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# Efficiency of different solarization-based ecological soil treatments on the control of Fusarium wilt and their impacts on the soil microbial community

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ABSTRACT

Fusarium wilt is considered one of the most destructive soil-borne diseases. Solarization-based soil treatments for the control of Fusarium wilt are considered ecological and are widely used. In this study, differences in the efficiency of Fusarium wilt control and the impact on the soil microbial community were investigated among three types of solarization-based soil treatments, including soil solarization (SS), soil solarization with flooding (SS-F), and soil solarization with organic amendment and flooding (SS-OA-F). The SS and SS-F treatments partially killed the *Fusarium oxysporum* over the first 6 days. However, these treatments had no or little effect on the deteriorated soil environment and soil microbial community. The remaining *Fusarium oxysporum* proliferated quickly after cucumber planting, and serious Fusarium wilt still occurred in the SS and SS-F treatments at the second planting. SS-OA-F significantly improved the efficiency of Fusarium wilt control. Soil *Fusarium oxysporum* were killed in greater numbers in a shorter time by SS-OA-F. The deteriorated soil environment was remediated over the 15-day treatment process due to increases in the acidified soil pH and reductions of the soil electrical conductivity. SS-OA-F also had a notably impact on the soil microbial community. The proportion of anaerobic microorganisms greatly increased, and that of aerobic microorganisms greatly decreased in SS-OA-F soil. The proliferation of soil *Fusarium oxysporum* was significantly inhibited after the SS-OA-F treatment. However, different organic matter types used in SS-OA-F resulted in different suppression durations. Compared with glucose, *Medicago sativa* amendment increased the soil bacterial diversity and prolonged the suppression duration.



## 1. Introduction

Fusarium wilt of cucumber is caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *Cucumberinum* (Cao et al., 2011). This disease was initially recorded in Crete, Greece, in 1989 and is now observed in many countries (Pavlou and Vakalounakis, 2005). Currently, Fusarium wilt is one of the most destructive diseases of greenhouse-grown crops and causes significant yield losses (Chen et al., 2010; Zhao et al., 2012). In China, this disease was initially reported in 2000 (Vakalounakis et al., 2004). Recently, many areas in China have been suffering from significant Fusarium wilt in crop outbreaks due to the increasing reliance of greenhouse crop cultures (Li et al., 2012).

Until now, the most common method to control Fusarium wilt has been soil disinfection with chemical disinfectants (Mao et al., 2012; Meszk and Malusà, 2014; Shi et al., 2009). However, the use of these disinfectants has generated considerable recent concern regarding food safety and environment pollution because of their high toxicity (Mao et al., 2012), although some new soil fumigants are said to be minimally toxic (Qiao et al., 2012; Wang et al., 2013). With the development of biological technology, many researchers

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have focused on ecologically controlling this disease using antagonistic microorganisms (Chen et al., 2011; Li et al., 2012; Shen et al., 2015a,b; Zhao et al., 2012). Successful suppression of soil pathogens and reduce of Fusarium wilt had been reported in some cases (Qiu et al., 2012; Zhang et al., 2008). However, the widespread application of these antagonistic microorganisms in the field remains challenging because these biocontrol agents cannot change the deteriorated soil environment, and the microorganisms often cannot colonize the soil (Alabouvette et al., 2009; Wei et al., 2011). Acidification and salinization of greenhouse soil are serious issues that favor the proliferation of soil pathogens (Triky-Dotan et al., 2005; Yao et al., 2015). The soil pH in most of southern China is less than 6.0 due to excessive chemical fertilizer application. Determining an alternative effective and ecological method to control Fusarium wilt is necessary.

Soil solarization is a non-chemical method of soil disinfection that results in pathogen control and nematode, insect, and weed management (Bonanomi et al., 2008;). Solarization is currently used all over the world. However, soil solarization is a climate-dependent process, typically lasting more than 4 weeks (Gilardi et al., 2014). This long duration decreases compatibility with intensive agricultural systems. For soil solarization to become a more widely adopted method, it is always combined with other methods, such as organic amendments and flooding (Ashworth et al., 2013; Gilardi et al., 2014; Klein et al., 2012). Soil solarization combined with organic amendments (SS-OA) shows great potential for controlling soil-borne pathogens and is considered one of the most effective methods because it is ecologically friendly and is associated with low costs (Gilardi et al., 2014). Soil solarization combined with flooding (SS-F) is commonly used in greenhouses in south China to control Fusarium wilt. SS-F is easily performed and efficiently reduces the incidence of Fusarium wilt in cucumber for that growing season. However, this disease can easily occur in the subsequent year, a process that, until recently, few reports have explained (Matheron and Porchas, 2010; Mauromicale et al., 2010). Finally, the combination of soil solarization with organic amendments and flooding (SS-OA-F) has been implemented in recent years in some area, but the differences in the suppression of Fusarium wilt among SS, SS-F, and SS-OA-F treatments have not been well demonstrated. Their impacts on soil microbial community were rarely investigated. Therefore, the durative effects of Fusarium wilt suppression using these techniques are unclear.

In this study, the efficiency of these three types of solarization-based ecological soil treatments for the control of Fusarium wilt, remediation of a deteriorated soil environment, and their impacts on the soil microbial community were investigated and compared in pot experiments. Soil pH, conductivity, and the number of pathogenic *Fusarium oxysporum* were measured during the process. Changes in the soil microbial community were analyzed using 454 high-throughput sequencing. The suppression durations of different methods on the control of cucumber Fusarium wilt were evaluated by twice-continuous cropping.

## 2. Materials and methods

### 2.1. Soil

We sampled the 0–20 cm layer of a pathogen-infested soil that had been planted with cucumber continuously for three years in a greenhouse at an agricultural cooperative in Jiaxing, Zhejiang Province, China. Serious Fusarium wilt infestation of cucumber occurred before sampling. Nearly 80% of cucumbers suffered from Fusarium wilt just before fruition, which resulted in the death of the plants and no harvest of cucumber. The sampled soil was passed through a 2-mm mesh sieve and totally mixed. The soil was purple clay, and the soil properties included 17.8% water content, 12.02 g/kg organic C, 1.27 g/kg total N, 4.98 pH (1:2.5 soil:$H_2O$), and 124.63 mg/kg available phosphorous.

