RESEARCH ARTICLE

Soil microbial characteristics and yield response to partial substitution of chemical fertilizer with organic amendments in greenhouse vegetable production

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Abstract

Greenhouse vegetable production has been characterized by high agricultural inputs, high temperatures, and high cropping indexes. As an intensive form of agriculture, nutrient cycling induced by microbial activities in the greenhouses is relatively different from open fields in the same region. However, the responses of soil microbial biomass carbon (MBC) and nitrogen (MBN), enzyme activities, microbial community composition, and yield to organic amendment are not well understood. Therefore, a 5-year greenhouse tomato (*Solanum lycopersicum* Mill.)-cucumber (*Cucumis sativus* L.) rotation experiment was conducted. The field experiment comprised 5 treatments: 4/4CN (CN, nitrogen in chemical fertilizer), 3/4CN+1/4MN (MN, nitrogen in pig manure), 2/4CN+2/4MN, 2/4CN+1/4 MN+1/4 SN (SN, nitrogen in corn straw) and 2/4CN+2/4SN. The amounts of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) were equal in the five treatments. Starting with the fourth growing season, the optimal yield was obtained from soil treated with straw. MBC, MBN, phospholipid fatty acid (PLFA) profiles, and enzyme activities were significantly changed by 5 years of substitution with organic amendments. Redundancy analysis showed that MBC accounts for 89.5 and 52.3% of the total enzyme activity and total community variability, respectively. The activities of phosphomonoesterase, N-acetyl-glucosaminidase, and urease, and the relative abundances of fungi, actinomycetes, and Gram-negative bacteria were significantly and positively related to vegetable yields. Considering the effects of organic amendments on soil microbial characteristics and vegetable yield, 2/4CN+1/4MN+1/4SN can improve soil quality and maintain sustainable high yield in greenhouse vegetable production.

Keywords: soil microbial biomass, enzyme activity, microbial community composition, organic amendment, greenhouse vegetable

1. Introduction

In China, greenhouse vegetable production has undergone rapid development from less than 7 000 hectares in 1980 to almost 3.7 million hectares in 2015 (Huang *et al.* 2016). The amount of inorganic nitrogen (N) fertilizer used on vegetable fields is greater than that used on cereal crops

(Ju *et al.* 2006; Min *et al.* 2011). This increased application of inorganic N can cause environmental pollution and soil degradation (Shen *et al.* 2010; Rezaei Rashti *et al.* 2015). To avoid these problems, applying chemical fertilizers that are partially substituted with organic amendments is an economical and effective practice (Syswerda *et al.* 2012; Aparna *et al.* 2014). However, as an intensive and unique form of agriculture, greenhouse vegetable production has been characterized by a high cropping index and high agricultural inputs (Li *et al.* 2001; Zhu *et al.* 2011; Yang *et al.* 2014), which is different from open fields in the same region. As a result, nutrient cycling induced by microbial activities undergoes large, relative changes in greenhouse vegetable production (Lin *et al.* 2004; Qin *et al.* 2016; Yao *et al.* 2016). Thus, a better understanding of how partial substitution with organic amendments affects activity and microbial community composition of soil is needed in order to determine the implications for managing greenhouse agroecosystems.

Soil microbes make important contributions to biologically- and biochemically-mediated processes in soil, e.g., the decomposition of soil organic matter and nutrient retention (Nannipieri *et al.* 2003; Köhl and van der Heijden 2016). Microbial parameters such as microbial biomass, enzyme activities, and activity and composition of the microbial community are sensitive and significant indicators for assessing changes in soil quality (Sharma *et al.* 2015). Soil microbial biomass, as a living part of soil organic matter, is an early indicator of soil management changes. Several long-term experiments have found that organic fertilization increases microbial biomass because the organic amendment delivers large amounts of external carbon (C) to soil (Kaschuk *et al.* 2010; Zhang *et al.* 2015). However, the response of the microbial biomass to organic C input depends upon the application rate and the chemical composition of the organic amendments used (Kallenbach and Grandy 2011). Therefore, more information is needed on the effects of different inputs of organic amendments as substitutes on soil microbial biomass in greenhouse vegetable production.

