J Soils Sediments (2016) 16:215–225
DOI 10.1007/s11368-015-1172-9

SOILS, SEC 4 • ECOTOXICOLOGY • RESEARCH ARTICLE

Effects of biological soil disinfestation and water regime on suppressing *Artemisia selengensis* root rot pathogens

Teng Wen [1,2,3,4] · **Xinqi Huang** [1,2] · **Jinbo Zhang** [1,2] · **Zucong Cai** [1,2]

Received: 11 March 2015 / Accepted: 28 May 2015 / Published online: 20 June 2015

Abstract
Purpose Biological soil disinfestation (BSD) is an effective non-chemical method to control soil-borne disease by incorporating organic amendments into soil under flooding conditions. For suppressing *Artemisia selengensis* root rot pathogens (*Fusarium oxysporum* f. sp. *cubense* (FOC), *Phytophthora* spp., and *Pythium* spp.), the effects of BSD treatment using maize straw as organic material and water regime were investigated by greenhouse experiments.
Materials and methods Pathogens infested soil was filled in greenhouse pots and incorporated with maize straw at rates of 0.2 %, 0.5 %, and 2 % (*w*/*w*) under flooded or water-saturated (100 % water-holding capacity) conditions at 25–35 °C for 25 days.
Results and discussion The population of *A. selengensis* root rot pathogens was effectively reduced after BSD treatments with all three maize straw application rates, and the largest reduction was reached up about 90 % at 2 % application rate. No obvious difference in suppressing effect was observed between flooded and water-saturated soil conditions with the same application rate of maize straw. BSD induced bacterial community structure shift and biodiversity enhancement. Two toxic organic acids producers, *Clostridium* and *Bacillus* spp., were found as predominant populations in both flooding alone and BSD treatments, while four types of organic acids, acetate, propionate, butyrate, and isovelerate were only detected in BSD treatments. Besides, increasing soil pH and organic matter with concomitantly decreasing nitrate occurred in BSD-treated soils.
Conclusions Maize straw is an effective BSD organic material, which might also provide a potential and environment-friendly disposal strategy of crop residues. Saturating soil to reach 100 % WHC was a good alternative to soil flooding in BSD. Bacterial community shifts, organic acids accumulation, and soil properties changes indicated multiple ways that might be involved in suppressing *A. selengensis* root rot pathogens during BSD treatment.

Keywords *Artemisia selengensis* root rot · Biological soil disinfestation (BSD) · Maize straw · Bacterial community shift · Organic acids

Responsible editor: Kirk T. Semple

✉ Teng Wen
wtjessy@163.com

✉ Zucong Cai
zccai@njnu.edu.cn

[1] School of Geography Sciences, Nanjing Normal University, Nanjing 210023, China

[2] Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, Nanjing 210023, China

[3] Jiangsu Provincial Key Laboratory of Materials Cycling and Pollution Control, Nanjing Normal University, Nanjing, China

[4] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

1 Introduction

Soil-borne disease caused by fungal, bacterial, and nematode pathogens has become a huge threat to agricultural production under intensive cultivation. For a long time, chemical disinfestation method, such as methyl bromide fumigation, is considered as the most effective and economical approach to control soil-borne disease. With the increasing concerns about human and environmental risks of chemicals, non-chemical methods, such as flooding, solarization, and organic amendment, have been widely investigated to search for alternatives

to chemical fumigation. Biological soil disinfestation (BSD) is one of alternatives controlling soil-borne disease. It accomplishes the goal of soil-borne pathogens suppression by incorporating decomposable organic material into soil under flooding conditions and then covering with plastic mulch. A broad spectrum of pathogens and nematodes has been markedly suppressed by this method (Kobara et al. 2007; Uematsu et al. 2007; Momma 2008; Momma et al. 2010).

It has been reported varieties of organic materials, such as ethanol, molasses, broccoli, cover crops, animal and green manure, and so on, are effective in BSD treatment (Shinmura 2002; Momma et al. 2011; Núñez-Zofío et al. 2011). However, these organic materials sometimes are not accessible and economical in fields. Crop residues, such as maize, wheat, and rice straw, are more easy to acquire due to the wide cultivation of these crops. Our previous studies have showed maize and rice straw, combined with soil flooding, could successfully suppress banana fusarium wilt in both laboratory and field experiments (Huang et al. 2015). Considering that the disposal of large quantities of crop residues has become an urgent environmental concern to government in China (Hrynchuk 1998; Buresh and Sayre 2007; Gadde et al. 2009), using crop residues as organic material in BSD might provide a new promising, potential, and environment-friendly strategy. Additionally, previous studies suggested that more incorporation rates of organic amendments in BSD might induce higher mortality of pathogens (Blok et al. 2000). Examining the effects of organic application rates on pathogens would help to figure out the optimal application rate of crop residues.

On the other hand, evidences from some researches pointed that flooding is not indispensible for BSD, and irrigating soil to reach 100 % water-holding capacity (WHC) could effectively inhibit pathogens as well (Momma et al. 2010; Muramoto et al. 2008). Because flooding soil demands vast amount of water and is not always practical, for instance in hilly mountain areas where standing water layer is hardly maintained and in field where soils have very large percolation rate, saturating soil would be a good alternative. Yet, some contrast results still exist, which regarded that merely saturating soil was not effective in suppressing some pathogens such as *Pythium* spp. (Snyder 1987). Thus, to promote practical application of BSD, a further understanding of water regime is essential.

Till now, the efficacy of BSD in soil-borne pathogen disinfestation has been confirmed by many studies, but its exact mechanism is still under exploration. Compared with other non-chemical methods (flooding and solarization), the temperature in BSD is obviously lower than the lethal level solarization requires (Katan 2000; Larkin and Griffin 2007; Yossen et al. 2008), and the anaerobic condition induced by flooding alone cannot result in as effective soil disinfestation as BSD does (Butler et al. 2012). Moreover, the soil redox potential in BSD experiences much more quick and dramatic drop than flooding alone. All of these made researchers assumed that it was such a strong reductive and anaerobic condition that led to final pathogens inactivation in BSD (Blok et al. 2000). In fact, this is a very complicated process, usually involving soil physicochemical changes, microbiota shifts, nutrients cycling, toxic compounds accumulation, and so on. Some researchers have reported apparent bacterial community changing from aerobic-dominated states to facultative and anaerobic-dominated states in BSD-treated soil (Momma et al. 2013). *Clostridium* spp., producers of some toxic organic acids, were identified to be engaged in the mechanism of BSD (Momma et al. 2013; Mowlick et al. 2013). High concentrations of acetate and butyrate directly suppressing pathogens have been detected in BSD-treated soil (Momma et al. 2006; Muramoto et al. 2008).

Artemisia selengensis Turcz, called "Li Hao" in Chinese, is a common perennial herbaceous edible weed, widely distributed along marshes, lakeshores, and riverbanks in Northeast China and Central China (Zhang and Kong 2005). Since its high nutrition and medicinal value, it has been favored as food and herbal medicine for thousands of years in China (Peng et al. 2010). Currently, development of intensive agriculture has improved the yield of *A. selengensis*, but its cultivation efficiency is hampered by some soil-borne diseases, particularly, root rot caused by *Fusarium oxysporum* f. sp. *cubense* (FOC), *Phytophthora* spp., and *Pythium* spp. (Yang 2007). The disease causes wilted leaves, stunted growth, vascular discoloration, and brown soft roots and, finally, leads to plant death.

