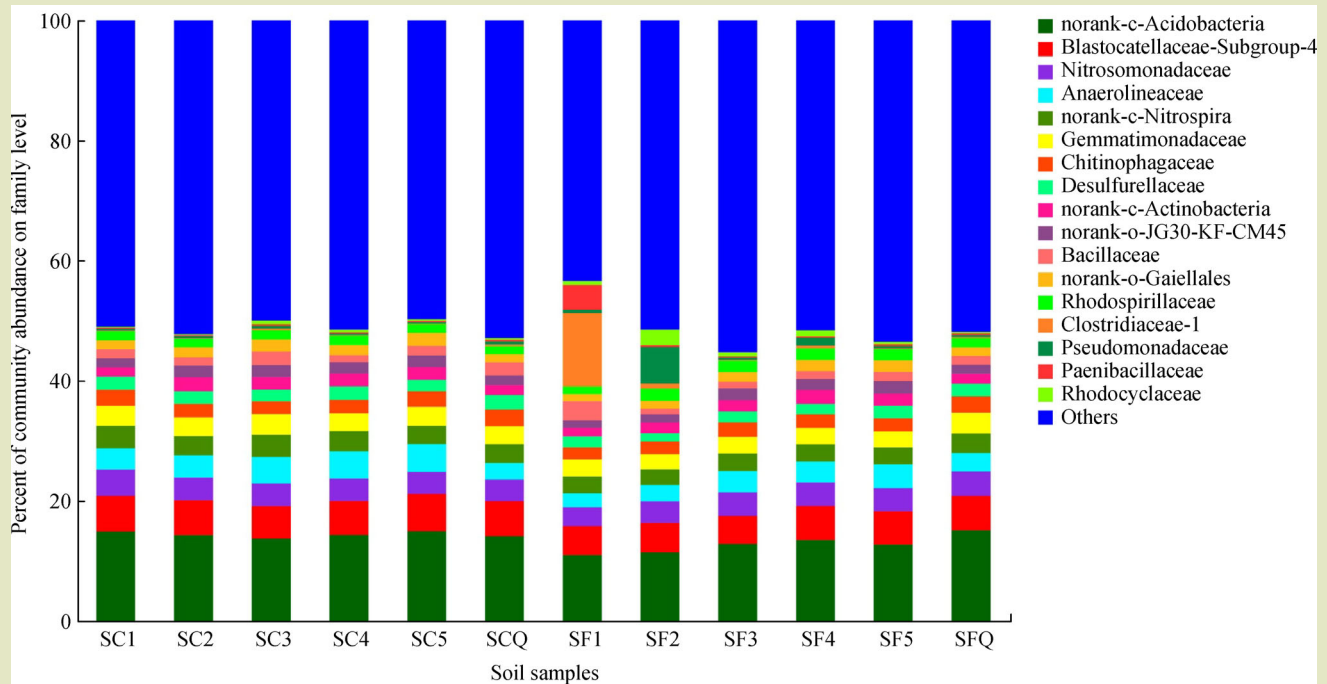
**Fig. 2** The relative abundances of operational taxonomic units classified at the phylum level. Abundances are percentages of the total amount of effective bacterial sequences in each sample. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1 to 5 for sampling on days 1, 5, 10, 15 and 20 after treatment.



**Fig. 3** The relative abundances of operational taxonomic units classified at the class level. Abundances are percentages of the total amount of effective bacterial sequences in each sample. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1 to 5 for sampling on days 1, 5, 10, 15 and 20 after treatment.



**Fig. 4** The relative abundances of operational taxonomic units classified at the order level. Abundances are percentages of the total amount of effective bacterial sequences in each sample. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1 to 5 for sampling on days 1, 5, 10, 15 and 20 after treatment.



**Fig. 5** The relative abundances of operational taxonomic units classified at the family level. Abundances are percentages of the total amount of effective bacterial sequences in each sample. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1 to 5 for sampling on days 1, 5, 10, 15 and 20 after treatment.

including the taxa Chloroflexi, Bacteroidetes, Firmicutes, Clostridiales, Bacillales, Bacilli, Proteobacteria, and Betaproteobacteria, etc. (Fig. 6).