Phospholipid fatty acid (PLFA) analysis, as an established technique, has been widely used to evaluate the influence of management practices or environmental factors on changes in the composition of microbial communities (Zelles 1999). The relative abundances of Gram-negative bacteria, saprophytic fungi, and actinomycetes were related to soil organic C (SOC) transformation or turnover in the forest soil of the Baotianman Nature Reserve (You *et al.* 2014). The application of chemical N fertilizer increased the total microbial biomass and fungal abundance but decreased the bacterial abundance in a fluvo-aquic soil in a wheat (*Triticum aestivum* L.)-maize (*Zea mays* L.) rotation (Ai

et al. 2012). The influence of management practices such as cover crops, continuous cropping, and compost on soil microbial community composition has attracted attention in greenhouse vegetable production (Maul *et al.* 2014; Willekens *et al.* 2014). Nonetheless, the effect of inorganic fertilizers with different percentages of organic amendments on soil microbial community composition has not been well documented.

Enzyme activity makes nutrients more available to plants and microorganisms by mineralizing organic C, N, sulphur (S), and phosphorus (P) from soil organic matter (Waring *et al.* 2014). Adding organic material often leads to an overall increase in enzyme activity (Bonanomi *et al.* 2014), but the response depends on changes in soil management and plant cover of soil (Nannipieri *et al.* 2012). Long-term application of horse manure compost to greenhouse vegetable soil enhanced α-galactosidase, β-galactosidase, α-glucosidase, and β-glucosidase activities of soil (Zhang *et al.* 2015). The activities of dehydrogenase, urease, and neutral phosphatase decreased significantly by increasing the rate of N application to a 2-year tomato (*Solanum lycopersicum* Mill.)-cucumber (*Cucumis sativus* L.) rotation in China's Yangtze River Delta (Shen *et al.* 2010). At present, most research is focused on enzyme activity related to N and C transformation in greenhouse vegetable production systems. Very few studies have explored the response of soil enzymes involved in the C, N, S, and P biochemical cycles as affected by different percentages of organic amendment substitutes to inorganic fertilizers in greenhouse vegetable production.

The objective of this work was to examine the effect of using different patterns of organic amendments as partial substitutes for chemical fertilizers on microbial biomass carbon (MBC) and nitrogen (MBN), soil microbial community composition, enzyme activity, and vegetable yields in greenhouse conditions. We hypothesized that changes in microbial properties would depend on different organic amendments (pig manure and straw), and these changes would be related to vegetable yield. To test our hypotheses, a 5-year field experiment was conducted with the following aims: (1) to investigate changes in chemical, biochemical, and microbial properties of soil and vegetable yield and to compare these patterns; and (2) to analyze the relationship between soil microbial properties and vegetable yield.

2. Materials and methods

2.1. Site description

The 5-year experiment was conducted at the Dahe Experimental Station belonging to the Hebei Academy of Agriculture and Forestry Sciences, Hebei Province,

China (38°08′N, 114°23′E). The study site has a warm, sub-humid continental monsoon climate with an average annual temperature of 11.5°C and annual precipitation of 540 mm.

The solar greenhouse employed in the test measured 8 m×48 m. The original surface soil was used for building the back wall. A crop rotation of winter-spring cucumber (Bomei 11) and autumn-winter tomato (Jinpeng 11) was used in the experiment from October 2009, and the field was left fallow between the growth periods of the two crops. The soil in the study has a clay loam texture and can be classified as calcareous cinnamon soil (FAO classification). The main soil properties (0–20 cm depth) are as follows: soil bulk density, 1.35 g cm^{-3}; electrical conductivity, 185.4 mS cm^{-1}; pH, 8.0; organic matter, 9.1 g kg^{-1}; nitrate N, 18.3 mg kg^{-1}; available P, 6.2 mg kg^{-1}; and available K, 98.2 mg kg^{-1}.

