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Full Length Article

Effects of Bio-organic Fertilizer on Soil Microbiome against *Verticillium dahliae*

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Abstract

The effects of three years continuous application of different rates of bio-organic fertilizer (BOF) were evaluated on three kinds of the counts of soil microbes, microbial metabolic activity and disease index of verticillium wilt through greenhouse pot experiments. The results showed that, the BOF not only reduced the occurrence of verticillium wilt in cotton and decreased the counts of *Verticillium dahliae*, but also improved the number of fungi, bacteria, actinomycetes, and microbial activity. The number of *V. dahliae* reduced gradually and fungi, bacteria, actinomycetes and average well color development (AWCD) firstly increased and then decreased with increasing the amount of fertilization in different organic matter content of soil. The number of *V. dahliae* was significantly increased by three years of fertilization. It showed that application of bio-organic fertilizer only delays the growth in the number of pathogen in soil within a certain time. The numbers of bacteria, actinomycetes were increased and the number of fungi was slightly reduced with increase of fertilization period. Application of bio-organic fertilizer made *AWCD* significantly higher than no fertilization (CK). By cluster analysis and principal component analysis, the results showed that the classification similar to disease index of various treatments, and it coincided with the number of microorganisms and *AWCD* values. BOF not only can control and improve the metabolic characteristics of soil microbial communities, but also maintain a high soil biological activity. © 2016 Friends Science Publishers

Keyword: BOF; BIOLOG; Cotton verticillium wilt; *V. dahliae*; Principal component analysis

Introduction

Soil microorganism is an important component part of it for maintaining soil microbial ecosystem's stability and sustainability (Pankhurst *et al.*, 1996; Schloter *et al.*, 2003). Studies have shown that soil microorganism take part in more or less 90% of the soil reaction (Coleman *et al.*, 1996). Distribution and activity of microorganisms in the soil was the combined result of the mutual influence and adaptation of the soil environment and microbial communities. Functional diversity of soil microbial community reflected the ecological characteristics of soil microbial communities; it was an important index of soil quality, maintenance of soil fertility and crop productivity (Hai *et al.*, 2010).

Fertilization was one of the most important measures that affected the soil quality and sustainable development. Application of fertilization regulated the storage and transformation of soil nutrients and impacted soil fertility and soil biota (Zelles, 1999). Different fertilization systems could indicate significant differences in community structure and soil microbial quantity. Applied nitrogen fertilizer in a long-term had reduced soil microbial activity (Fauci and Dick, 1994), however, added manure and plant residues to the soil to maintain soil fertility and microbial stability of the system (Marschnera *et al.*, 2003). Straw returning could increase the soil organic matter content, improved soil structure, reduced failure of soil fertility (Hooker *et al*., 2005), conducive to growth and reproduction of soil microbe, and increased soil microbial diversity (Marschnera *et al*., 2003).

Many studies indicate that application of organic (class) of fertilizer plays a part in prevented soil-borne diseases (Hoitink *et al*., 1996; Serra-Wittling *et al*., 1996). In particular the application of bio-organic fertilizer would be more effective which combined organic fertilizer with functional microorganisms (Chen *et al*., 1995; Hoitink and Boehm, 1999). Both Srivastava *et al.* (2010) and Singh *et al.* (2007) reported that use of biocontrol agents control soil-borne diseases had certain influence, but the effect was very unstable and the control rate was 50% generally. The reason may be that biocontrol agents have died before playing the roles and soil environment was not conducive to the growth of biocontrol agents. Application of biocontrol agents could take advantage of the nutrients in organic fertilizer to promote rhizosphere colonization, thus play a biological role after the combination of biocontrol agents and organic

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fertilizer. Efficiency to control soil-borne disease could be achieved more than 70% in some microbial organic fertilizer (Zhang *et al.*, 2008; Ling *et al.*, 2010). Although microbial organic fertilizer was less efficient than chemical pesticides; it was clearly a better choice from the perspective of the safety of national agricultural products. Efficiency to control soil-borne disease of other micro-organic fertilizer could reach more than 80% by improving the micro-organic fertilizer formulation and production process and optimization of application method (Yogev *et al.*, 2009; Wu *et al.*, 2009).

Different researchers had different views on the control mechanism of bio-organic fertilizer for controlled soil-borne diseases (Craft and Nelson, 1996; Zhang *et al.*, 1998). In this study, we used new bio-organic fertilizer (BOF) with the help of second solid-state fermentation, composed of antagonistic bacteria and suitable organic fertilizer. By this way, it had significant results to control soil-borne disease of cotton, cucumber, watermelon and so on (Zhang *et al.*, 1996; Wu *et al.*, 1998; Luo *et al.*, 2010). The effect of bio-organic fertilizer (BOF) on the number of soil microorganism, microbial metabolic activity and disease index of verticillium wilt in three cotton growing seasons were evaluated. We also provide a reference that revealed mechanism of microbial ecology for bio-organic fertilizer (BOF) to control soil-borne disease.