### 2.2. Solarization-based soil treatment experiments

The soil treatment pot experiments were carried out in a greenhouse. Three types of solarization-based soil treatments were performed including soil solarization (SS; let the soil stand still without any treatment), soil solarization combined with flooding (SS-F), and soil solarization combined with organic amendments and flooding (SS-OA-F). Two types of organic matter were selected as the organic amendment agents used in SS-OA-F treatments according our previous study: *Medicago sativa* (SS-OA (M)-F) and glucose (SS-OA(G)-F). Glucose was considered a single carbon source, and *Medicago sativa*, a complex carbon source. *Medicago sativa* was directly purchased as commercial forage grass from Zhejiang Mufeng Agriculture Science and Technology Development Co., LTD, Hangzhou, China. The organic matter content and water content of Medicago sativa was 402.61 g/kg and 5.4%, respectively. The dried Medicago sativa was crushed into a powder before the experiment. Glucose of analytical grade was purchased from Sangon Biotech (Shanghai) Co.,Ltd, China. Pots with a 30-cm diameter and height were used in this study with holes in the bottom covered with plastic film to prevent water run-off during the experiment. Six kilograms of prepared soil as mentioned above were added into each pots, The *Medicago sativa* powder and glucose were mixed with the soil at a concentration of 10 g/kg soil in SS-OA(M)-F and SS-OA(G)-F pots, respectively. Then tap water was added to all treatments until the water was 3 cm higher than the soil surface except SS. The soil treatment experiment lasted for 15 days. During the 15-day treatment, the water remained 3 cm higher than the soil surface in all flooding-treatments. Each pot was considered a replicate, and each treatment consisted of 5 replicates. The experiment was performed from July 15 to July 30, 2012. The mean minimum air temperature and maximum air temperature during the experiment were 26 °C and 36 °C, respectively. The air temperature in greenhouse during the experiment ranged from 30 to 50 °C, with an average of approximately 45 °C during the day. Soil samples were collected every 3 days after the beginning of the experiment. The samples were divided into three portions: one portion was used to count *Fusarium oxysporum*, another was naturally dried to analyze soil character, and the third was stored at −80 °C for molecular analysis of the microbial community.

### 2.3. Cucumber planting

Following soil treatment, the water in the flooding-treatment pots was drained. These pots were then used for planting. Two continuous seasons of cucumber planting were performed from August to November in 2012 and from April to July in 2013. Cucumber seeds (Zhexiu 302, produced by Zhejiang Wuwangnong Seeds Shareholding Co. Ltd., Zhejiang, China)were initially sown in seedling plug trays (plug size: 3.4 × 3.4 × 5 cm; 50 plugs per tray). The cucumber seeds were homogeneously mixed to minimized the effect potentially caused by these seeds before seeding, since they could have introduced *Fusarium oxysporum* theoretically. Two weeks after emergence, the seedlings were removed from the trays, and three seedlings were planted in each pot. Disease was monitored and quantified as the total percentage of plants showing symptoms of Fusarium wilt, which included yellowing and wilting of the leaves, followed by general chlorosis and complete wilting. Soil samples were collected at an interval of 15 d at the first planting and at the initial and end of second planting for counting of *Fusarium oxysporum* in soil.

### 2.4. Analysis of soil characteristics

Soil pH and conductivity were measured using a pH and conductivity meter (Mp521 Lab pH/conductivity meter, Shanghai Sanxin Meter Factory, Shanghai, China). We weighed 10 g of soil and placed it into a beaker with 25 mL of distilled water. After mixing, the soil was allowed to rest for 30 min, and then the pH and conductivity were measured. Soil oxidation-reduction potential was detected using a CD-1 platinum composite electrode (Nanjing Chuandi Equipment Corporation, Nanjing, China).

### 2.5. Abundance of *cultivable* Fusarium oxysporum

The total *Fusarium oxysporum* population was determined. On each sampling date, 10 g of soil was suspended in 90 mL of sterile water with 30 glass marbles (5 mm in diameter) and shaken for 30 min at 120 rpm. Ten-fold serial dilutions were conducted, and suspensions (100 μL) of $10^{-2}$ dilution were distributed on Komada's Fusarium-selective medium to determine the *Fusarium oxysporum* counts (Komada, 1975). Colony forming units (CFU) were counted, and the population per gram of dry soil (CFU/g dry soil) was calculated.

### 2.6. DNA extraction and purification for pyrosequencing-based microbial community analysis

Soil samples at the start and end of the soil treatment experiment were used to analyze the changes in the microbial community. Only one soil sample was collected at the start of the experiment because the soil in different treatments was from the same place and was well mixed before the experiment. Genomic DNA was extracted from each soil sample using a PowerSoil® DNA Isolation Kit following the manufacturer's protocol (MO BIO Laboratories Inc., Carlsbad, CA, USA). The extracted genomic DNA from each treatment replicate was equally mixed.

### 2.7. PCR amplification, amplicon quantitation, pooling, and pyrosequencing

Changes in the microbial communities, including bacteria and fungi, during the experiment were analyzed using 454 pyrosequencing analyses. A fragment covering the V1–V3 region of the 16S rDNA gene and a fragment covering the V4 region of the 18S rDNA gene were selected to construct bacterial and fungal community libraries, respectively, through tag pyrosequencing. Polymerase chain reaction (PCR) amplification was performed in triplicate. The barcoded, broadly conserved primers 27F and 533R amplified the V1–V3 region of the 16S rDNA gene, and the 3NDF and V4_euk_R2 primers amplified the V4 region of the 18S rDNA gene (Brate et al., 2010; Wu et al., 2012). The sequences are listed in Table 1. Replicate PCR products of a sample were assembled within a PCR tube. The samples were then examined on agarose gels (2% in TBE buffer) and purified using an AxyPrep DNA gel extraction kit (Axygen, Union City, CA, USA). Prior to sequencing, the DNA concentration of each PCR product was checked for quality using PicoGreen® dsDNA Quantitation Reagent (Fisher Scientific, Schwerte, Germany) and with a QuantiFluor™-ST Handheld Fluorometer (Promega-GloMax Promega QuantiFluor, Madison, USA).

Following quantification, the amplicons from each reaction mixture were pooled in equimolar ratios based on concentration and subjected to emulsion PCR to generate amplicon libraries using Roche GS FLX Titanium emPCR Kits (Basel, Switzerland). Amplicon pyrosequencing was performed from the A-end using a Roche GS FLX+ Sequencing Method Manual_XLR70 kit on a Roche Genome Sequencer GS FLX Titanium platform at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China.

### 2.8. Bioinformatic analyses

Data preprocessing was performed in Mothur following the standard operating procedure (SOP) (http://www.mothur.org/wiki/454_SOP) (Schloss et al., 2011). The resulting pyrosequencing reads were screened for effective sequences that contained the barcode sequence. These effective reads were defined as 'raw reads'. The shhh.flows command in Mothur was used to de-noise raw reads. De-noised sequences were further optimized by removing the adaptor, polyA/T, barcode sequence and forward primer sequence using the program Sequence Cleaner (http://sourceforge.net/projects/seqclean/). Sequences that were shorter than 200 bp, had ambiguous bases, or had an average quality lower than 25 were also discarded. The optimized sequence data were then used for statistical analysis. These pyrosequencing reads were simplified using the 'unique.seqs' command to generate a unique set of sequences, aligned using the 'align.seqs' command, and compared with the Bacterial and Eukaryotic SILVA Database (SILVA version SSU111; http://www.arb-silva.de/) (Quast et al., 2013)

The aligned sequences were further trimmed, and redundant reads were eliminated using the 'screen.seqs', 'filter.seqs', and 'unique.seqs' commands in order. The 'chimera.slayer' command was used to determine chimeric sequences with a default score cutoff of 0.28 (http://drive5.com/uchime) (Haas et al., 2011). The 'dist.seqs' command was performed, and unique sequences were clustered into operational taxonomic units (OTUs) defined by 97% similarity (http://www.mothur.org/wiki/Cluster). To test differences in microbial community richness between samples, alpha diversity measures (Chao1 and Shannon index) were calculated at 97% OTU sequence identity (http://www.mothur.org/wiki/Chao and http://www.mothur.org/wiki/Shannon). Clustered heatmaps were generated with the R pheatmap function (pheatmap-package.r) with default setting parameters (Kolde, 2013). The raw reads produced in this experiment have been deposited in the NCBI database (Accession Number: SRP063816).