2.2. Experimental design

A randomized block design was used with 3 replications and 5 different treatments as follows: (1) 4/4CN (CN, N in chemical fertilizer); (2) 3/4CN+1/4MN (MN, N in pig manure); (3) 2/4CN+2/4MN; (4) 2/4CN+1/4MN+1/4SN (SN, N in corn straw); and (5) 2/4CN+2/4SN. The amount of nutrient inputs (N, P$_2$O$_5$, and K$_2$O) for the five treatments was the same. The nutrient inputs were determined based on soil tests and nutrient requirements for target yields (150 t ha^{-1} for cucumber and 90 t ha^{-1} for tomato) (Huang *et al.* 2017). The nutrient inputs of N, P$_2$O$_5$, and K$_2$O used in the winter-spring cucumber season were 600, 300, and 525 kg ha^{-1}, respectively, whereas those in the autumn-winter tomato season were 450, 225, and 600 kg ha^{-1}, respectively.

The chemical fertilizers used in the experiment included urea, calcium superphosphate, and potassium chloride. In order to keep the fertilizer treatments comparable with only small interannual changes every year, fresh commercial pig manure was used, which contained (33.2±4.5)% of water, (1.67±0.13)% of N, (1.30±0.12)% of P$_2$O$_5$, (1.04±0.12)% of K$_2$O, and (142.6±6.8) g kg^{-1} of C. The fresh straw used contained ((13.7±1.6)% of water, (0.75±0.08)% of N, (0.20±0.03)% of P$_2$O$_5$, (1.21±0.22)% of K$_2$O, and (324.1±12.3) g kg^{-1} of C. In the winter-spring cucumber season, 9 t ha^{-1} of fresh commercial pig manure was used in the treatments 3/4CN+1/4MN and 2/4CN+1/4MN+1/4SN, of which 25% of the N came from pig manure; 20 t ha^{-1} of fresh straw was used in the 2/4CN+1/4MN+1/4SN treatment, of which 25% of the N came from straw. In the autumn-winter tomato season, 6.75 t ha^{-1} of fresh commercial pig manure was used in the treatments 3/4CN+1/4MN and 2/4CN+1/4MN+1/4SN, of which 25% of the N came from pig manure; and 15 t ha^{-1} of fresh straw was used in the

2/4CN+1/4MN+1/4SN treatment, of which 25% of the N came from straw. When 50% N was substituted, the consumption of pig manure and straw doubled. Inputs of N, P$_2$O$_5$, and K$_2$O for each organic amendment treatment were calculated by each nutrient concentration in pig manure and corn straw and the organic amendment investments. The amounts of N, P$_2$O$_5$, K$_2$O, and C from chemical fertilizer, manure, and straw for each treatment are given in Tables 1 and 2.

All the pig manure and 20% N, 100% P, and 40% K of the chemical fertilizer were evenly broadcast onto and mixed into the soil through rotary tillage. The crop straw was cut into short pieces and scattered within the 20–25 cm soil layer and then was covered with soil. In the winter-spring cucumber season, the remaining 80% of the N and 60% of the K was divided equally into 10 parts and top-dressed according to the nutrient requirements of the crop. In the autumn-winter tomato seasons, the rest of the fertilizer (80% N; 60% K) was divided into four parts and top-dressed at the expanding stage of the first to fourth fruit spikes when the diameter of the fruits reached 3–4 cm.

The experimental plot corresponded to an area of 14.4 m^2 (2.4 m wide×6 m long). In each plot, 4 rows with 20 plants per row were planted (0.6 m between rows, 0.30 m between plants). Polyvinyl chloride (PVC) plates were embedded into the soil between plots to a depth of 100 cm, which extended above the ground by 5 cm. These were used to prevent lateral and transverse migration of nutrients and water between plots. Furrow irrigation was employed in the experiment. A soil probe (WITU technology Inc., Shenyang, China) was inserted into the soil to a depth of 20 cm to monitor and control the moisture content at 75–95% of the field capacity.

2.3. Soil sampling and analyses

Sampling protocol Soil samples were collected from each plot at 0–20 cm depth during the uprooting stage of the fifth crop cycle (10 July 2014). Fresh soil samples were immediately transported to the laboratory in an ice-box for further processing. Gravel and residual roots were removed, and the soil samples were sieved (2-mm). A portion of each sample was then stored at 4°C to be analyzed for enzyme activities, soil microbial biomass, and dissolved organic C (DOC) and N (DON). Another part was stored at –70°C for microbiological PLFA analysis. The rest of each sample was air-dried for chemical analysis.