Given the concerns above, the aim of this study was to determine the potential of maize straw as organic amendments for BSD in suppressing *A. selengensis* root rot caused by FOC, *Phytophthora* spp., and *Pythium* spp. Different maize straw addition rates and two soil irrigation states were evaluated to test its efficiency and provide practical guidelines for BSD field promotion. Furthermore, the mechanism of suppressing pathogens was examined by investigating bacterial community structure changes and toxic organic acids productions.

2 Materials and methods

2.1 Soil and crop residues

The soil used in greenhouse experiment was collected from the farm in Baguazhou, Nanjing. The farm has planted *A. selengensis* for more than 12 years. In 2013, root rot incidence has spread most of the areas of the farm and its yield has been greatly reduced. Because some fields are no longer suitable for growing *A. selengensis*, farmers have to plant other crops such as maize as alternative. Soil samples were collected from the fields with the most serious root rot disease. The soil was clay loamy in texture, with organic matter content of

1.8 %. The soil was seriously acidified into pH 4.2 due to intensively cultivation. Locally, the natural soil is neutral. All soil was sieved with 2-mm sieve before experiment. Maize straw was collected locally as organic materials for BSD, with organic C 42.5 %. Before amended into soil, the straw were cut into sections, dried at 40 °C, and grounded into powder.

2.2 Experiment design

All experiments were conducted in greenhouse. Pots (20× 10 cm) were filled with 500-g soil sample, and treatments were set up as follows: (1) CK: no amended and flooded; (2) flood: flooded but no amended; (3) flood+0.2 %: flooded and amended with 0.2 % (*w*/*w*) maize straw; (4) flood+0.5 %: flooded and amended with 0.5 % (*w*/*w*) maize straw; (5) flood+2 %: flooded and amended with 2 % (*w*/*w*) maize straw; (6) saturate+0.5 %: saturated and amended with 0.5 % (*w*/*w*) maize straw. The pots were randomly arranged in a greenhouse with temperature ranging between 25–35 °C. Three replicates were set for each treatment. Before experiments, each pot was thoroughly mixed with designed rate of maize straw; then tap water was irrigated into soil to reach 1:1 ratio in flooded pots and to reach the maximum water-holding capacity (100 % WHC) in saturated pots; finally, each pot was sealed with a plastic film for 25 days incubation in greenhouse.

2.3 Soil properties analysis

The soil redox potential in each pot was measured in situ by an ORP meter (FJA-16, ISSAS, China) on days 1, 2, 3, 5, 10, 15, 20, and 25. Soil samples for pH and nitrate analyzing were collected on days 5, 10, 15, and 25. Soil samples for pathogen evaluation and PCR-DGGE analysis were respectively collected on days 25 and 15, and samples for organic acid analysis were collected on days 5, 10, and 15. The plastic film in each pot was carefully removed when sampling, five subsamples in each pot were collected, and then the plastic film was used to seal the pot again. After fully mixed the five subsamples obtained from each pot, the sample was prepared for analysis. Soil pH was measured in slurry (1:1 with deionized water) by a pH electrode (Mettler Toledo, FE20-FiveEasy, USA). Soil nitrate were extracted by 2 M KCl in a solution (soil:water=1:5) after 1-h 250 r/min shaking and then measured by a continuous-flow analyzer (Skalar, Netherlands). At the start and end of the experiment, organic matter of soil was determined by Walkly-Black wet digestion method. The soil maximum water-holding capacity was determined as follows: First, about 100-g soil with three replicates were used to determine soil water-holding capacity. After fully irrigating and flooding soil, the water was allowed to stand on the soil surface (about 2 cm) for 2 h. The soil container was sealed by cling film to prevent evaporation. Then the water was seeped out of soil for 6 h, and the weight of soil with 100 % WHC was obtained. The excess water was removed by drying the soil at 105 °C to constant mass.

2.4 Soil pathogen evaluation

Soil-borne pathogens were calculated by a standard 10-fold dilution method. Here, oat medium was used for the growth of *Pythium* spp., corn medium was used for *Phytophthora* spp. growth, and modified komada medium was for FOC growth. Diluted soil suspensions were inoculated into plates and incubated at 28 °C for 72 h. The total number of pathogens were determined by counting the number of colony-forming units (CFU) and transformed as log10 (CFU g^{-1} dry soil).

2.5 Soil DNA extraction and PCR amplification

According to previous researches, microorganism shift in BSD soil could be clearly observed during the first 2 weeks (Momma et al. 2013; Mowlick et al. 2013). About 0.25-g soil from each treatment was taken after 15-day incubation, and DNA was carefully extracted according to the manual instructions (MO BIO Ultra Clean® Soil DNA Isolation Kit, MO BIO Laboratories Inc., USA). The DNA quality and yield were checked on 1.2 % agarose gel (ethidium bromide stained) and visualized by UV trans-illumination. PCR was carried out with a CRX-96 thermocycler (Bio-Rad Laboratories Inc., Hercules, CA, USA). The primer systems used for amplification of bacteria 16S rDNA fragments were listed in Table 1 (Zoetendal et al. 1998). The 25 μl PCR mixture contained 12.5 μl master mix (including Taq DNA Polymerase, dNTP, Tris-HCl, KCl, and $MgCl_2$, 2× Taq PCR Master Mix, Tiangen Biotech, Beijing, China), 1 μl of each primer, 5 μl DNA template, and 5.5 μl sterilized dd-H_2O. Thermal cycling conditions were as follows: an initial denaturing step at 94 °C for 4 min followed by 35 cycles consisting denaturation at 94 °C for 30 s, 30 s for primer annealing at 57 °C and 45 s at 72 °C for primer elongation. Cycling was finally extended at 72 °C for 10 min and cooled to 4 °C. The PCR products obtained from PCR reactions were checked by 1.2 % agarose gel electrophoresis.

Table 1 Primers used in the experiment

Primers[a]	Sequence (5′–3′)[b]	Product size (bp)	Reference
GC-U968 (F)	GC-AACGCGAA GAACCTTAC	490	Zoetendal et al. (1998)
L1401 (R)	GCGTGTGTACA AGACCC	400	Zoetendal et al. (1998)

[a] F forward primer, R reverse primer

[b] A GC-rich sequence (GC.-) attached to the 5' end of sequences is indicated

2.6 DGGE analysis and DNA sequencing

PCR amplified 16S DNA fragments were fingerprinted using a D-GENE System (Bio-Rad Laboratories, Hercules, CA, USA). PCR products were loaded onto 6 % (*w*/*v*) polyacrylamide gels (40 % acrylamide/bis-solution, 37.5:1, Bio-Rad) containing a linear denaturing gradients ranging from 50 to 70 %. Gels were performed electrophoresis for 16 h at 60 °C. After the electrophoresis, gels were stained with SYBR® Gold nucleic acid gel stain (Molecular Probes, Eugene, OR) and digitally photographed, and analyzed by image analysis (Quantity One 4.6.3, Bio-Rad).

The target DGGE bands were carefully excised by a sterilized scalpel and each band was eluted into 20 μl sterilized dd-H_2O at 4 °C overnight. Then 2 μl DNA elution was taken to re-amplification by the same PCR conditions listed above. The PCR products were analyzed by same DGGE program to confirm the expected products isolation. Only samples displaying a single band which co-migrated with its original sample were excised. After overnight elution, they were amplified with the primers without GC clamp, purified and cloned to pEASY-T1 vector (Transgen, Beijing, China), and sequenced in Genscript Company (Nanjing, China). The recovered sequences were aligned with bacteria gene fragments available from the National Center for Biotechnology Information databases (NCBI). The closest known relatives of the partial bacteria sequences were found by BLAST searches in GenBank.