### 2.9. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA; Version 12.0) and Excel 2007. When applicable, values are presented as the mean ± standard error and were analyzed by one-way analysis of variance (ANOVA). To

**Table 1**
Primer sequences used to amplify the V1–V3 and V4 regions of the 16S rDNA and 18S rDNA genes, respectively.

| | Primer name | Sequence |
|---|---|---|
| V1-V3 region of 16S rDNA | 27F | 5′-*CCTATCCCCTGTGTGCCTTGGCAGTCTCA*GAGAGTTTGA TCCTGGCTCAG-3′ |
| | 533R | 5′-*CCATCTCATCCCTGCGTGTCTCCGACTCAG*NNNNNNNNNNTTACCGCGGCTGCTGGCAC |
| V4 region of 18S DNA | 3NDF | 5′-*CCTATCCCCTGTGTGCCTTGGCAGTCTCAG*GGCAAGTCTGGTGCCAG-3′ |
| | V4_euk_R2 | 5′-*CCATCTCATCCCTGCGTGTCTCCGACTCAG*NNNNNNNNNNNACGGTATCT(AG)ATC(AG)TCTTCG-3′ |

*Note*: The sequence of the B adaptor in the forward primers 27F and 3NDF is shown in italics and underlined. The sequence of the A adaptor in the reverse primers 533R and V4_euk_R2 is shown in italics and underlined, and the Ns represent an eight-base sample-specific barcode sequence.

detect the significance of differences between the means of observations, Tukey's multiple comparison test was performed.

## 3. Results

### 3.1. Changes in soil pH during the experiment

The change of soil pH is shown in Fig. 1A. The initial soil was acidic, with a value of only 4.84, similar to soils observed in most of southern China (Guo et al., 2010). The pH of the SS soil did not change significantly during the experiment, and a small decrease was observed after 15 days. A small increase of approximately 0.31 was observed in the SS-F soil after 15 days. The soil pH values of the SS-OA(M)-F and SS-OA(G)-F treatments were 6.87 and 6.30, respectively, after 3 days. The most increase of soil pH was observed in the SS-OA(M)-F treatment. The pH of the SS-OA(M)-F soil reached 6.89 after 15 days. The statistical analysis showed that the soil pH of the SS-OA(M)-F and SS-OA(G)-F treatments was significantly higher than those in the SS or SS-F treatments ($p < 0.01$) (Table 2).

### 3.2. Soil electrical conductivity

Secondary soil salinization is a major issue for continuous cropping (Zhou et al., 2013). Soil electrical conductivity reflects the degree of soil salinization. The changes in soil electrical conductivity during the experiment are shown in Fig. 1B. The initial soil conductivity was 898.8 μs/cm, although this value was observed where the soil had been planted for only three years. A decrease in soil electrical conductivity was observed in all treatments and controls after 15 days. However, the highest decrease in electric conductivity was observed in the SS-OA(M)-F and SS-OA(G)-F soils during the first 3 days. The electrical conductivity values of SS-OA(M)-F, SS-OA(G)-F, and SS-F soil at 15 days were markedly lower than that of the SS soil. The largest decrease, 85.16%, was observed in the SS-OA(G)-F soil. The soil electrical conductivity of the SS-OA(G)-F soil decreased from $898.80 \pm 9.23$ μs/cm to $133.380 \pm 17.68$ μs/cm over 15 days. The statistical analysis indicated a significant difference between the different treatments ($p < 0.01$) (Table 2).

### 3.3. Soil oxidation-reduction potential

The change in soil oxidation-reduction potential (Eh) is shown in Fig. 1C. The Eh of SS was relatively constant during the experiment. A small decrease was observed in the SS-F soil over the initial 3 days of the experiment, and the Eh remained higher than 100 mV after day 3. The Eh significantly decreased to less than 0 in the organic amendment treatments. The largest decrease was observed in the SS-OA(G)-F soil, in which the Eh decreased to −485.33 mV by day 3. Subsequently, the Eh of the SS-OA(G)-F soil increased to approximately −250 mV after 6 days. The Eh of the SS-OA(M)-F soil decreased quickly during the initial 6 days to approximately −287.33 mV on day 6, after which the Eh changed