Soil analyses Both MBC and MBN were determined using the fumigation-extraction technique (Wu *et al.* 1990), while the filtrates were analyzed using a TOC/TN analyzer (Analytik Jena, Multi N/C 3100). Both of the efficiency

Table 1 Amounts of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) used in each treatment during the winter-spring cucumber season

Nutrient	Treatment[1]	Chemical fertilizer (kg ha⁻¹)	Pig manure (kg ha⁻¹)	Straw (kg ha⁻¹)	Total (kg ha⁻¹)
N	4/4CN	600.0	0	0	600.0
	3/4CN+1/4MN	450.0	150.0	0	600.0
	2/4CN+2/4MN	300.0	300.0	0	600.0
	2/4CN+1/4MN+1/4SN	300.0	150.0	150.0	600.0
	2/4CN+2/4SN	300.0	0	300.0	600.0
P_2O_5	4/4CN	300.0	0	0	300.0
	3/4CN+1/4MN	183.3	116.7	0	300.0
	2/4CN+2/4MN	66.7	233.3	0	300.0
	2/4CN+1/4MN+1/4SN	143.0	116.7	40.3	300.0
	2/4CN+2/4SN	219.5	0	80.5	300.0
K_2O	4/4CN	525.0	0	0	525.0
	3/4CN+1/4MN	431.0	94.0	0	525.0
	2/4CN+2/4MN	337.0	188.0	0	525.0
	2/4CN+1/4MN+1/4SN	190.1	94.0	240.9	525.0
	2/4CN+2/4SN	43.1	0	481.9	525.0
C	4/4CN	0	0	0	0
	3/4CN+1/4MN	0	1 283.2	0	1 283.2
	2/4CN+2/4MN	0	2 566.4	0	2 566.4
	2/4CN+1/4MN+1/4SN	0	1 283.2	6 482.8	7 766.0
	2/4CN+2/4SN	0	0	12 965.5	12 965.5

[1] CN, N in chemical fertilizer; MN, N in pig manure; SN, N in corn straw.

Table 2 Amounts of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) used in each treatment during the autumn-winter tomato season

Nutrient	Treatment[1]	Chemical fertilizer (kg ha⁻¹)	Pig manure (kg ha⁻¹)	Straw (kg ha⁻¹)	Total (kg ha⁻¹)
N	4/4CN	450.0	0	0	450.0
	3/4CN+1/4MN	337.5	112.5	0	450.0
	2/4CN+2/4MN	225.0	225.0	0	450.0
	2/4CN+1/4MN+1/4SN	225.0	112.5	112.5	450.0
	2/4CN+2/4SN	225.0	0	225.0	450.0
P_2O_5	4/4CN	225.0	0	0	225.0
	3/4CN+1/4MN	137.5	87.5	0	225.0
	2/4CN+2/4MN	50.0	175.0	0	225.0
	2/4CN+1/4MN+1/4SN	107.3	87.5	30.2	225.0
	2/4CN+2/4SN	164.6	0	60.4	225.0
K_2O	4/4CN	600.0	0	0	600.0
	3/4CN+1/4MN	529.5	70.5	0	600.0
	2/4CN+2/4MN	459.0	141.0	0	600.0
	2/4CN+1/4MN+1/4SN	348.8	70.5	180.7	600.0
	2/4CN+2/4SN	238.6	0	361.4	600.0
C	4/4CN	0	0	0	0
	3/4CN+1/4MN	0	962.4	0	962.4
	2/4CN+2/4MN	0	1 924.8	0	1 924.8
	2/4CN+1/4MN+1/4SN	0	962.4	4 862.1	5 824.5
	2/4CN+2/4SN	0	0	9 724.2	9 724.2

[1] CN, N in chemical fertilizer; MN, N in pig manure; SN, N in corn straw.

constants required for MBC and MBN calculation, k_{EC} and k_{EN}, were taken to be 0.45 (Wu *et al.* 1990; Joergensen 1996). The SOC content was determined by oxidation with potassium dichromate and titration with ferrous ammonium sulfate. The nitrate-N level (NO_3^--N) was determined using the dual-wavelength ultraviolet spectrophotometric method (Norman *et al.* 1985). DOC and DON were analyzed as described by Ghani *et al.* (2007).