2.7 Toxic organic acids determinations

1 ml soil solution sample was collected from each pot to detect toxic organic acids by small size of soil water samplers (Daiki, Japan, contributed by Prof. Yuso Kobara) on days 5, 10, and 15. Then they were centrifuged at 3000 rpm for 5 min and identified by an HPLC system (Agilent 1260, USA) according to the modified method of Ling (Ling et al. 2011). XDB-C18 column (4.6×250 mm, Agilent, USA) with a gradient solution of 2.5 mM H_2SO_4 (A) and methanol (B) were used to isolate organic acids. The gradients were set as follows: first 95 % A plus 5 % B at the flow rate of 1 ml min^{-1}/5 min, then 95 % A plus 5 % B at the rate of 1 ml min^{-1}/8 min, 85 % A plus 15 % B at the rate of 1 ml min^{-1}/40 min, 85 % A plus 15 % B at the rate of 1 ml min^{-1}/stop. The wavelength of UV detector was 210 nm. Organic acid components were isolated and identified through comparing retention times and peak areas with standards (Sigma, USA).

2.8 Data analysis

All data collected were checked for normality and homogeneity and appropriately transformed before performing statistical analysis. The ANOVA module in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and SNK tests ($p \leq 0.05$) was used to analyze the variances among different treatments.

3 Results

3.1 Soil Eh and pH

During the first 3 days, soil redox potentials (Eh) exhibited sharp drop in all BSD treatments (Fig. 1a). The speeds of Eh drop decreased in the following order: flood+2 %>flood+0.5 %>flood+0.2 %>saturate+0.5 %. The lowest Eh was observed in the treatment of flood+2 % at day 3, in which Eh decreased from 300 to −416 mv. Although the Eh drop varied very significantly ($p<0.001$) across BSD treatments at the beginning of experiment, their differences were smaller at the end of experiment (about −150~200 mv) and even totally diminished between the treatments of flood+0.5 %, flood+2 %, and saturate+0.5 % ($p>0.05$). On the contrast, the Eh in flooding alone treatment was only slightly decreased to 88 mv, and the Eh in CK treatment was kept around 300 mv through the whole experiment.

Compared with CK, the soil pH was apparently elevated in all flooded or water-saturated treatments (Fig. 1b). The pH in CK was maintained around 4.3 throughout the experiment, while it was rapidly increased beyond 4.7 in other treatments on day 5 and further raised above 5.3 on day 25. The highest pH was in the treatment of flood+0.5 % (pH=5.60). No statistical difference in the final pH was observed between each BSD treatment ($p>0.05$) and between flooding alone and BSD treatments ($p>0.05$).

3.2 Soil-borne pathogen

The initial populations of three kinds of pathogens in the soil were above 3×10^4 CFU g^{-1} soil. Incorporating maize straw into flooded or saturated soil did result in significant mortality of soil-borne pathogens (*Phytophthora* spp., *Pythium* spp., and FOC) (Fig. 2). The flood+2 % treatment exhibited the most pronounced inactivation of pathogens, up to 90 % for all three kinds of pathogens. Although the final pathogens populations of the other three BSD treatments (flood+0.2 %, flood+0.5 %, and saturate+0.5 %) were still remarkably higher than that in the flood+2 % treatment, they also experienced sharp reduction of pathogens (about 75~80 %) and the differences of their final pathogens populations were smaller or not significant. Flooding alone did inactivate pathogens as well, but the effect was quite insignificant when compared with BSD treatments. Less than 65 % pathogens were reduced in flooding alone treatment. For CK, its viable pathogens were persisted over 2×10^4 CFU g^{-1} soil till the end of the experiment.

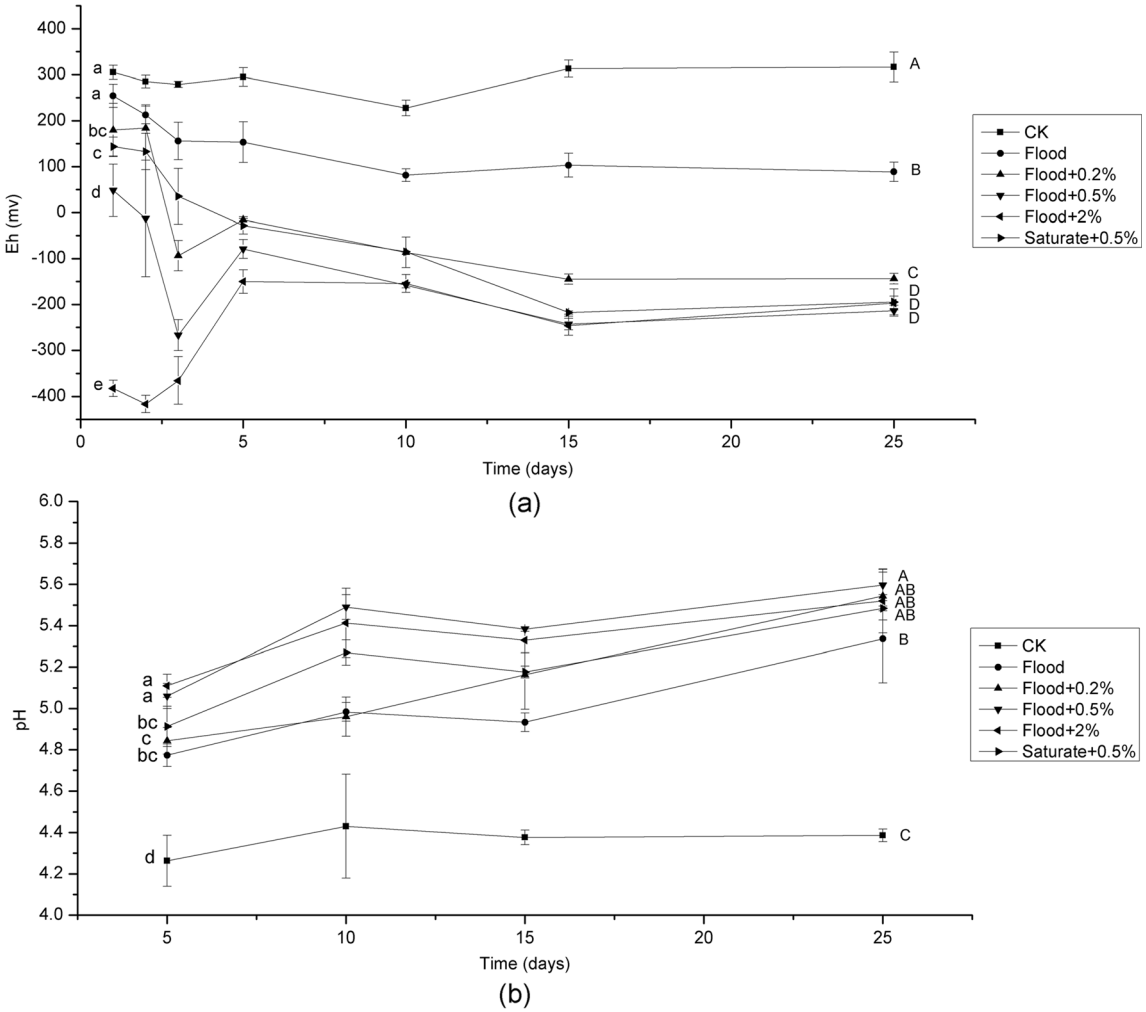
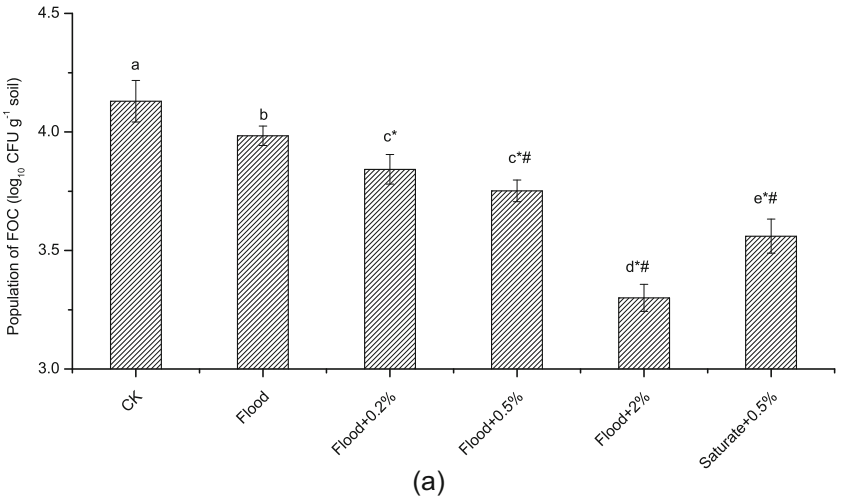