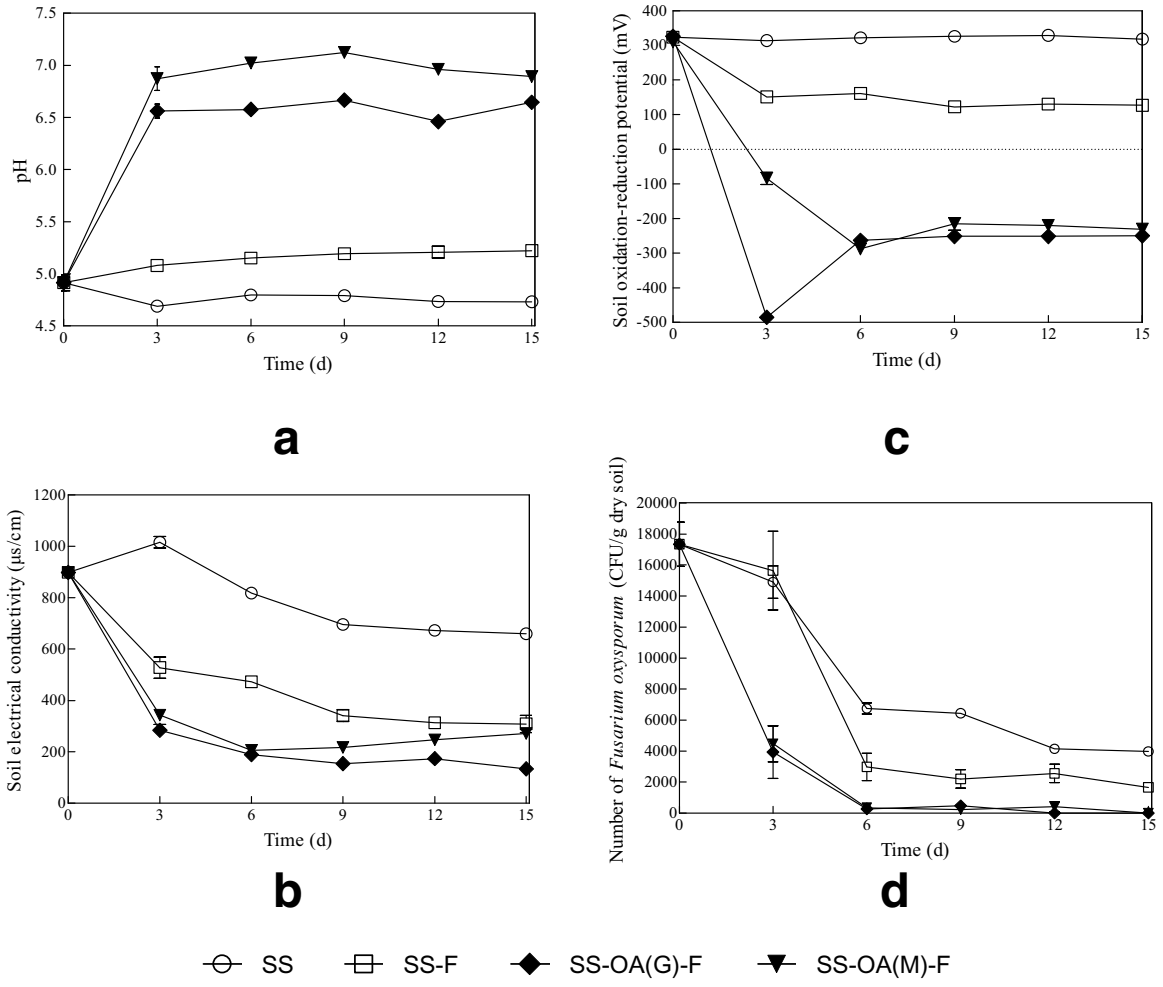


**Fig 1.** Variations in soil pH (a), electrical conductivity (b), oxidation-reduction potential (c) and the number of *Fusarium oxysporum* (d) during the soil treatment experiment.

**Table 2**
Statistical analysis of the soil characteristics after 15 days.

| | pH | Soil electrical conductivity (μs/cm) | Soil oxidation-reduction potential (mV) | Number of *Fusarium oxysporum* (CFU/g dry soil) |
|---|---|---|---|---|
| SS | 4.73 ± 0.04D | 659.60 ± 19.37A | 317.67 ± 4.03A | 3968.7 ± 500.8A |
| SS-F | 5.22 + 0.11C | 308.20 ± 74.45B | 127.11 ± 4.66B | 1663.8 ± 357.0B |
| SS-OA(G)-F | 6.65 ± 0.16B | 133.32 ± 7.80C | −249.71 ± 4.86D | 0C |
| SS-OA(M)-F | 6.89 ± 0.08A | 271.20 ± 14.32B | −231.01 ± 5.88C | 0C |

Different letters indicate significant differences among the samples at the 1.0% significance level.

little until the end of the experiment. A Tukey multiple comparison test demonstrated that the Eh of the SS-OA-F treatments was significantly lower than that of the SS or SS-F treatments ($p < 0.01$) (Table 2).

### 3.4. Variation in Fusarium oxysporum in the soil

Fusarium wilt of cucumber was induced by *Fusarium oxysporum*. Selective media were adapted to detect variations in the total number of *Fusarium oxysporum* during the experiment (Fig. 1D). The total number of *Fusarium oxysporum* was high in the initial soil, reaching 17,336 ± 2461 CFU/g dry soil. The *Fusarium oxysporum* counts in the soil of the SS and SS-F treatments decreased over the first 6 days but changed minimally after 6 days in these treatments. At the end of the study period, the SS and SS-F soils featured 3969 ± 501 and 1664 ± 357 CFU/g dry soil, respectively. *Fusarium oxysporum* counts in the glucose- and *Medicago sativa*-amended soils significantly decreased in the first 6 days and were under the detection limit after 12 days. A Tukey multiple comparison test showed that the *Fusarium oxysporum* counts in the SS-OA(M)-F and SS-OA(G)-F soils were significantly lower than those in the SS or SS-F soils ($p < 0.01$) and that the counts in the SS-F soil were significantly lower than in the SS soil.

### 3.5. Change in the soil microbial community before and after soil treatment

High-throughput sequencing has been applied to recover the environmental microbial community structure. This technology greatly improves the knowledge of the microorganism environment and was used in this study to identify changes in the soil microbial community.

#### 3.5.1. Soil bacteria community structure and soil bacterial diversity

The 16S rDNA V1-V3 regions were amplified using PCR, and the products were used for 454 high-throughput sequencing. In total, 49480 unique sequences were obtained from 68063 raw reads of the 5 samples. The average sequence length was 475 bp. The sequences were divided by sample according to the barcode and clustered into different OTUs at 97% similarity. In total, 20,141 OTUs were obtained from the five samples. The sequence analyses are listed in Table 3.

Based on the OTU clusters, the bacterial community composition was further analyzed at the class level, as shown in Fig. 2a. The dominant bacteria in the initial soil mainly included *Actinobacteria* (21.3%), *Gammaproteobacteria* (17.7%), *Alphaproteobacteria* (13.3%), *Acidobacteria* (5.7%), no_rank_*Actinobacteria* (5.4%), *Betaproteobacteria* (4.1%), and *Ktedonobacteria* (4.0%), accounting for 71.5% of the total microorganisms. The bacterial composition of the SS soil at 15 days was nearly identical to day 0 and changed only slightly in proportion. The solarization with flooding treatment had little effect on the composition of the microorganism community. The ratios of *Acidobacteria* and *Alphaproteobacteria* notably decreased, and *Clostridia* and *Sphingobacteriia* became the dominant microorganisms, accounting for 9.0 and 8.2% of the total microorganisms, respectively. The *Medicago sativa* and glucose soil amendments had significant effects on the bacterial community. The proportions of *Clostridia*, *Bacilli*, and *Bacteroidia* increased, and *Actinobacteria*, *Gammaproteobacteria*, and *Gemmatimonadetes* became the dominant microorganisms. *Clostridia* became the absolutely dominant microorganism, accounting for 29.8% and 34.0% in the SS-OA(G)-F and SS-OA(M)-F soils, respectively. However, the effect of the *Medicago sativa* amendment on the microorganism community differed slightly from the glucose amendment. The ratio of *Bacilli* in the SS-OA(G)-F soil was higher than in the SS-OA(M)-F soil, whereas the ratio of *Bacteroidia* in the SS-OA(M)-F soil was higher than that in the SS-OA(G)-F soil.