Materials and Methods

Greenhouse Experiments

This study selected three representative cropping soil of cotton with different organic matter content (severe incidence of verticillium wilt): the high organic matter content of marsh soil (Soil A) derived from Shihezi Nong 8 shi experimental farm 4; the medium organic matter content of gray desert soil (Soil B) derived from Shihezi University College of Agriculture Experiment Station; the low organic matter content of gray desert soil (Soil C) derived from Shihezi Nong 8 shi 149 farm 4.

The experimental design consisted of five treatments design with five replicates (Table 1). The soil nutrient was showed in Table 2. Cotton seeds (*Gossypium hirsutum* L. Xinluzao No. 8) provided by Xinjiang Shihezi University. Each pot planted ten cottonseeds and reserved the best one plant after emergence cotton seedlings, did not add fertilization during the processing of cotton grown. The same pot experiment was repeated in three growing years: from August 22 to November 5, 2009; from May 3 to June 24, 2010; and from April 25 to July 2, 22, 2011.

The Bio-organic fertilizer (BOF), reached 10^8 cfu/g *Bacillus subtilis* and containing 30.4% organic matter, 2.01% total N, 3.7% P_2O_5 and 1.1% K_2O, including the amino nitrogen account for 60% of the total nitrogen, was provided by Jiangsu Tianniang Ltd, China.

Table 1: Design of the experiments in the pot experiments

Treatments	CK	T1	T2	T3	T4
Applied BOF (g/kg)	0	10	20	30	40

Table 2: Properties of soil in the experiments

Soil	Organic matter (g/kg)	Total N (g/kg)	Available N (mg/kg)	Available P (mg/kg)	Available K (mg/kg)	pH
Soil A	38.6	1.26	78.1	37.1	250	8.44
Soil B	23.4	0.93	116.5	66.9	233	8.32
Soil C	10.1	0.75	39.9	9.4	302	8.75

Analytical Methodology

Collect soil surface to the pelvic floor with homemade earth soil devices, remove debris and rapid screening. Soil samples of the pot experiments conducted over the three growing years were analyzed for microorganism after harvest of every year i.e., the flowering of cotton. The soil was transferred to a 200 mL polypropylene tube and then kept at 4℃ until analysis within 48 h for microorganism and BIOLOG analysis.

About 10 g of soil were added to 90 mL sterile distilled water and shaken on a rotary shaker at 200 rpm for 30 min. From the suspensions subsequent ten-fold dilutions were prepared and spread-plated on suitable media i.e. beef extract medium (beef extract 3.0 g, peptone 10.0 g, NaCl 5 g, H_2O 1000 ml, pH 7.2) for bacteria, Gause's No. 1 medium for actinomycetes, and Martin's Rose Bengal medium for fungi (Martin, 1950), and an improved selective medium (K_2HPO_4 1 g, KCl 0.5 g, $MgSO_4$ 0.5 g, Na-EDTA 0.01 g, pentachloronitrobenzene 0.05 g, L-asparagine 2 g, bile salt from ox 0.5 g, L-sorbose 2 g, sodium tetraborate 1 g, dH_2O 1000 mL, pH 5.4, and 1% streptomycin stock solution 0.3 mL after autoclaving sterilization when the mixture cooled to 55°C) for *V. dahliae* (Ausher *et al.*, 1975). Plates with bacteria, actinomycetes, fungi, or *V. dahliae* were incubated in the dark at 28°C for 4 to 7 days; each of the counting items had three replicates. CFUs were adjusted to soil dry weight.

Soil samples (5.0 g d.w.) were serially diluted to a 10^{-3} suspension in sterile 0.9% NaCl solution, and then adjusted to pH 7.0 for inoculation of BIOLOG plates at 28°C in the dark. Due to the limited time from sample collection to inoculation, the supernatants were not standardized for inoculum density before inoculation. Substrate utilization was monitored every 12 h at 590 nm for 8 days. The data were collected by Microlog Release 4.20 software. The readings for individual substrates were corrected and each absorbance value was subtracted by the control well (0.9% NaCl solution) for the next analysis step. The 168 h absorbance values were used to calculate diversity index and factor analyses. The parameter was estimated using the equation below:

$$(Shannon\ index)\ H' = -\sum P_i \ln p_i \quad (Simpson\ index)\ H = 1 - \sum p_i^2$$

The P_i is the measure of ith species proportional to the total measure of all species.

Cotton disease index is observed in cotton per plant flowering incidence and morbidity levels to determine the number of trees. Severity of disease classification criteria: 0, no symptoms; 1, 1/4 leaf disease; 2, 1/2 leaf disease; 3, 3/4 leaf disease; 4, the whole plant died.

$$\text{Disease index} = \sum \frac{\text{number of levels of diseased plants} \times \text{the corresponding series}}{\text{the total number of plants survey}} \times 100\%$$

Statistical Analysis

Change in diseases incidence and amounts of Verticillium wilts, bacteria, actinomyces and fungi were statistically determined with Microsoft EXCEL and SPSS Base Ver17.0 statistical software (SPSS, Chicago, IL, USA). Duncan's multiple range test was applied when the two way ANOVA showed obvious differences ($p<0.05$, $p<0.01$). The Shannon index (H) and Simpson index were calculated to designate this diversity and the multivariate methods such as principal component analysis (PCA) and hierarchical clustering analysis (CLUSTER) were employed to determine how the samples were different as a whole.