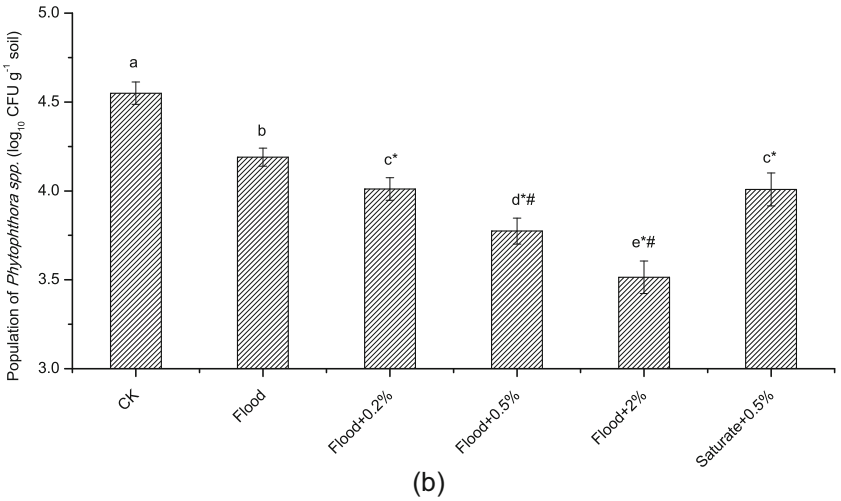
Fig. 1 **a**, **b** Change in soil Eh and pH during the 25-day incubation. *Bars* refer to one standard deviation. The treatments are (1) CK: no amended and flooded, (2) flood: flooded but no amended, (3) flood+0.2 %: flooded and amended with 0.2 % (*w*/*w*) maize straw, (4) flood+0.5 %: flooded and amended with 0.5 % (w/w) maize straw, (5) flood+2 %: flooded and amended with 2 % (*w*/*w*) maize straw, and (6) saturate+0.5 %: saturated and amended with 0.5 % (*w*/*w*) maize straw. Within days 1 and 25, *means indicated by the same letter* are not significantly different, $p>0.05$

3.3 Community composition of bacteria

The DGGE banding patterns of bacterial community from the soils are shown in Fig. 3. Clear variability in bands position and intensity could be visually inspected from different treatments. Upon the DGGE profiles, the community structure of bacterial populations in CK was represented by a series of weak, resolved but still distinguishable bands, with two or three dominant intense bands. However, an apparent increasing bacterial diversity was evidenced in both flooded alone and BSD treatments accompanied by the occurrence of more intense and dominant bands (8~12). The bacterial diversity peaked in samples from flood+2 % treatment, with about 51 bands, while there are only 39 and 45 bands in CK and flooding alone, respectively. The majority of dominant bands were localized in the upper part of the gel in saturated treatment, whereas they were in the middle part of the gel in flooded treatments.

In the gel, three replicates profiles of each treatment were highly repetitive; therefore, cluster analysis of DGGE banding patterns by the unweighted pair-group method using arithmetic averages (UPGMA) was utilized to generate similarity dendrogram (Fig. 4). The clustering profile showed apparent two broad clusters (CK; flooded or saturated treatments), indicating that CK was most distinct from the other treatments. Nearly all samples from different treatments could be identified from each other and their replicates were grouped into the same clusters, which substantiated the visual inspection results of DGGE profile. Samples from saturated treatment were grouped on a separate cluster sharing a 50 % similarity with other flooded treatments, which further confirmed the visual results. Among the three flooded treatments, two BSD





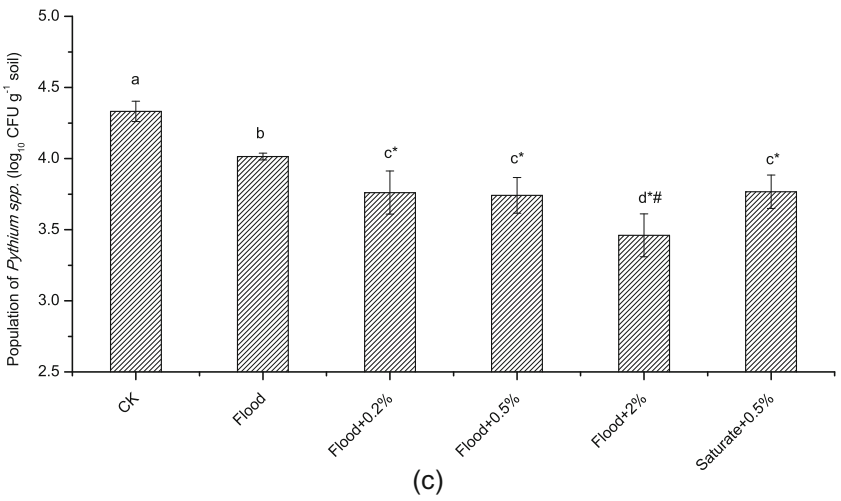


Fig. 2 **a**–**c** Suppressing effects on *Artemisia selengensis* root rot pathogens (FOC, *Phytophthora* spp., and *Pythium* spp.) after 25-day incubation. Data presented are averages of treatments with standard deviation. Column indicated by same *letters* are not significantly different ($p>0.05$). *Bars marked with* * or # are very significantly different with the CK or flood treatments, respectively ($p<0.001$)

treatments were more alike, while the flooded alone treatment shared a 60 % level similarity with them.