A cluster analysis of the bacterial communities from the different samples showed that the bacterial community in the soil amended with glucose and *Medicago sativa* differed from that of the day 0, SS, and SS-F soils (as shown in Fig. 2b). The day 0 and SS communities were clustered into one group and further clustered with SS-F. The SS-OA(G)-F and SS-OA(M)-F soils were clustered into one group. The above results demonstrate that the bacterial community changed little in the SS soil and was nearly identical on days 0 and 15. The bacterial community changed under the SS-F treatment but was similar to the day 0 and SS soils. The bacterial community changed greatly and differed from the day 0, SS, and SS-F soils following treatment with *Medicago sativa* and glucose, demonstrating that amendment with organic matter had a remarkable regulatory effect on the soil microorganism community.

The OTU number illustrates the species richness of the samples. The Chao1 and Shannon indices indicate community differences. The bacterial diversity of each sample is shown in Table 4. Relative to the day 0 sample, minimal decreases in the Chao1 and Shannon indices were observed in the SS and SS-F soils. Obvious decreases in both indices were observed in the SS-OA(G)-F soil. *Medicago sativa* amendment increased soil bacterial community diversity and richness. The Chao1 and Shannon indices were higher for the

**Table 3**
An analysis of the soil bacterial sequence data.

| Sampling time | Sample | Raw reads | Reads after denoising | Unique sequences | Bacterial sequences | Bacterial OTUs |
|---|---|---|---|---|---|---|
| 0 d | 0d | 13222 | 10855 | 9944 | 9944 | 4046 |
| 15 d | SS | 13145 | 10530 | 9390 | 9390 | 3910 |
| 15 d | SS-F | 13644 | 10983 | 9845 | 9845 | 3940 |
| 15 d | SS-OA(G)-F | 12891 | 10260 | 9610 | 9610 | 3569 |
| 15 d | SS-OA(M)-F | 15161 | 11880 | 10691 | 10691 | 4676 |

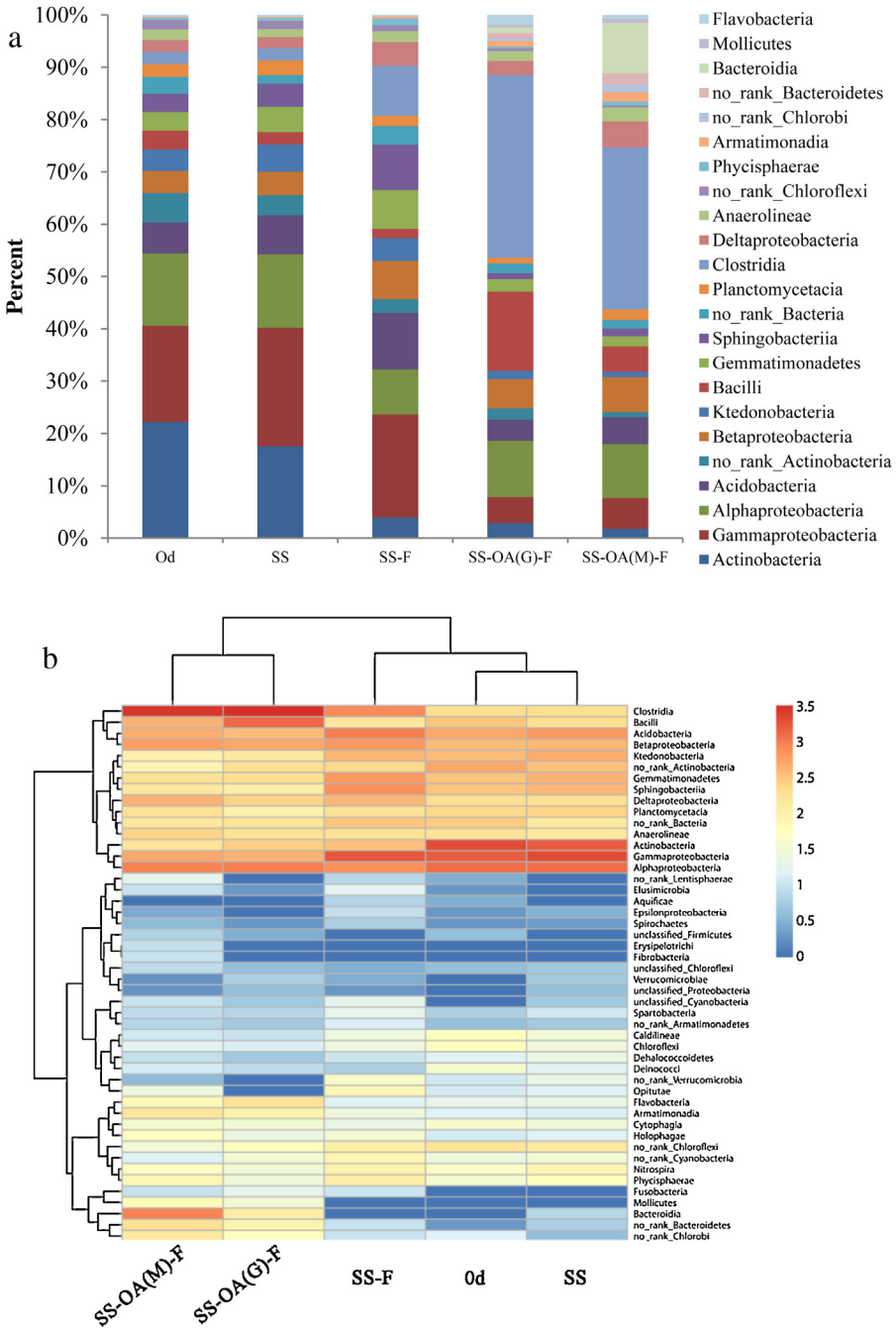


**Fig. 2.** (a) The abundances of different bacterial classes in each sample. Twenty-three dominant bacterial classes, representing at least 94.14% of the total community in each sample, were selected. (b) Heat map of bacterial classes in each sample. The color intensity (log-scale) in each panel shows the percentage of each class in each treatment based on the color key on the right.

**Table 4**
An analysis of the bacterial diversity indices of each sample under a similarity of 0.97.

| Sample | Chao1 | Shannon |
|---|---|---|
| 0 d | 10944.61 | 7.54 |
| SS | 10272.04 | 7.38 |
| SS-F | 10876.69 | 7.39 |
| SS-OA(G)-F | 9224.86 | 7.12 |
| SS-OA(M)-F | 12001.90 | 7.73 |

SS-OA(M)-F soil than for the day 0 soil and other treatments after 15 days.

### 3.5.2. Soil fungi community structure

The fungal 18S rDNA V4 regions were amplified using PCR, and the products were used in 454 high-throughput sequencing. In total, 36739 unique sequences were obtained from 43878 raw reads of the 5 samples. The average sequence length was 442 bp. In total, 3 591 OTUs were obtained from the five samples. According to the taxonomic results, these sequences included fungi, Metazoa, and other Eukaryota. Because we focused on soil fungi variation, the fungal sequence OTUs were selected for further analysis. The sequences are summarized in Table 5.