Results

Disease Incidence of Verticillium Wilts in the Three Kinds of Soil

Disease index reflected to disease resistance of plant, the higher of disease index and the greater the degree of plant infection and the severer the disease occurred. Analysis of the disease index from the three trials showed that application of the BOF significantly reduced verticillium wilt disease symptoms on cotton (Table 3). The disease index in the continuous fertilization three years has different changes in different organic matter content of soil. With increasing of amount of fertilizer, disease index showed an earlier raised and later decreased state in different types of soil within the same year. In Soil A, disease index first increased then decreased with the delaying of fertilization period. Compared the third year of fertilization with the first year disease index had a significant decrease at the same time, which was minimum in T2 treatment compared to other treatments in three years; in Soil B, change tendency of disease index was similar to Soil A, Compared the third year of fertilization with the first year disease index both had an increase when there was no fertilizer (CK) and application of a relatively small amount of bio-organic fertilizer (T1, T2). Furthermore, T2 treatment in 2010 was the lowest in all treatment, and T3 treatment was the lowest in all treatment in 2009, 2011 years; The disease index of Soil C had a similar trend to Soil B, while it was relatively high in the third year of fertilization compared with the first year in no fertilizer treatment (CK), the situation of fertilizer treatment was in adverse. Besides, disease index was the lowest in T3 treatment in the three years.

Table 3: Disease incidence of Verticillium wilts in the three kinds of soil under different treatments of three years

Soil types	Treatments	Disease index/%		
		2009	2010	2011
Soil A	CK	77.5±12.4a	90.0±13.69a	30.0±11.18a
	T1	42.5±12.4b	75.0±17.68a	10.0±13.69b
	T2	40.0±10.5b	70.0±11.18a	0 b
	T3	52.5±16.8ab	75.0±17.68a	5.0±11.18b
	T4	57.5±23.7ab	80.0±20.92a	10.0±13.69b
Soil B	CK	40.0±14.3a	45.0±11.18a	40.0±22.36a
	T1	20.0±10.5ab	35.0±13.69ab	30.0±20.92ab
	T2	17.5±16.8ab	10.0±13.69c	25.0±17.68ab
	T3	10.0±10.5b	15.0±13.69bc	0 c
	T4	17.5±14.3ab	20.0±20.92bc	10.0±13.69bc
Soil C	CK	30.0±12.7a	40.0±13.69a	37.5±14.43a
	T1	17.5±6.8ab	35.0±13.69ab	15.0±22.36b
	T2	15.0±6.3ab	25.0±17.68ab	5.0±11.18b
	T3	12.0±6.8b	15.0±13.69b	0 b
	T4	17.5±10.5ab	20.0±11.18ab	0 b

"±"Values are means ± standard deviation (n=5).Values with different lowercase letters within a column are statistically significantly different at $P<0.05$

Number of Soil Microorganism in the Three Kinds of Soil

Application of bio-organic fertilizer can significantly increase the number of bacteria. From Table 4, number of bacteria shows an earlier raised and later decreased state in different organic matter content of soil within three year. Compared the third year of fertilization with the first year it increased at different degrees, and the amount increased by soil A: 4.25%, Soil B: 110%, Soil C: 148.45%. Number of bacteria has a slight change in different treatments of different kinds of soil. number of bacteria in T2 treatment (66.7×10^5 cfu/g) was the highest in Soil A and significantly different from other treatments; Number of bacteria in T2, T3 treatment (71.04×10^5 cfu/g, 74.96×10^5 cfu/g) in Soil B were both more than other treatments, had significant and highly significant change; in Soil C, there are significant changes compare with T3 treatment (62.44×10^5 cfu/g) and other treatments in Number of bacteria.

Number of actinomyces change was various among different types of soil, while it showed an increasing trend after the application of bio-organic fertilizer within three consecutive years (Table 5). The number of actinomyces increases as fertilizer year's increase and no fertilizer treatment (CK) and fertilizer treatments were highly significantly different, reached the highest level in third year ($48.8*10^5$cfu/g) in soil A; number of actinomyces first increases then decrease among three years, and the third year of fertilization significantly increased by 59.84% than the first year in soil B. There were significant differences between T3 and other treatments; Number of actinomyces in soil C, the third year of fertilization significantly increased by 46.72% than the first year and the change was similar to soil B. There were highly significant differences between no fertilizer (CK) and fertilizer treatments, whereas no obvious difference among fertilizer treatments was observed.