Furthermore, DNA sequences of the recognized different bands in the gel showed that *Verrucomicrobiaceae sp*., *Clostridium sp*., and *Bacillus sp*., were detected in flooded or saturated treatments (Table 2). DGGE bands 1 and 2 both showed 99 % similarities to *Verrucomicrobiaceae sp*., which was a typical bacteria species engaged in fermentation process in the soil. Band 3 exhibited 100 % similarity to uncultured *Candidatus Saccharibacteria bacterium* (EF016808), a

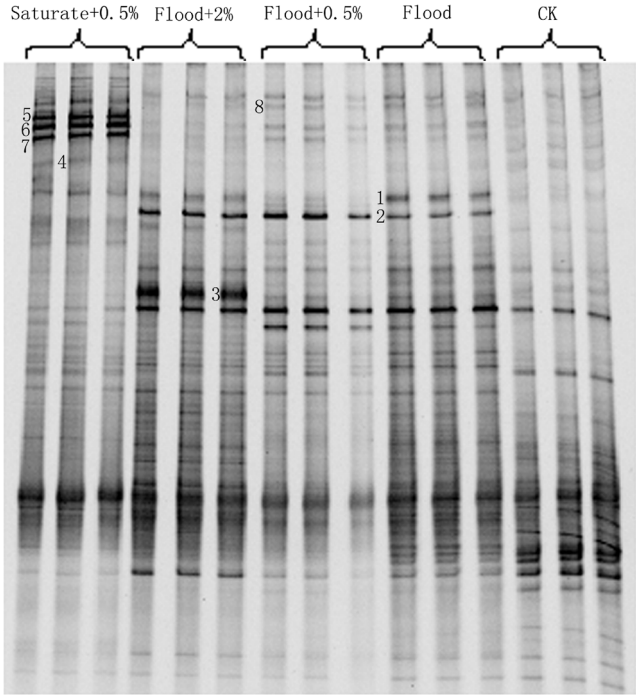


Fig. 3 The original DGGE profile of the bacterial community in different treatments on day 15 (CK, flood, flood+0.2 % maize straw, flood+2 % maize straw, saturate+0.5 % maize straw). *Bands indicated with numbers* were excised, re-amplified, and sequenced

candidate TM7 species usually found in sludge or rainforest soil. In addition, bands 4, 5, 6, and 7 all showed high nucleotide similarity to *Clostridium sp*. (from 97 to 98 %), which included many species producing organic acids under anaerobic soil environment.

3.4 Organic acids produced in BSD

Soil solution samples were collected and analyzed by HPLC to investigate whether organic acids were produced in the treatments (Fig. 5). Of the four organic acids tested, acetic acid was the major part in all BSD treatments, while isovaleric acid was the minor one. In both flood+0.2 % and flood+0.5 % treatments, four organic acids were not detected until 10 days after the start of treatments in most cases and, generally, followed by an obvious decline on day 15. Change of organic acids in the saturate+0.5 % treatment largely mirrored this trend, in spite of that they were detectable on day 5. In the flood+2 % treatment, a different trend was observed, with gradual increasing concentrations of organic acids from day 5 to day 15. Across the four BSD treatments, the highest concentrations of the four organic acids were all in the flood+2 % treatment, with 7.84, 2.30, 2.16, and 1.75 mM for acetic acid, propionic acid, butyric acid, and isovaleric acid, respectively. The second one was in the saturate+0.5 % treatment, and the lowest one was displayed by the flood+0.2 % treatment. None of the four organic acids was detected in CK and flooding-alone treatments.

4 Discussion

Biological soil disinfestation is a promising, effective, and non-chemical method, which achieves soil disinfestation and controls soil-borne disease by incorporating decomposable organic material into flooded soil. In this study, different rates of maize straw were amended into flooded or water-saturated soil to investigate the potential of this method on suppressing *A. selengensis* root rot caused by FOC, *Phytophthora* spp., and *Pythium* spp. The soil bacterial community changes and organic acids dynamics were analyzed to help in clarifying the mechanism of this method.

Addition of crop residues, like maize, rice, or wheat straw in the soil has been reported to remarkably reduce several kinds of fusarium wilt disease (Shinmura 2002; Yossen et al. 2008; Huang et al. 2013). In the study, a sharp decline in three kinds of viable *A. selengensis* root rot pathogens (up to 90 %) were found in all maize straw amended treatments under either flooded or saturated conditions. This confirms that maize straw was an effective organic material in suppressing a wide spectrum of soil-borne disease. Of the three addition rates of maize straw, the highest one (2 %, *w*/*w*) resulted in the greatest mortality of pathogens. This agrees with what Momma et al. (2010) have found that improving organic material addition rates could reduce more pathogens and even shorten the BSD period. No statistical distinction was noted in the other two lower addition rates (0.2 and 0.5 %, *w*/*w*). Admittedly, compared with CK and flooding-alone treatments, the two lower rates have effectively decreased pathogens. Moreover, they are relatively less than common addition rates of other BSD organic material (Subbarao et al. 1999; Momma et al. 2006; Momma 2008; Butler et al. 2012; Momma et al. 2013), indicating their higher efficacy in BSD.

In spite of that, the drop of Eh in saturated treatment was not as dramatic as that in flooded treatment at the beginning of the experiment, and the difference between their reductive states was gradually diminished and become unobservable at the end of experiment. Nearly equal pathogens mortalities were noted in flooded and saturated treatments with same addition rates of maize straw. This is accorded with previous reports that irrigating soil to reach its maximum water-holding capacity (WHC) was sufficient to establish a strong anaerobic and reductive environment to reduce pathogens (Snyder 1987; Kobara et al. 2007). Besides, some researches proposed that even 20~30 % moist soil was capable to effectively suppress pathogens when paired with organic material and covered with plastic films (Blok et al. 2000; Butler et al. 2012b; Momma et al. 2013).