**Table 5**
An analysis of the fungal sequence data.

| Sampling time | Sample | Raw reads | Reads after denoising | Unique sequences | Fungal sequences | Fungal OTUs |
|---|---|---|---|---|---|---|
| 0 d | 0d | 9611 | 8602 | 8575 | 6489 | 298 |
| 15 d | SS | 9190 | 7454 | 7248 | 4980 | 360 |
| 15 d | SS-F | 8540 | 7153 | 7009 | 3408 | 301 |
| 15 d | SS-OA(G)-F | 8942 | 7764 | 7660 | 3909 | 250 |
| 15 d | SS-OA(M)-F | 7595 | 6418 | 6247 | 2529 | 253 |

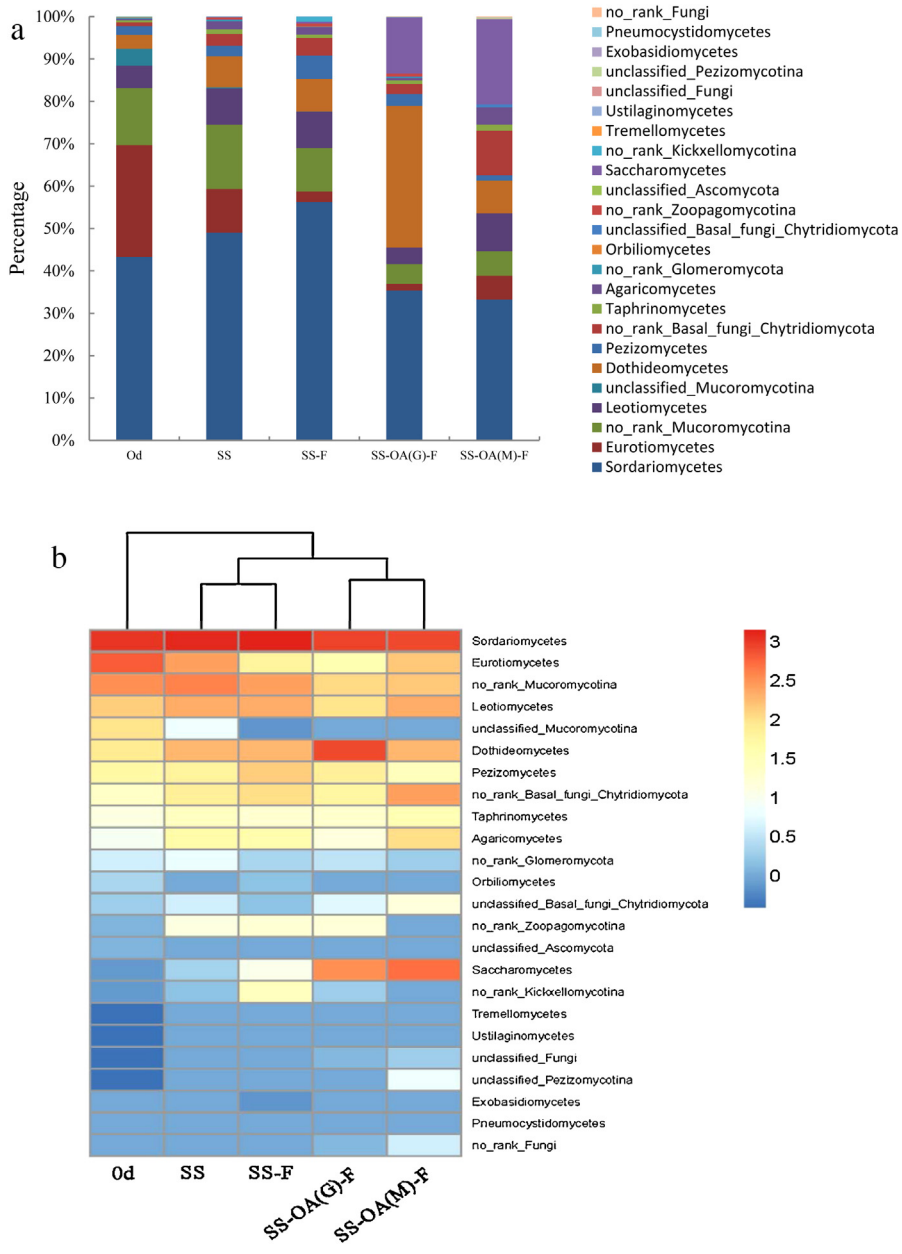


**Fig. 3.** (a) Abundances of different fungal classes in each sample. Twenty-four dominant fungal classes, representing at least 94.14% of the total community in each sample, were selected. (b) Heat map of fungal classes in each sample. The color intensity (log-scale) in each panel shows the percentage of each class in each treatment based on the color key on the right.

Based on the OTU clusters, the fungal community composition was further analyzed at the class level. In total, 24 fungal classes were observed, including partially unclassified fungi, as shown in Fig. 3a. The dominant fungi in the initial soil mainly included *Sordariomycetes* (43.32%), *Eurotiomycetes* (26.38%), no_rank_*Mucoromycotina* (13.47%), *Leotiomycetes* (5.24%), unclassified *Mucoromycotina* (4.01%), *Dothideomycetes* (3.25%), and *Pezizomycetes* (2.07%), which accounted for 97.73% of the total fungi. The fungal composition of the SS soil at 15 days changed slightly. The ratio of *Eurotiomycetes* decreased to 10.30%, and the proportions of *Sordariomycetes,* no_rank_*Mucoromycotina*, *Leotiomycetes*, and *Dothideomycetes* increased to 49.02, 15.20, 8.55, and 7.29%,

respectively. The solarization combined with flooding treatment had an obvious effect on the composition of the fungal community. Compared with the day 0 and SS samples, the proportions of *Sordariomycetes, Pezizomycetes,* no_rank_Basal_fungi_*Chytridiomycota*, and no_rank_*Kickxellomycotina* were higher, and the proportions of *Eurotiomycetes* and no_rank_*Mucoromycotina* were lower. The *Medicago sativa* and glucose amendments had significantly different effects on the fungal community. The proportions of *Sordariomycetes*, *Eurotiomycetes*, and no_rank_*Mucoromycotina* dramatically decreased, whereas the proportion of *Saccharomycetes* greatly increased. However, the effects of *Medicago sativa* on the fungal community were not identical to those of glucose. The proportion of *Dothideomycetes* in the SS-OA (G)-F soil was much higher than in the SS-OA(M)-F soil, whereas the proportions of no_rank_Basal_fungi_*Chytridiomycot*, *Agaricomycetes*, and *Saccharomycetes* in the SS-OA(M)-F soil were much higher than those in the SS-OA(G)-F soil.