### 4 DISCUSSION

Studies show that heat can be used to control pest populations and different pest species have different lethal temperatures<sup>[21]</sup>. For example, control of *Trogoderma variabile* was achieved when this pest was exposed to 56 °C for 6 min<sup>[22]</sup>. Exposure to 65 °C for 10 min can completely kill all *Leptinotarsa decemlineata* larvae<sup>[23]</sup>. Thermal treatment of *B. cellarum* adults, eggs, larvae, and pupae at 40 °C for 3 h gave mortalities of 100%, 100%, 100% and 81%, respectively<sup>[14]</sup>, suggesting that *B. cellarum* is quite sensitive to elevated temperatures. Another study shows that the optimal developmental temperature for

*B. cellarum* ranges from 20 to 25 °C, and survival declines as temperatures rise above that range<sup>[7]</sup>. Cheng et al<sup>[24]</sup> show that the fecundity of *B. cellarum* significantly decreased with increasing temperature and exposure time, completely inhibiting egg-laying at 37 °C for 2 h. These data are consistent with our results which further indicate that *B. cellarum* infestations can be controlled by thermal treatment.

Given that *B. cellarum* has multiple life stages (larvae, pupae and eggs) in the top 5 cm of the soil profile and the adults also spend time near the soil surface<sup>[6]</sup>, raising the temperature of the soil to 5 cm depth to a lethal level is a plausible strategy to control this pest. Our results show that the solarization applied by heating *B. cellarum*-inhabited soil with a 0.10-mm-thick LBADF on a sunny day achieved a maximum temperature of 48.7 °C, and temperatures exceeded 40 °C for 8.5 h. More importantly, the