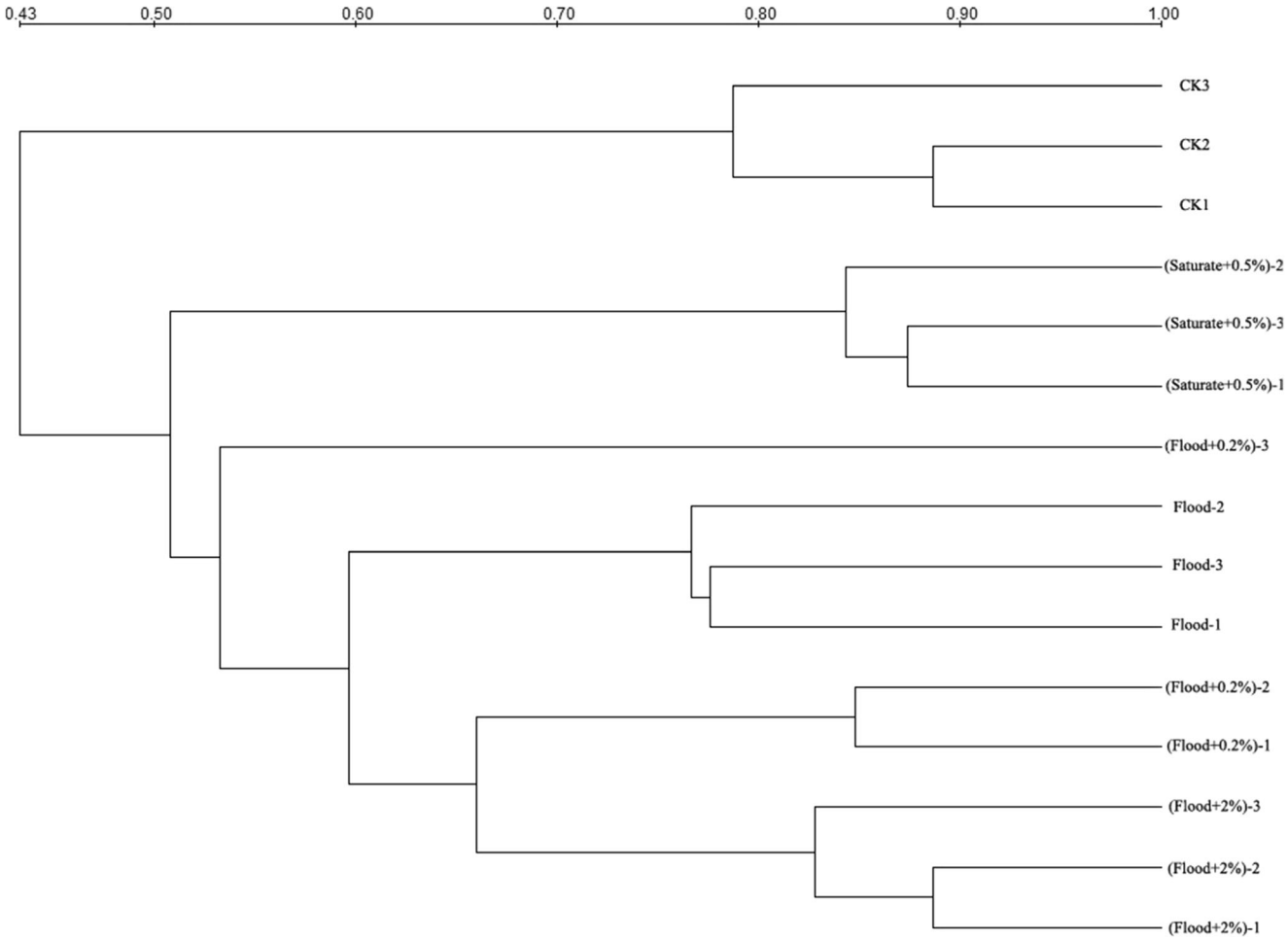


Fig. 4 Similarity dendrograms (UPGMA, Dice coefficient of similarity) of bacterial banding patterns of different treatments. The similarity dendrograms (ranging from 0–1) were calculated based on PCR-DGGE profiles in Fig. 3

Our molecular results illustrated that a marked indigenous soil bacterial communities shifted in all flooded and saturated soil. As we have known, flooding used to reducing fungi populations while leaving bacteria unaffected or even increasing it (Drenovsky et al. 2004; Huang et al. 2015). Organic amendments such as compost or manure have been illustrated that they could affect natural soil microorganisms, especially bacteria community, and provide biological control to soilborne pathogens (Bulluck et al. 2002; Tiquia et al. 2002; Gelsomino and Cacco 2006). In BSD, the anaerobic decomposition of organic matter stimulates growth and proliferation of anaerobic bacteria (Blok et al. 2000; Mowlick et al. 2013). Some of them, such as *Clostridium* and *Bacillus* spp., are well-known toxic producers and have antagonistic activity

Table 2 Phylogenetic relationships of bands sequence from bacterial PCR-DGGE profiles (Fig. 3)

Representative sequence (GenBank accession number)	Best match database (GenBank accession number)	Similarity (%)
1(KT157526)	Uncultured *Verrucomicrobia bacterium* (AJ629850)	99
2(KT157526)	Uncultured *Verrucomicrobia bacterium* (AJ629850)	99
3(KT157527)	Uncultured *Candidatus Saccharibacteria bacterium* (EF016808)	100
4(KT157528)	Uncultured *Clostridium* sp. (AB286217)	100
5(KT157529)	Uncultured *Clostridium* sp. (AY330123)	97
6(KT157530)	Uncultured *Clostridium* sp. (JX230409)	97
7(KT157531)	Uncultured *Clostridium* sp. (HE575392)	98
8(KT157532)	*Bacillus subtilis* (AY219900)	96

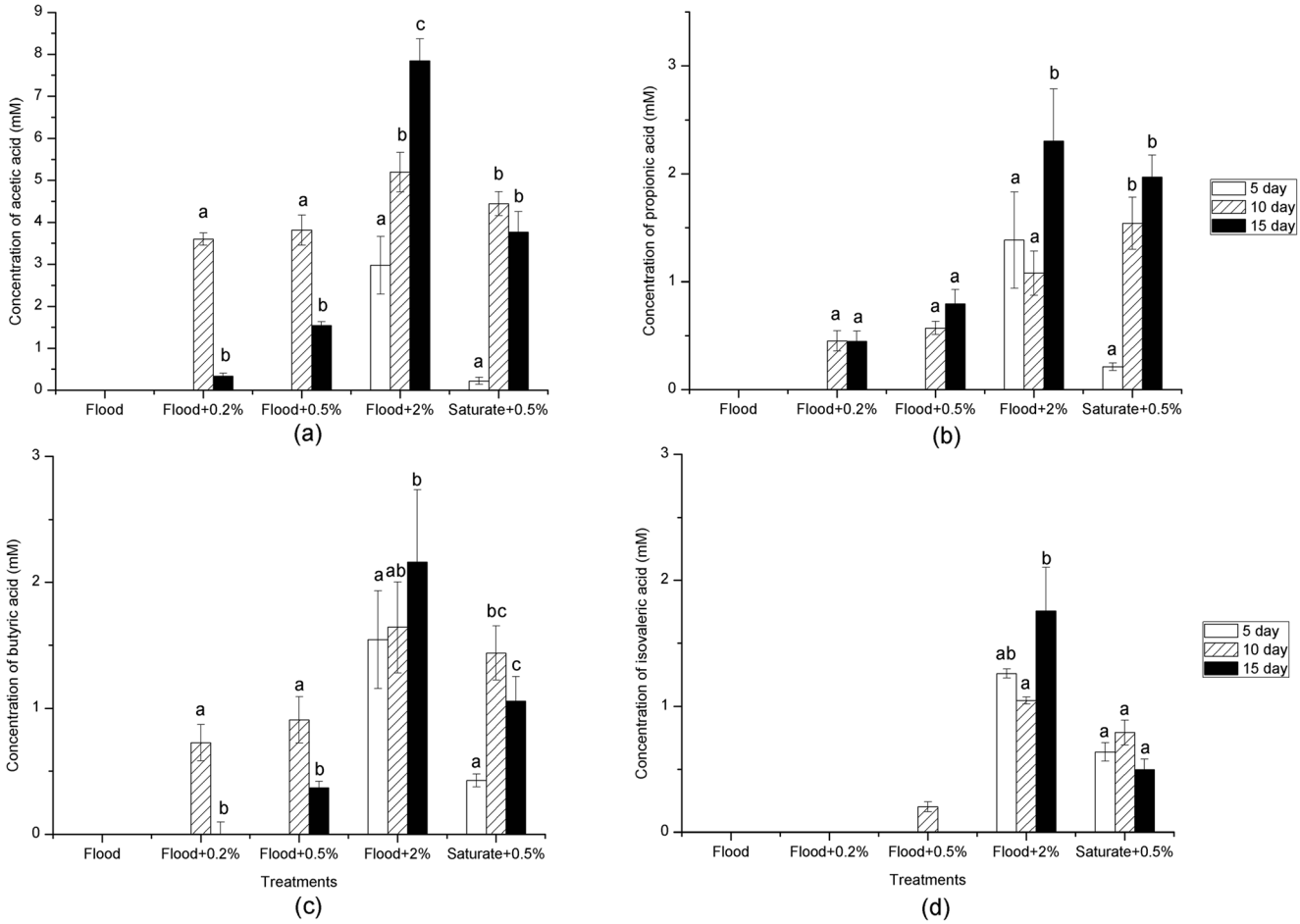


Fig. 5 **a**–**d** The changes of acetic acid, propionic acid, butyric acid, and isoveleric acid concentrations from 0–15 days in treatments of flood, flood+0.2 %, flood+0.5 %, flood+2 %, and saturate+0.5 %. Data presented are averages of treatments with standard deviation. Within each treatment, *bars indicated by the same letter* are not significantly different, $p>0.05$