Table 4: number of bacteria in the three kinds of soil under different treatments of three years (× 10^5 cfu/g)

Soil types	Years	CK	T1	T2	T3	T4	Mean
Soil A	2009	11.33±8.36	29.78±9.26	60.78±2.83	54.44±6.85	50.89±3.84	41.44bB
	2010	20.67±4.73	81.00±12.86	72.33±7.81	69.67±15.10	38.67±19.00	56.47aA
	2011	30.00±2.00	56.00±6.00	67.00±11.00	41.00±9.00	22.00±4.00	43.20bB
	Mean	20.67dC	55.59bA	66.70aA	55.04bA	37.19cB	
Soil B	2009	12.22±2.59	34.78±3.75	30.44±4.54	41.56±13.40	33.67±4.60	30.53bB
	2010	20.00±1.73	88.00±17.16	92.67±4.00	73.33±4.04	51.67±4.93	65.13aA
	2011	30.00±4.00	50.00±10.00	90.00±10.00	110.00±10.00	40.00±20.00	64.00aA
	Mean	20.74dD	57.59bB	71.04aA	74.96aA	41.78cC	
Soil C	2009	15.78±2.14	24.33±1.71	26.56±5.69	19.67±6.35	18.33±0.84	20.93cC
	2010	22.67±8.50	67.00±9.45	80.00±12.66	91.67±9.00	53.00±6.03	62.87aA
	2011	20.00±12.00	34.00±14.00	50±10.00	76.00±4.00	80.00±20.00	52.00bB
	Mean	19.48dC	41.78cB	52.19bAB	62.44aA	50.44bcAB	

Values with different lowercase letters within a column are statistically significantly different at P <0.05, and the capital letters within a column are statistically significantly different at P <0.01

Table 5: Number of actinomyces in the three kinds of soil under different treatments of three years (× 10^5 cfu/g)

Soil types	years	CK	T1	T2	T3	T4	Mean
Soil A	2009	13.44±2.36	26.56±2.34	58.22±10.77	64.22±16.33	51.78±6.74	42.84aA
	2010	22.67±4.16	48.22±8.44	54.00±6.00	46.44±6.30	39.11±9.10	42.09aA
	2011	38.00±18.00	73.00±15.00	47.00±19.00	50.00±10.00	36.00±2.00	48.80aA
	Mean	24.70cB	49.26abA	53.07abA	53.55aA	42.30bA	
Soil B	2009	12.33±4.55	23.56±9.53	35.78±9.31	50.22±13.42	32.00±1.00	30.78bB
	2010	26.67±5.70	62.89±8.88	64.00±8.51	53.11±5.18	51.33±8.35	51.60aA
	2011	38.00±18.00	43.00±19.00	45.00±5.00	62.00±6.00	58.00±20.00	49.20aA
	Mean	25.67cB	43.15bA	48.26abA	55.11aA	47.11abA	
Soil C	2009	9.67±2.60	11.00±4.36	16.89±5.42	26.11±1.39	27.00±3.11	18.13cB
	2010	41.33±4.06	66.00±2.91	81.11±20.93	54.00±6.11	50.89±4.44	58.67aA
	2011	15.00±1.00	25.00±9.00	25.00±7.00	38.00±10.00	30.00±18.00	26.60bB
	Mean	22.00bB	34.00aA	41.00aA	39.37aA	35.96aA	

Table 6: Number of fungi in the three kinds of soil under different treatments of three years (× 10^2 cfu/g)

Soil types	years	CK	T1	T2	T3	T4	Mean
Soil A	2009	198.89±7.03	648.89±10.36	525.56±10.80	502.22±14.59	475.56±24.40	470.22aA
	2010	48.83±25.82	95.00±26.89	119.33±24.54	100.50±27.12	53.00±20.48	83.33bB
	2011	54.00±6.00	90.00±3.00	84.00±9.00	105.00±42.00	61.50±10.50	78.90bB
	Mean	100.57dD	277.96aA	242.96bB	235.91bB	196.69cC	
Soil B	2009	167.78±1.35	238.89±5.17	332.22±5.83	195.56±6.50	185.56±2.71	224.00aA
	2010	46.67±2.31	88.00±6.93	99.33±9.61	90.33±10.02	59.67±9.02	76.80bB
	2011	28.50±1.50	33.00±6.00	40.50±1.50	58.50±1.50	31.50±1.50	38.40cC
	Mean	80.98dD	119.96bB	157.35aA	114.80bB	92.24cC	
Soil C	2009	161.11±4.60	181.11±7.07	260.00±4.84	210.00±13.30	212.22±2.22	204.89aA
	2010	60.33±11.02	93.00±7.94	112.33±15.87	130.33±1.15	87.00±13.53	96.60bB
	2011	37.50±4.50	40.50±1.50	49.50±10.50	57.00±3.00	58.50±7.50	48.60cC
	Mean	86.31dD	104.87cC	140.61aA	132.44aA	119.24bB	

The number of fungi reduced gradually in varying degrees among three years (Table 6), in the second year and the third year it reduced more significantly than the first year in different types of soil, which respectively reduced by 82.28%, 83.22% in soil A and 65.71%, 82.87% in soil B and 52.85%, 76.28% in soil C compare with the first year, furthermore, fertilizer treatments and no fertilizer treatment (CK) were highly significant different. Among fertilizer treatments, the number of fungi decreased as increasing the amount of fertilization and had a significant change in T1 and other treatments in soil A, while it first increased and then decreased and both reached the highest in T2 treatment in soil B and soil C.