A heat map was used to directly show changes in the fungal community based on a cluster analysis of the samples. Fig. 3b shows obvious changes in the fungal community before and after the treatments and among different treatments. The cluster analysis showed that the five samples were divided into three groups. The day 0 sample was clustered into one group. The SS and SS-F samples were clustered into a second group, and the SS-OA (G)-F and SS-OA(M)-F samples were clustered into a third group. All treatments affected the dominant fungi, unlike for soil bacteria. The SS and SS-F treatments had similar effects on the dominant fungus, and *Medicago sativa* and glucose amendments had similar effects on the dominant fungus, demonstrating that organic matter amendments had remarkably different effects on the soil fungal community.

### 3.6. Variation in Fusarium oxysporum counts during two plantings of cucumber

Two cucumber plantings were performed after the soil treatment, and *Fusarium oxysporum* variations were measured (Fig. 4). The initial number of *Fusarium oxysporum* was high in the SS and SS-F soils because *Fusarium oxysporum* was not efficiently killed. The number increased quickly following cucumber planting, reaching the highest values of $9616 \pm 1201$ and $7146 \pm 2728$ CFU/g dry soil in the SS and SS-F soils, respectively, on day 45. The *Fusarium oxysporum* counts in the SS-OA(G)-F and SS-OA(M)-F soils were low during the first 60 days, particularly in the SS-OA(M)-F soil. Although *Fusarium oxysporum* proliferated after 60 days in the SS-OA(G)-F and SS-OA(M)-F soils, the *Fusarium oxysporum* counts remained much lower than those in the SS and SS-F soils.

*Fusarium oxysporum* counts before and after the second planting of cucumber were also observed. *Fusarium oxysporum* counts in the SS and SS-F treatments were greater than $10^3$ CFU/g dry soil on day 0, whereas those observed in the SS-OA(G)-F and SS-OA(M)-F soils were lower than $10^3$ CFU/g dry soil. On day 90, the *Fusarium oxysporum* counts in SS-OA(M)-F soil remained low, whereas the counts in the SS, SS-F, and SS-OA(G)-F treatments increased significantly. The average number of *Fusarium oxysporum* was greater than $2.0 * 10^4$ CFU/g dry soil, which was 14.73, 9.21, and 36.84 times greater than the number of *Fusarium oxysporum* on day 0 in the SS, SS-F, and SS-OA(G)-F treatments, respectively.

### 3.7. Ratio of Fusarium wilt in cucumber

The occurrence of Fusarium wilt in cucumber was recorded during the two cucumber plantings. During the initial planting, no Fusarium wilt was observed in any treatment, which may be due to the relatively low number of soil *Fusarium oxysporum* and normal low incidence in autumn. Fusarium wilt was observed in 50%, 30% and 40% of the SS, SS-F, and SS-OA(G)-F treatments, respectively, during the second planting (Fig. 5). However, no Fusarium wilt was observed in the SS-OA(M)-F treatment.

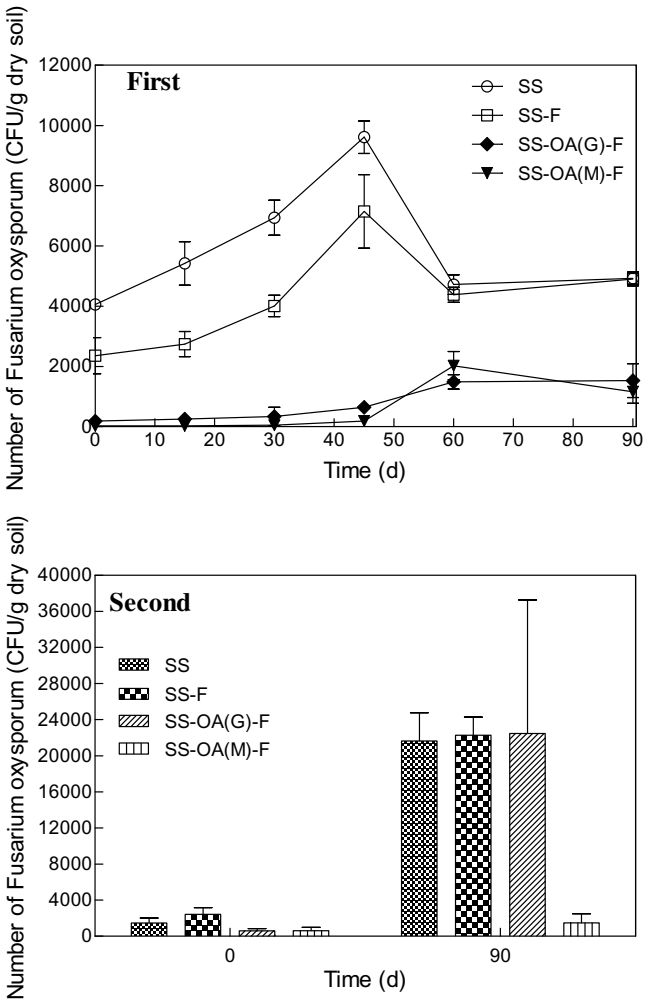


**Fig. 4.** The number of *Fusarium oxysporum* in the soil during cucumber planting.

## 4. Discussion

The excessive use of chemical fertilizers in continuous cropping and the minimal elution by rain results in the acidification and

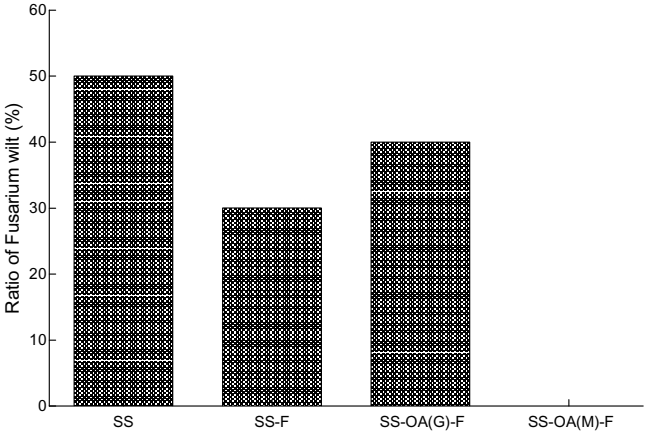


**Fig. 5.** The percentage of Fusarium wilt in cucumber at the second planting.

salinization of soil and the accumulation of pathogens and harmful substances (Guo et al., 2010). The deteriorated soil environment results in a high incidence of Fusarium wilt in cucumber. The soil in this experiment was acidic and contained a large number of *Fusarium oxysporum*. Thus, the deteriorated soil environment favored the proliferation of the pathogen, and resulted in Fusarium wilt.