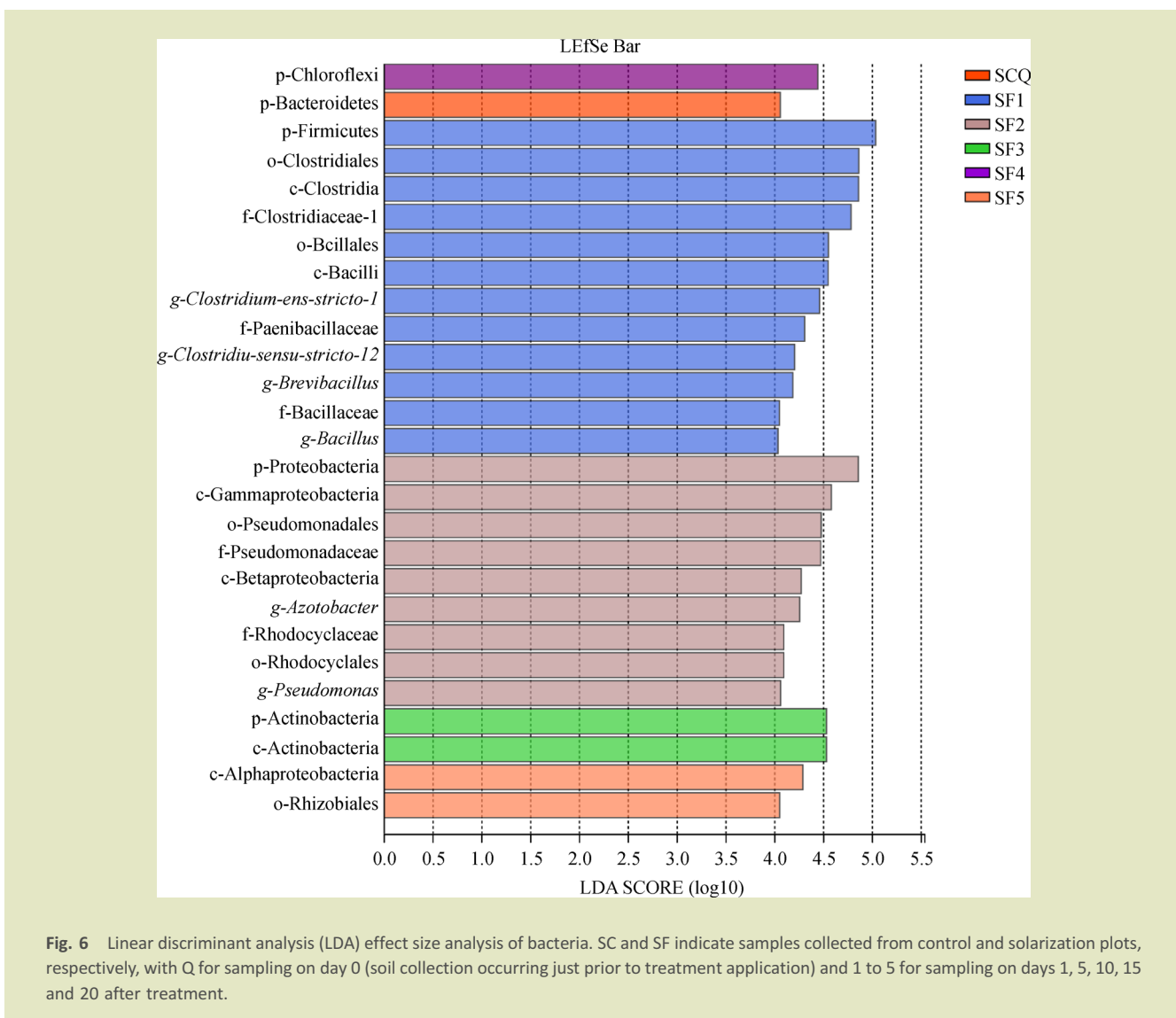


Fig. 6 Linear discriminant analysis (LDA) effect size analysis of bacteria. SC and SF indicate samples collected from control and solarization plots, respectively, with Q for sampling on day 0 (soil collection occurring just prior to treatment application) and 1 to 5 for sampling on days 1, 5, 10, 15 and 20 after treatment.



solarization treatment achieved 100% control of *B. cellarum*, supporting this pest control strategy. Our results also show that the growth of Chinese chive was significantly lower in solarized plots than in control plots during the initial 10 DAT. However, by day 20 after treatment, the mean leaf height and yield were equivalent to the control treatment. These results indicate that the growth of Chinese chive is suppressed by soil solarization but this suppression does not last. Chive growth in solarized soil eventually caught up to that of the control. If growth was monitored beyond 20 days, chive growth in the heat-treated plots might surpass that in the untreated plots.

The positive recovery of plant growth from day 10 after solarization may be caused by control of the targeted pest population or other positive side-effects of solarization. Similar studies have indicated that heated soil apparently stimulates a phenomenon reported as an increased growth response of plants<sup>[25,26]</sup>. For example, soil solarization increased the yields of *Arachis hypogaea*, *Solanum tuberosum* and *S. melongena* by 123%, 35% and 215%, respectively<sup>[27]</sup>. Heated soil can also benefit plant growth by increasing micro- and macro-elements in the soil solution, eliminating minor plant pathogens and stimulating the activity of mycorrhizae or other microorganisms beneficial to plants<sup>[27,28]</sup>. For example, solarization has been used to heat soils prior to planting in order to eliminate soil-borne pathogens and weeds<sup>[29–31]</sup>. In addition, numerous studies have shown that soil solarization can decompose organic matter and enhance the quantities of soluble nutrients such as nitrogen, potassium, magnesium and calcium<sup>[32,33]</sup>. In *Abelmoschus esculentus*, for example, soil solarization increased the contents of magnesium, potassium, nitrogen and carbon in the leaf tissues<sup>[34]</sup>. However, these studies do not explain why we observed an initial suppression of growth followed by a recovery

in Chinese chive in response to solarization. Further investigation is needed to elucidate the underlying cause of this shift from suppression to recovery of plant growth.

Whether or not soil solarization altered soil microbial diversity was investigated. Our results show that alpha diversity of the solarization soil, based on Chao, ACE, and Shannon indices, initially decreased and then gradually increased over time until there was no significant difference from the untreated soil. These observations suggest that solarization will not destroy the microbial ecological balance in the long-term. However, several studies have shown that soil solarization can greatly affect microbial species and soil structure<sup>[35]</sup>. Our research shows that all bacterial communities overlapped except for communities in the soil one and five days after solarization, indicating that solarization is likely to have killed some microorganisms that could not tolerate high temperatures. However, the abundances of beneficial microorganisms one and five days after solarization, such as members of the taxonomic groups *Bacillus*, Proteobacteria, Chloroflexi and Firmicutes, increased significantly. The potential mechanism or microbial function that may be associated with plant growth needs further study by examining the soil metagenome.

## 5 CONCLUSIONS

Soil solarization is a promising strategy to control *B. cellarum*. It is simple to implement, pesticide-free and non-destructive to soil microbial diversity, and it may also promote the abundance of beneficial microorganisms. Soil solarization is practical and worth promoting as a new method of control of *B. cellarum* infestations in Chinese chive-growing regions.

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### Compliance with ethics guidelines

Caihua Shi, Linlin Shi, Qingjun Wu, Shaoli Wang, Baoyun Xu, and Youjun Zhang declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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