Application of the BOF significantly reduced number of *V. dahliae* (Table 7). With increasing the amount of fertilization the number of *V. dahliae* gradually reduce to be specific it had significant and highly significant change among three kinds of soil and five treatments. Number of *V. dahliae* increased at different level among three years and it in the second year and third year separately increased by 5.35%, 9.35% in soil A and 56.13%, 58.50% in soil B and 77.73%, 127.27% in soil C compare with first year.

Soil Microbial Functional Diversity in the Three Kinds of Soils

AWCD could judge the carbon metabolism activity of soil microbial community with incubation time (Choi and Dobbs, 1999). The Fig. 1 shows that microbial metabolic activity was affected by different kinds of soil obvious at

Table 7: Number of V. dahliae in the three kinds of soil under different treatments of three years (×10 cfu/g)

Soil type	years	CK	T1	T2	T3	T4	Average
Soil A	2009	141.00±7.00	97.50±0.50	87.50±5.50	54.00±2.00	69.00±7.00	89.80aA
	2010	144.50±4.50	101.00±16.00	82.00±6.00	76.00±4.00	69.50±20.50	94.60aA
	2011	159.00±25.00	107.50±27.50	75.00±7.00	75.50±14.50	74.00±6.00	98.20aA
	Average	148.17aA	102.00bB	81.50cC	68.50cC	70.83cC	
Soil B	2009	51.50±6.50	38.50±8.50	6.00±2.00	12.50±4.50	18.00±2.00	25.30bB
	2010	56.00±11.00	46.00±0.00	39.00±4.00	21.00±5.00	35.50±7.50	39.50aA
	2011	65.00±4.00	40.50±9.50	33.50±2.5.00	32.50±2.50	29.00±9.00	40.10aA
	Average	57.50aA	41.67bB	26.17cC	22.00cC	27.50cC	
Soil C	2009	40.50±8.50	30.50±5.50	15.50±6.50	14.50±2.50	9.00±2.00	22.00cC
	2010	51.00±16.00	44.00±12.00	40.50±5.50	33.00±10.00	27.00±6.00	39.10bB
	2011	79.50±7.50	60.00±13.00	51.50±13.50	37.00±14.00	22.00±1.00	50.00aA
	Average	57.00aA	44.83bB	35.83bcBC	28.17cdCD	19.33dD	

different level and its maximum value appeared in soil C, while the lowest in soil B. AWCD tended towards stabilize and change significantly in various treatments of different types of soil with incubation time at 168 h. The AWCD values were the highest both in the T1 treatment in soil A and soil B. In soil C, T1 treatment had lower AWCD values than no fertilizer treatment (CK), because the soil texture was sandy soil, and lower fertilized soil did not result in any significant change in microbial metabolic activity and corresponding weakness in carbon-source utilization capability. In T3 treatment AWCD values was the highest. The Shannon index and Simpson index were shown in Fig. 2, the microbial community from fertilization treatments showed slightly lower Shannon index and Simpson index over no fertilizer treatment (CK), the results of all treatments had the same characters in soil A; though Shannon index and Simpson of microbial community had no significant change, they were from fertilization treatments higher than no fertilizer treatment (CK) in soil B; in soil C, Shannon index and Simpson index had a significant increase compared with fertilization treatments and no fertilizer treatment (CK), and were the highest in T3 treatment.

The study of cluster analysis was about carbon-source utilization capability from Fig. 3. Carbon-source utilization capability from five treatments could be divided into three groups. In soil A, The T1 treatment and T2 treatment respective belonged to the first and second group, the CK, T3 and T4 treatments were the third groups; in soil B, the T4 treatment and CK treatment respective belonged to the first and third group, the T1, T2 and T3 treatments were the second group; in soil C, the T4 treatment was an independent group, the CK and T1 treatments were the second group, the T2 and T3 treatments were the third group.

The PCA (Principal Component Analysis) of absorbance of the 31 carbon sources in the Biology Eco-plants was shown in Fig. 4. The results showed that the no fertilizer treatment (CK) was different from fertilization treatments in three kinds of soil, indicating that bio-organic fertilizer could enhance the carbon sources utilization.

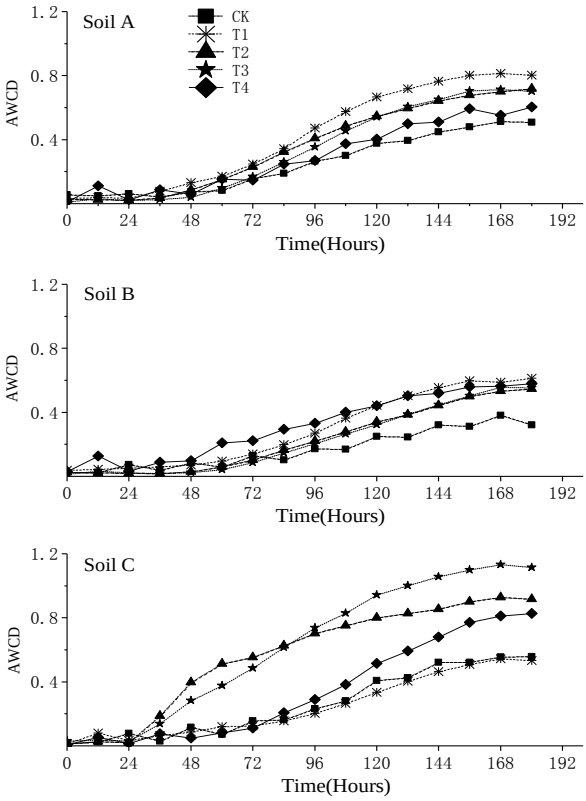

Fig. 1: AWCD variations of soil microbial communities after three consecutive years for application of bio-organic fertilizer