(Mowlick et al. 2013; Mowlick et al. 2014). Consistently, although distinguishes in DGGE patterns were observable between each BSD treatment, especially between the saturated and flooded BSD treatments, the bands representing the two bacterial species were very intense and well resolved in all of the BSD treatments, indicating that they were dominant bacterial types in BSD soil. Ironically, same bands also appeared in the flooding-alone treatment, but flooding alone never reduced pathogens as effectively as BSD did. According to our DGGE profile, the bacterial biodiversity in BSD treatments was apparent higher than that in flooding alone, indicating many other bacterial species proliferated in BSD soils, especially in the highest maize straw addition treatment. Multiplication of these indigenous microorganisms might compete with pathogens in nutrients and niches and play a role in pathogens suppression. Since functions of these species are not clear, further research is needed to provide more evidences.

It has been proven that the dominated clostridial species in BSD is tightly connected with accumulation of acetate and butyrate, which could greatly suppress pathogens (Momma et al. 2006; Momma et al. 2010). Here, rather high concentrations of acetic acid were detected in all BSD treatments, together with lower amounts of butyric, propionic acid, and isoveleric acids, while none of them was detected in CK and flooding-alone treatments. The four detected organic acids were known to be generated via anaerobic decomposition of straw (Gotoh and Onikura 1971). Their amount enhanced with the added rates of straw and peaked during 10 and 20 days (Rao and Mikkelsen 1976; Shan et al. 2008). Our experiments verified that the highest production of organic acids appeared in the largest addition rates of maize straw. Accumulation of organic acids gradually decreased after 10 days incubation, except the highest maize straw addition treatment. Momma et al. (Momma et al. 2006) suggested that anaerobic bacteria such as *Clostridium* spp. might proliferate using organic material as growth substrate immediately after the establishment of reductive and anaerobic condition, but with the consumption of substrate, less organic acids would be generated. Pathogens mortality coincided well with the change of organic acids, with most dramatic decrease of pathogens occurring in the first 10~15 days in BSD (Momma 2008; Klein et al. 2011, 2012). It is worth to noting that saturated BSD treatments seemed to produce higher concentrations of organic

acids than flooded ones at the same addition rate of maize straw. Since less water was added in saturated soil, it is one possible reason to explain their higher concentrations of organic acids than flooding treatments. Overall, our results emphasize again the important roles of toxic organic acids (acetate, propionate, butyrate, and isovelerate) in pathogen suppression. Since only four organic acids were examined in our experiments, other unknown volatile fatty acids and compounds are possibly engaged in BSD process as well.

Besides a direct killing of pathogens, effects of BSD on soil microbiota might also directly or indirectly result from the changes in soil physical and chemical properties, such as soil pH, Ec, nutrients, and soil structure (Ferreras et al. 2006; Gelsomino and Cacco 2006; Gopinath et al. 2008; Butler et al. 2014). In this study, the original soil pH (4.2) was very acidic and not suitable for general plant growth and microbial activity, but it increased up to 6.0 after BSD treatment. However, rather contrasting results were reported regarding the soil pH change in BSD: in some cases the soil pH was found to be reduced (Momma et al. 2006; Huang et al. 2015), whereas in others increasing pH was reported (Butler et al. 2012; Butler et al. 2014). In fact, the change of soil pH depends on the original pH in soil. That is, after BSD treating, pH value tends to decrease in soil with higher original pH (>6.0), but to increase in soil with lower original pH (<5.0). Therefore, BSD plays an important role in soil pH amelioration.

The total organic matter in soil was greatly improved; for example, it was increased from 1.81 to 2.68 % in flood+2 % treatment, but soil nitrate concentration decreased to nearly zero level (data not shown). Soil provides a habitat for living organisms, including plants and microbiota, whose activities are tightly coupled with the physicochemical properties of soil (Paul 2006). Improvement of soil properties frequently induce positive impact in microbiology communities and crop growing (Tejada et al. 2006; Stark et al. 2007; Gopinath et al. 2008). As we have known, increasing soil microorganism diversity is likely to be antagonistic to pathogens or beneficial to crop growth (Gelsomino and Cacco 2006). The promoting growth of crop, in turn, may influence the abundance and activities of soil microorganism by releasing exudates and lysates in the rhizosphere (Grayston et al. 1998) that might further show biological control on pathogens. Thus, to fully explore the mechanism of BSD, additional experiments must be undertaken to clarify the inherent interactions between soil physicochemical properties, crop growth, and microbiology communities and how these mutual relationships influence the survival and growth of soil-borne pathogens.

5 Conclusions

In this study, effects of maize straw as BSD organic material was clearly observed in suppressing *A. selengensis* root rot pathogens via greenhouse experiments under flooded and water-saturated conditions. All three addition rates of maize straw ranged from 0.2 to 2 % could effectively inactivate FOC, *Phytophthora* spp., and *Pythium* spp., although the largest amount of maize straw addition did result in significantly higher pathogens' mortality than the other two rates. Saturating soil to reach 100 % WHC was a good alternative to soil flooding in BSD, because no obvious difference appeared between them at the same rates of organic amendments. BSD induced clear bacterial community shifts with enhanced biodiversity, and two well-known toxic organic acids producers, *Clostridium*, and *Bacillus* spp., were dominant taxonomic groups. Four toxic acids, acetate, propionate, butyrate, and isovelerate were accumulated in BSD, but gradually diminished after 10 to 15 days. Soil pH and organic matter increased, while NO_3^- disappeared after BSD treatment. All the factors observed would contribute to the inactivity of FOC to different extent.

Acknowledgments The authors are very grateful to the editors and reviewers for their invaluable comments and suggestions. This work was supported by the Research Fund of State Key Laboratory of Soil and Sustainable Agriculture, Nanjing Institute of Soil Science, Chinese Academy of Science (Y412201404), National Natural Science Foundation of China (41222005, 41330744, 413301335), and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

Blok WJ, Lamers JG, Termorshuizen AJ, Bollen GJ (2000) Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253–259

Bulluck LR, Brosius M, Evanylo GK, Ristaino JB (2002) Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. Appl Soil Ecol 19:147–160

Buresh R, Sayre K (2007) Implications of straw removal on soil fertility and sustainability. Expert consultation on biofuels. IRRI, Los Banos, Philippines: 34

Butler DM, Kokalis-Burelle N, Muramoto J, Shennan C, McCollum TG, Rosskopf EN (2012) Impact of anaerobic soil disinfestation combined with soil solarization on plant-parasitic nematodes and introduced inoculum of soilborne plant pathogens in raised-bed vegetable production. Crop Prot 39:33–40

Butler DM, Kokalis-Burelle N, Albano JP, McCollum TG, Muramoto J, Shennan C, Rosskopf EN (2014) Anaerobic soil disinfestation (ASD) combined with soil solarization as a methyl bromide alternative: vegetable crop performance and soil nutrient dynamics. Plant Soil 378:365–381

Drenovsky R, Vo D, Graham K, Scow K (2004) Soil water content and organic carbon availability are major determinants of soil microbial community composition. Microb Ecol 48:424–430