Enzyme activity All enzyme activities with the exception of urease, phenol oxidase, and peroxidase activities were determined using the microplate fluorometric assay (DeForest 2009; Ai *et al.* 2015). The fluorescence was quantified using a microplate fluorometer (Scientific Fluoroskan Ascent FL, Thermo, USA) with 365 nm excitation

and 450 nm emission filters (Saiya-Cork *et al.* 2002). The enzyme activities were expressed in nmol h^{-1} g^{-1}. Urease activity was determined using urea as the substrate as described by Kandeler and Gerber (1988) and was expressed as mmol NH$_4^+$ g^{-1} dry soil h^{-1}.

PLFA analysis The composition of the soil microbial community was determined *via* PLFA analysis according to the procedure described by Wu *et al.* (2009). The abundance of individual PLFAs was indicated by its % mole abundance in each sample. PLFAs were divided into various taxonomic groups based on previously published PLFA biomarker data (Ai *et al.* 2012; Moeskops *et al.* 2012). Specifically, we used i14:0, a15:0, i15:0, 16:0, i16:0, 17:0, a17:0, cy17:0, i17:0, and cy19:0ω8c as bacteria biomarkers; i14:0, a15:0, i15:0, i16:0, a17:0, and i17:0 as Gram-positive bacteria biomarkers; and cy17:0 and cy19:0ω8c as Gram-negative bacteria biomarkers. The unsaturated PLFA 18:1ω9c was used as a fungal biomarker. The fatty acids 16:0 (10Me), 17:0 (10Me), and 18:0 (10Me) were used as markers for actinomycetes.

2.4. Statistical analysis

Statistical analysis was carried out using the SAS software package (ver. 9.1). A two-way randomized block ANOVA test was performed to analyze each variable using Fisher's least significant difference (P=0.05) to compare the treatment means. Pearson correlation analysis was performed to determine the relationship of MBC and MBN, enzyme activity, microbial community composition, and yield (5 different treatments of the fifth year, three replicates each). Principal component analysis (PCA) and redundancy analysis (RDA) with the Monte Carlo permutation test (499 permutations) were performed to determine if soil enzyme activity or community composition was correlated with soil properties, as implemented in Canoco 5.0.

3. Results

3.1. Changes in vegetable yield

The cucumber and tomato yields from 2010 to 2014 are shown in Table 3. The 4/4CN treatment had higher vegetable yield than treatments using organic amendment substitutions in the first three growing seasons. Since the autumn-winter tomato season in 2012, straw treatment (2/4CN+2/4SN) significantly increased tomato yield compared with 4/4CN treatment (except in the autumn-winter tomato season in 2013). For cucumber yield, straw treatment (2/4CN+1/4MN+1/4SN, 2/4CN+2/4SN) induced a slight increase in the fifth growing season in 2012, however, a significant increase was observed in the following winter-spring cucumber seasons in 2013 and 2014. However, there was no significant difference between 2/4CN+1/4MN+1/4SN and 2/4CN+2/4SN.

3.2. Changes in microbial biomass C and N

Both MBC and MBN were significantly affected by the organic amendments in the ninth growing season (except 3/4CN+1/4MN) (Fig. 1). The MBC and MBN values in organic-amended soil (i.e., 3/4CN+1/4MN, 2/4CN+2/4MN, 2/4CN+1/4MN+1/4SN, and 2/4CN+2/4SN), were in the ranges of 137.0–290.2 and 36.2–57.2 mg kg^{-1}, respectively. These values are much higher than those in 4/4CN treatment, which were in the ranges of 10.5–134.1 and 28.1–102.1%, respectively (Fig. 1).