The T1 treatment was different from other fertilization treatments in soil A, and exactly 35.7% and 35.15% of total data variability were explained by the first (PC1) and second (PC2) principal components; the fertilization treatments had no significant change both in soil B and soil C, and the variability explained by PC1 and PC2 were 36.24, 22.99% and 41.31, 28.59%, respectively. This reflects the different treatments had similar community structure and metabolic characteristics of the same soil by adding at the same fertilizer.

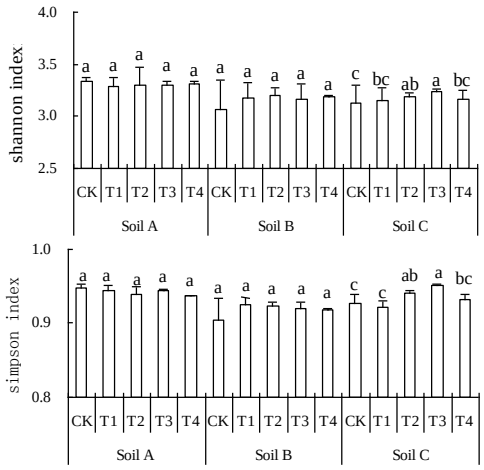

Fig. 2: Diversity indices of soil microbial communities after three consecutive years for application of bio-organic fertilizer

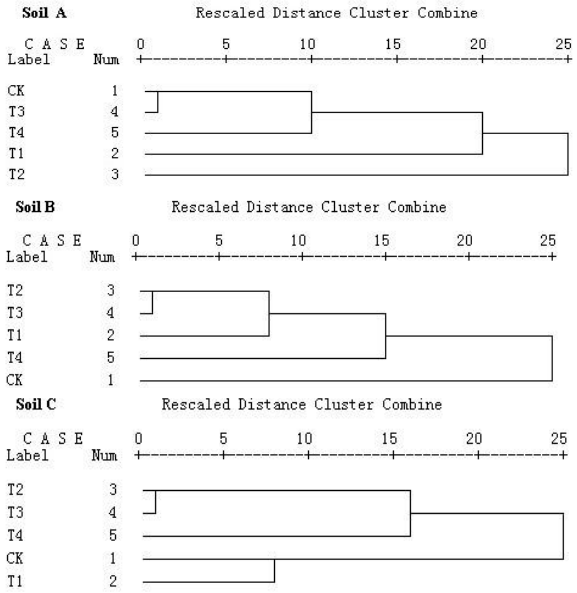

Fig. 3: Cluster analyses for carbon utilization of soil microbial in different treatments after three consecutive years for the application of bio-organic fertilizer

By examining the correlation of the original variables to the PCs (principal components), the most useful carbon sources in different samples could be established. The substrates with high correlation coefficients to PCs for the three soils are shown in Table 8, respectively. Substrates greatly affecting PC1 were amino acids and for PC2 were carbohydrates in soil A. In soil B, PC1 and PC2 were mainly affected by carboxylic acids and carbohydrates, respectively. Carbohydrates and amino acids affected PC1 significantly while carbohydrates affected PC2 greatly in soil C.

Discussion

The use of soil microorganism to prevent soil-borne diseases of plant had great value and attracted the attentions of the domestic and foreign researchers relevant microbial agents were constantly emerging (Berg *et al*., 2001; Uppal *et al*., 2007; Antomopoulos *et al*., 2008). The use of combined organic fertilizer or the organic fertilizer of the method of secondary fermentation with antagonist showed the good effect (Luo *et al*., 2010). Results of this study showed that application of bio-organic fertilizer could significantly reduce the incidence of Verticillium wilt in cotton. Disease index of *V. dahliae* was the lowest when the high organic matter content of soil applied organic fertilizer 10 g/kg and it was also the lowest both in medium and low organic matter content of soil applied organic fertilizer 30 g/kg. Fertilization treatments played an obvious part in prevented disease in three kinds of soil.