Ferreras L, Gomez E, Toresani S, Firpo I, Rotondo R (2006) Effect of organic amendments on some physical, chemical and biological properties in a horticultural soil. Bioresource Technol 97:635–640

Gadde B, Bonnet S, Menke C, Garivait S (2009) Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environ Pollut 157:1554–1558

Gelsomino A, Cacco G (2006) Compositional shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. Soil Biol Biochem 38:91–102

Gopinath KA, Saha S, Mina BL, Pande H, Kundu S, Gupta HS (2008) Influence of organic amendments on growth, yield and quality of wheat and on soil properties during transition to organic production. Nutr Cycl Agroecosys 82:51–60

Gotoh S, Onikura Y (1971) Organic acids in a flooded soil receiving added rice straw and their effect on the growth of rice. Soil Sci Plant Nutr 17:1–8

Grayston SJ, Wang S, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378

Hrynchuk L (1998) Rice straw diversion plan. California Air Resources Board Sacramento, California

Huang X, Wen T, Zhang J, Zhu T, Cai Z (2013) The inhibitory effect of quickly and intensively reductive soil on *Fusarium oxysporum*. Acta Ecol Sin 34(16):4526–4534 **(in Chinese)**

Huang X, Wen T, Zhang J, Meng L, Zhu T, Cai Z (2015) Toxic organic acids produced in biological soil disinfestation mainly caused the suppression of *Fusarium oxysporum* f. sp. *cubense*. BioControl 60: 113–124

Katan J (2000) Physical and cultural methods for the management of soilborne pathogens. Crop Prot 19:725–731

Klein E, Katan J, Gamliel A (2011) Combining residues of herb crops with soil heating for control of soilborne pathogens in a controlled laboratory system. Crop Prot 30:368–374

Klein E, Katan J, Gamliel A (2012) Soil suppressiveness to *Meloidogyne javanica* as induced by organic amendments and solarization in greenhouse crops. Crop Prot 39:26–32

Kobara Y, Uematsu S, Tanaka-Miwa C, Sato R, Sato M (2007) Possibility of the new soil fumigation technique with ethanol solution. http://www.mbao.org/2007/PDF/Preplant/PP15. Proceedings of 2007 annual international research conference on methyl bromide alternatives and emissions reductions

Larkin RP, Griffin TS (2007) Control of soilborne potato diseases using Brassica green manures. Crop Prot 26:1067–1077. doi:10.1016/j.cropro.2006.10.004

Ling N, Huang Q, Guo S, Shen Q (2011) *Paenibacillus polymyxa* SQR-21 systemically affects root exudates of watermelon to decrease the conidial germination of *Fusarium oxysporum* f. sp. *niveum*. Plant Soil 341:485–493

Momma N (2008) Biological soil disinfestation (BSD) of soilborne pathogens and its possible mechanisms. Jan Agr Res Q 42:7–12

Momma N, Yamamoto K, Simandi P, Shishido M (2006) Role of organic acids in the mechanisms of biological soil disinfestation (BSD). J Gen Plant Pathol 72:247–252

Momma N, Momma M, Kobara Y (2010) Biological soil disinfestation using ethanol: effect on *Fusarium oxysporum* f. sp. *lycopersici* and soil microorganisms. J Gen Plant Pathol 76:336–344

Momma N, Kobara Y, Momma M (2011) Fe^{2+} and Mn^{2+}, potential agents to induce suppression of *Fusarium oxysporum* for biological soil disinfestation. J Gen Plant Pathol 77:331–335

Momma N, Kobara Y, Uematsu S, Kita N, Shinmura A (2013) Development of biological soil disinfestations in Japan. Appl Microbiol Biot 97(9):3801–3809

Mowlick S, Takehara T, Kaku N, Ueki K, Ueki A (2013) Proliferation of diversified clostridial species during biological soil disinfestation incorporated with plant biomass under various conditions. Appl Microbiol Biot 97:8365–8379

Mowlick S, Inoue T, Takehara T, Tonouchi A, Kaku N, Ueki K, Ueki A (2014) Usefulness of Japanese-radish residue in biological soil disinfestation to suppress spinach wilt disease accompanying with proliferation of soil bacteria in the *Firmicutes*. Crop Prot 61:64–73

Muramoto J, Shennan C, Fitzgerald A, Koike S, Bolda M, Daugovish O, Rosskopf E, Kokalis-Burelle N, Butler D (2008) Effect of anaerobic soil disinfestation on weed seed germination. http://mbao.org/2008/Proceedings. Proceedings of the annual international research conference on methyl bromide alternatives and emissions reductions

Núñez-Zofío M, Larregla S, Garbisu C (2011) Application of organic amendments followed by soil plastic mulching reduces the incidence of *Phytophthora capsici* in pepper crops under temperate climate. Crop Prot 30:1563–1572

Paul EA (2006) Soil microbiology, ecology and biochemistry. Elsevier, Oxford

Peng L, Jia X, Wang Y, Zhu H, Chen Q (2010) Ultrasonically assisted extraction of rutin from *Artemisia selengensis* Turcz: comparison with conventional extraction techniques. Food Anal Methods 3: 261–268

Rao DN, Mikkelsen D (1976) Effect of rice straw incorporation on rice plant growth and nutrition. Agron J 68:752–756

Shan Y, Johnson-Beebout S, Buresh R (2008) Crop residue management for lowland rice-based cropping systems in Asia. Adv Argon 98: 117–199

Shinmura A (2002) Studies on the ecology and control of Welsh onion root rot caused by Fusarium redolens. J Gen Plant Pathol 68:265–265

Snyder GH (1987) Agricultural flooding of organic soils. http://ufdc.ufl.edu/UF00026741/00001 Agricultural Experiment Station, Institute of Food and Agricultural Sciences, University of Florida

Stark C, Condron LM, Stewart A, Di HJ, O'Callaghan M (2007) Influence of organic and mineral amendments on microbial soil properties and processes. Appl Soil Ecol 35:79–93

Subbarao KV, Hubbard JC, Koike ST (1999) Evaluation of broccoli residue incorporation into field soil for Verticillium wilt control in cauliflower. Plant Dis 83:124–129

Tejada M, Garcia C, Gonzalez JL, Hernandez MT (2006) Use of organic amendment as a strategy for saline soil remediation: influence on the physical, chemical and biological properties of soil. Soil Biol Biochem 38:1413–1421

Tiquia SM, Wan H, Tam NF (2002) Microbial population dynamics and enzyme activities during composting. Compost Sci Util 10:150–161

Uematsu S, Tanaka-Miwa C, Sato R, Kobara Y, Sato M (2007) Ethyl alcohol as a promising material of reductive soil disinfestation for controlling root knot nematode and soilborne plant diseases. http://www.mbao.org/2007/Proceedings Proceedings of 2007 annual international research conference on methyl bromide alternatives and emissions reductions

Yang Z (2007) Cultivation techniques to increase Artemisia selengensis yields. Hangzhou Agricultural Sci 1:32–33 **(in Chinese)**

Yossen V, Zumelzu G, Gasoni L, Kobayashi K (2008) Effect of soil reductive sterilisation on Fusarium wilt in greenhouse carnation in Cordoba, Argentina. Australas Plant Pathol 37:520–522. doi:10.1071/ap08039

Zhang J, Kong L-y (2005) Study on chemical constituents from *Artemisia selengensis* leaves. Chin Pharm J 40:1778 **(in Chinese)**

Zoetendal EG, Akkermans AD, De Vos WM (1998) Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl Environ Microb 64:3854–3859