Soil solarization is a non-chemical method of soil disinfestation and is widely used all over the world (Doğan et al., 2013). Soil solarization is thought to control the presence of pathogens either directly, through physicothermal action, or indirectly, by stimulating antagonists and/or weakening the pathogen's resting structures present in the soil (Gilardi et al., 2014). Our research suggests that soil solarization only partially kills *Fusarium oxysporum*, though appropriate controls allowing a definite inference of effects by solarization were impractical in this experiment. The remaining *Fusarium oxysporum* exceeded $10^3$ CFU/g dry soil, explaining why Fusarium wilt in cucumber can continue to occur after solarization treatment. From our experiment, we also observed that heat treatment had no effect on soil pH recovery and a minimal effect on soil salinization. Soil solarization alone also had a minimal effect on the soil bacterial community and little effect on the soil fungal community. Soil solarization is commonly combined with flooding in southern China to lessen problems associated with continuous cropping. It was thought that high temperatures kill pathogens, weed seeds, and pests and that soil quality is effectively improved by flooding. Based on our study, solarization combined with flooding treatments increased the soil pH by 0.6, decreased the number of *Fusarium oxysporum*, and decreased the soil salinization relative to the soil solarization treatment. The microbial community after SS-F treatment was also minimally different from the SS and initial samples. The proportion of anaerobic microorganisms and facultative anaerobes, such as *Clostridia* and *Saccharomycetes*, increased. Thus, the oxygen concentration in the soil was low, and the environment became partially anaerobic, as reflected by the redox potential. Because the pathogens were aerobic, the anaerobic environment was not suitable for survival, which has been well documented (Blok et al., 2000). This observation may explain why many more *Fusarium oxysporum* were killed in the SS-F soil. All of these above factors increased the efficiency of SS-F treatment on the control of Fusarium wilt. However, soil acidification did not significantly improve, and the number of *Fusarium oxysporum* remained high after SS-F treatment. The remaining *Fusarium oxysporum* may persist as spores (Vakalounakis and Chalkias, 2004) and resists relatively harmful conditions, allowing them to proliferate quickly after a return to suitable conditions. This unremediated environment allowed the quick proliferation of pathogens during the first and second plantings of cucumber, resulting in a higher frequency of Fusarium wilt in the second planting.

Soil solarization combined with organic amendments is more effective in the control of soilborne disease than soil solarization alone (Gilardi et al., 2014; Kaşkavalci, 2007; Klein et al., 2011; Ndiaye et al., 2007). Our results were also consistent with this result. Organic amendments in this experiment provided an easily utilized carbon source. The rapid decomposition of organic matter by soil microorganisms depleted the oxygen levels in the soil and rapidly created low redox potential conditions over the first six days. This shift corresponds to the rapid decrease in the number of *Fusarium oxysporum* during the first 6 days in the SS-OA(M)-F and SS-OA (G)-F soils. The number of *Fusarium oxysporum* was significantly lower and was under the detection limit in soils treated with either SS-OA(M)-F or SS-OA(G)-F at the end of the soil treatment experiment. Compared with the physicothermal action generated by soil solarization, extremely low oxygen levels and redox potentials generated in organic amendment treatment killed pathogens more effectively and had a significant impact on the soil microbial community. The proportions of *Clostridia* and *Saccharomycetes* in the microbial community increased markedly and became dominant. The proportions of *Sordariomycetes*, *Eurotiomycetes*, and no_rank_*Mucoromycotina* dramatically decreased. *Clostridia* are strictly anaerobic, and *Saccharomycetes* are facultative anaerobes; in contrast, *Sordariomycetes*, *Eurotiomycetes*, and no_rank_*Mucoromycotina* are aerobic fungi. The pathogen, *Fusarium oxysporum* f. sp. *cucumberinum*, belongs to *Sordariomycetes*. This result corresponds to the number of *Fusarium oxysporum* f. sp. *cucumberinum* grown on selective media. Though the chosen V1-V3 region of the 16S rRNA gene in this study might not provide maximum possible coverage of the present bacterial diversity according to recent research (Klindworth et al., 2013; Sapp et al., 2015), it clearly demonstrated the effect of SS-OA-F treatments on the soil community. In another term, organic amendment significantly increased the pH of the acidified soil and decreased the soil salinization, which remediated the deteriorated soil environment. The increase in soil pH under flooding conditions with organic amendment was well documented in the previous study. A decrease of soil electrical conductivity may be achieved by removing $SO_4^{2-}$ and $NO_3^-$ and decreasing the mobility of partial metal ions under reductive status (Meng et al., 2015; Sun et al., 2007). Improvement of the soil environment restrained the proliferation of the pathogen in soil because the proliferation of *Fusarium oxysporum* was slowed in neutral and alkaline soil (Yao et al., 2015). The proliferation rate of the *Fusarium oxysporum* in organically amended soil at the first planting was significantly lower than that in SS and SS-F treated soil. All these factors may explain why the efficiency of SS-OA-F treatment on the control of Fusarium wilt was greatly improved.

Different types of organic matter, such as compost and crop residues, have shown potential in controlling soil-borne pathogens (Gilardi et al., 2014; Hadar and Papadopoulou, 2012; Klein et al., 2011). However, different organic matter types have different suppression durations (Klein et al., 2011). It was calculated that the effect of OM amendments was found to be suppressive in 45% and a significant increase of disease incidence was observed in 20% of the cases (Bonanomi et al., 2007). In our study, we observed that the suppression duration of the *Medicago sativa* amendment was longer than that of glucose, although these two amendments had nearly identical effects on soil remediation and pathogen killing during treatment. The obvious difference between the *Medicago sativa* amendment and the glucose amendment was their effect on soil microbial diversity. According to high-throughput sequencing, the *Medicago sativa* amendment increased bacterial diversity and richness, whereas the glucose amendment reduced the bacterial diversity and richness. The bacterial diversity of SS-OA(G)-F treatment was even much lower than that of SS and SS-F treatments. It was reported that higher bacterial diversity is associated with disease suppression (Shen et al., 2015b; van Bruggen et al., 2015). An increase in soil microbial diversity would enhance disease suppression (Bruggen et al., 2006; Qiu et al., 2012), while a decrease in soil microbial diversity was thought to be responsible for the development of soil-borne plant diseases (Qiu et al., 2012).The decrease of soil bacterial diversity in glucose amended soil coincided with the previous study, which might weaken the suppression of *Fusarium oxysporum* proliferation. The number of *Fusarium oxysporum* in the SS-OA(G)-F soil during the second planting was nearly 15 times than that in the SS-OA(M)-F soil. The high number of pathogens led to a high incidence of Fusarium wilt.

## 5. Conclusion

The efficiency of three types of solarization-based ecological soil treatments for the control of Fusarium wilt and their impacts on soil microbial community were compared each other in this study. Soil solarization and soil solarization with flooding were less effective in the control of Fusarium wilt of cucumber, because of their deficiency in the decrease and suppression of *Fusarium oxysporum*. Soil solarization combined with organic amendment and flooding was much more effective on the restriction of pathogen proliferation and significantly impacted the soil microorganism community. However, the pathogen suppression duration differed between the different organic matter types in the SS-OA-F treatments. *Medicago sativa* amendment increased the soil bacterial diversity and prolonged the suppression duration. Soil solarization combined with *Medicago sativa* amendment and flooding was recommended for the efficient control of Fusarium wilt.

## Conflict of interest

The authors have no conflicts of interest to declare.

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