Research experiments indicated that it was important to control the number of soil pathogens, because of incidence rates or disease index was significantly correlated with the number of soil pathogens (Harris and Ferris, 1991). Bio-organic fertilizer could inhibit growth of the number of pathogens in soil. By taking the method of added *Bacillus subtilis* and *Trichoderma* to organic fertilizer for searched control effect of cucumber blight, the plate colony calculation results showed that the number of *Fusarium* significantly reduced compared with the control after bio-organic fertilizer application 60 days in the rhizosphere soil of cucumber, which has been verified by quantitative PCR method (Cao *et al*., 2011; zhao *et al*., 2011). Some research used compost of garden waste could significantly reduce the incidence of tomato root rot in the soil, but there were no significant correlation between the soil microbial activity, number of microorganisms and incidence of tomato root rot (Hasna *et al*., 2007). In our research it was shown that as fertilizer volume increases, the number of *V. dahliae* gradually reduced and all treatments showed a significant change in high, medium, and low organic matter content of the soils. This may be due to a direct inhibitory effect or the physiological and biochemical role of antagonist (Wu *et al*., 2009). It may be due to the organic matter addition to soil improved the microbial activity of original antagonist, thereby reduced the density of soil pathogens, inhibited activities of soil pathogen and reduced the incidence of disease (Ouhdouch, 2001). It controlled the number of pathogens in the soil, increased the number of beneficial microorganisms, and changed the soil microflora to be a healthy state gradually in a certain extent by applied bio-organic fertilizer; so it protected the plants from infection and played the role of controlling soil-borne diseases.

Table 8: Main substrates with high correlation coefficients for PC1 and PC2 in PCA of diversity patterns for each site of upper layer

Soil A		Soil B		Soil C	
PC1 (35.7%)	r	PC1 (36.237%)	r	PC1 (41.314%)	r
L-Phenylalanine	0.970	D-Galacturonic Acid	0.998	L-Phenylalanine	0.977
Pyruvic Acid Methyl Ester	0.926	Pyruvic Acid Methyl Ester	0.987	Itaconic Acid	0.957
Phenylethyl-amine	0.923	L-Serine	0.955	D-Mannitol	0.952
D-Galactonic Acid y-Lactone	0.844	Phenylethyl-amine	0.901	Putrescine	0.921
4-Hydroxy Benzoic Acid	0.810	D-Malic Acid	0.842	Phenylethyl-amine	0.890
D,L-a-Glycerol	0.804	Itaconic Acid	0.835	4-Hydroxy Benzoic Acid	0.873
Tween 80	0.685	D,L-a-Glycerol	0.641	D-Galacturonic Acid	0.815
D-Mannitol	0.668			Tween 40	0.804
Itaconic Acid	0.660	PC2 (22.985%)	r	D-Cellobiose	0.789
		N-Acetyl-D-Glucosamine	0.940	L-Asparagine	0.782
PC2 (35.145%)	r	L-Asparagine	0.887	L-Serine	0.778
N-Acetyl-D-Glucosamine	0.969	D-Cellobiose	0.784	D,L-a-Glycerol	0.715
Tween 40	0.959	a-D-Lactose	0.751	N-Acetyl-D-Glucosamine	0.711
Glucose-1-Phosphate	0.880	L-Arginine	0.717	L-Arginine	0.701
D-Cellobiose	0.850	L-Phenylalanine	0.693	ß-Methyl-D-Glucoside	0.632
D-Malic Acid	0.832	Glycogen	0.680		
a-D-Lactose	0.793			PC2 (28.594%)	r
ß-Methyl-D-Glucoside	0.779			D-Galactonic Acid y-Lactone	0.960
D-Galacturonic Acid	0.721			D-Malic Acid	0.869
D-Glucosaminic Acid	0.697			I-Erythritol	0.860
L-Asparagine	0.671			Glycyl-L-Glutamic Acid	0.763
				2-Hydroxy Benzoic Acid	0.717
				D,L-a-Glycerol	0.667

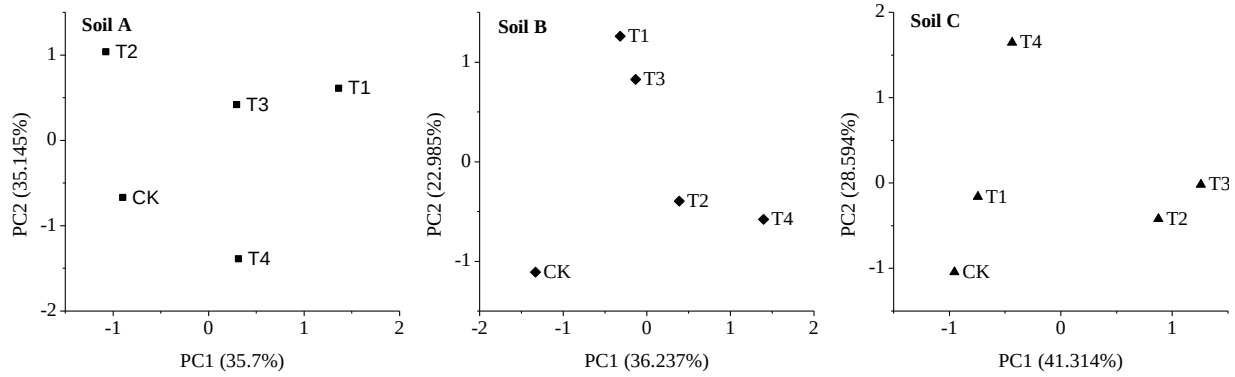

Fig. 4: Principal component analyses for carbon utilization of soil microbial in different treatments after three consecutive years for the application of bio-organic fertilizer

The incidence of disease increased significantly when irises were planted in ordinary soil and soil treated by fungicide or waterlogging. The number of pathogen (*Pythium* sp) significantly increased with continuous cultivation of irises (Van Os and Van Ginkel, 2001). Similarly, the fertilization of consecutive three years could cause enrichment of *V. dahliae* and significant increase in the number of *V. dahliae* in high, medium, and low organic matter content of soils, indicated that application of bio-organic fertilizer (BOF) delayed the growth of the number of verticillium in soil within a certain time.

Soil microorganisms played an important role in transformation of soil nutrients and formation of humus. The diversity of soil microorganisms had an influence on structure, function and process of soil ecosystem and it was an important component to maintain soil productivity (Wardle, 1992; Kaye and Hart, 1997). Some studies showed that application of organic fertilizer or organic-inorganic fertilizer could improve the number of soil bacteria, fungi and actinomyces (Ndayeyamiye and Cote, 1986). Shukla *et al*. (2008) reported that the bio-organic fertilizer which was compounded with *Trichoderma* and *Gluconaceto-bacter* sp significantly increased the number of microorganisms in the rhizosphere soil of sugarcane. According to another study the application of micro-organic fertilizer significantly increased the number of nitrogen-fixing microorganisms (*Azotobacter sp*, *Azospirillum sp* and *Azoarcus sp*) in the rhizosphere soil of corn. It indicated that bio-organic fertilizer increased the number of some beneficial microorganisms in the soil (Jilani *et al*., 2007). This study showed that application of bio-

organic fertilizer (BOF) could significantly improve the number of bacteria and actinomycetes. The number of bacteria and actinomycetes increased first and then decreased with the amount of fertilization increased within treatments. And it increased and the number of fungi decreased with fertilizer year increased. The number of fungi was the highest when the high organic matter content of soil applied organic fertilizer 10 g/kg, and it was the biggest both in medium and low organic matter content of soils applied organic fertilizer 20 g/kg. The accumulation of carbohydrates and amino acids in root exudates provided necessary energy for the microorganisms, constituted a specific soil environment and promoted growth and reproduction of beneficial rhizosphere microorganisms in short-term.

Different fertilization had different effects on carbon source utilization ability of soil microbial community and AWCD could be used to characterize the level of utilization rate of carbon source. When applied manure, green manure and other organic fertilizer to help maintain the soil microbial diversity and activity (Dick, 1992). On the one hand the amount of stubbles were increased, on the other hand a lot of soil organic matter was put into soil and promoted soil microbial activity through application of organic fertilizer and straw returning (Bohme *et al*., 2005; Bossi *et al*., 1998). The AWCD of applied bio-organic fertilizer significantly higher than no fertilization indicated that the bio-organic fertilizer provided rich organic matter for the soil microbes and increased microbial activity.

Some scholars believed that outside interference (such as added exogenous material) had a bigger effect in low organic matter content of soil on soil microbial community diversity compared with high organic matter content of soil (Zhou *et al*., 2002). In this study, the *Shannon index* and *Simpson index* of fertilization treatments slightly higher than no fertilization in the middle and low organic matter content of soils, they first increase and then decrease with the amount of fertilization increased and the opposite conclusion in high organic matter content of soil. The reason may be have inductive effect on soil microbes when the same fertilizer was added to three organic matter content of soil. The inductive effect related to original organic matter of soil and significantly increased soil microbial community metabolic diversity in the low organic matter content of soil. Some studies also showed that the microbial community metabolic diversity in high background level of soil organic matter lower than control when applied straw of ryegrass, while it was significantly higher than control in the low background level of soil organic matter (Bending *et al*., 2002). Changes in microbial community diversity not only affected on the application of exogenous substances, but also affected on background level of soil organic matter. Microbial species richness index did not show regularity with the background level of soil organic matter changed, which may be related to soil texture, ability to retain water and nutrient of soil, soil temperature and so on.

By the method of cluster analysis and principal component analysis, we studied that the soil microbes of different amount of fertilization for impacts of utilization of carbon source in high, medium and low organic matter content of soils and made a classification. The results showed that the classification similar to disease index of various treatments and it coincided with the number of microorganisms and AWCD values. The carbohydrates, amino acids and carboxylic acids affected the main carbon source of microorganisms using in different treatments in metabolism of different carbon sources.

Conclusion

Different amount of fertilization and soil factors affected the metabolic activity of soil microbial community, in-depth study of these factors for soil health, cultivation of ecological fertility and sustainable use of soil resources had important theoretical and practical significance. In this study, application of bio-organic fertilizer (BOF) not only controlled and improved the metabolic characteristics of soil microbial communities but also maintained high microbial activity of soil. With the development of research and improvement and optimization of testing techniques and combined with other research method of soil microbial community, such as fatty acid methyl ester (FAME), molecular biology techniques (PCR-DGGE). It would help to improve the understanding of microbial community structure and function, so as to provide a theoretical basis for controlling and improving the function of sick soil microbial communities, soil ecological environment and controlling soil-borne diseases of cotton.

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