Both MBC and MBN also increased with higher amounts of added pig manure or straw (Fig. 1). The MBC and MBN in the 2/4CN+2/4MN treatment were, on average, 25.2 and 32.8% higher, respectively, than those in the 3/4CN+1/4MN treatment. Similarly, MBC and MBN in the 2/4CN+2/4SN treatment were, on average, 27.9 and 6.7%

Table 3 The effects of different partial replacements of inorganic fertilizer (t ha^{-1}) on the yields of winter-spring cucumber and autumn-winter tomato in the greenhouses from 2010 to 2014

Year	Crop	Treatment[1]				
		4/4CN	3/4CN+1/4MN	2/4CN+2/4MN	2/4CN+1/4MN+1/4SN	2/4CN+2/4SN
2010	Cucumber	141.6±2.0	140.3±10.3	133.4±4.8	140.8±12.0	137.4±6.6
	Tomato	96.2±8.4 a	93.5±4.3 a	91.1±3.6 a	80.4±6.0 b	80.5±4.4 b
2011	Cucumber	178.9±5.3 a	170.8±7.0 ab	169.9±1.5 ab	165.8±6.0 b	170.4±4.6 ab
	Tomato	70.5±2.9 bc	65.8±1.5 c	72.5±1.4 b	72.1±6.0 bc	79.5±6.3 a
2012	Cucumber	144.3±1.9 ab	139.1±3.7 b	136.8±6.3 b	147.4±3.8 ab	152.2±10.0 a
	Tomato	70.1±8.6 b	71.6±7.6 b	78.4±6.7 a	80.8±6.5 a	81.0±10.7 a
2013	Cucumber	103.2±3.3 d	105.1±3.6 cd	107.3±2.9 bc	110.0±2.9 ab	112.1±2.8 a
	Tomato	72.5±3.8	73.6±2.8	74.7±3.7	75.8±6.2	74.1±5.5
2014	Cucumber	125.3±4.0 b	131.7±8.6 ab	133.9±6.6 ab	138.0±5.4 a	136.2±7.1 a

[1] CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw.
Data are mean±SE, n=3. Different letters in the same row for each growing season indicate significant differences among the fertilization regimes at the P<0.05 level.
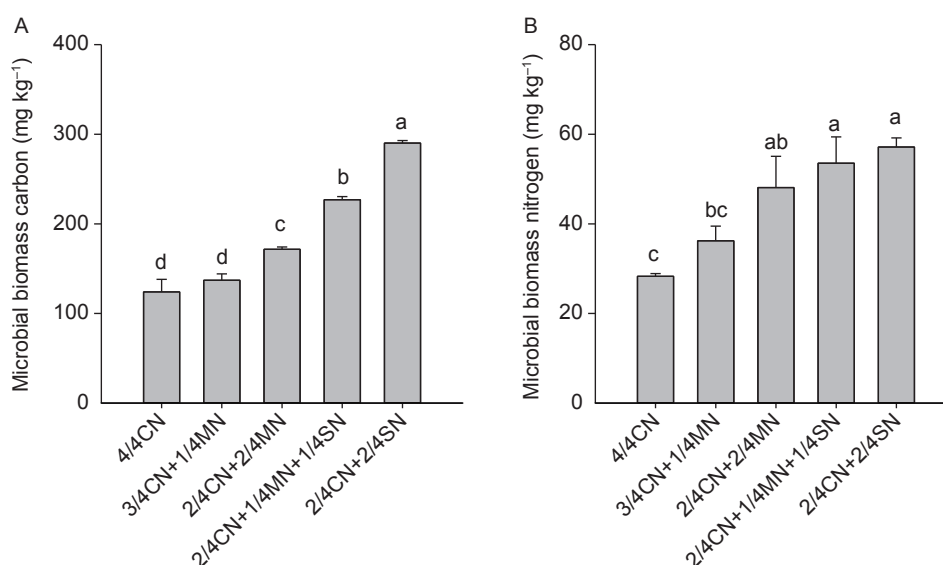
Fig. 1 The effects of different partial organic-amendment replacements on microbial biomass carbon (A) and nitrogen (B) in the soil in the uprooting stage of the 9th growing season (winter-spring cucumber). CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw. Bars indicate standard error, $n=3$. Different letters indicate significant differences between treatments (at the $P<0.05$ level).

higher, respectively, than those in the 2/4CN+1/4MN+1/4SN treatment.

3.3. Changes in soil enzyme activity

Enzymes involved in C, N, and P cycling were significantly increased in activity in the organic-amended soil. The largest soil enzyme activities were observed in soils amended with straw (Fig. 2). Compared with 4/4CN, the activities of β-glucosidase, β-cellobiosidase, β-xylosidase, and α-glucosidase (involved in the C cycle) were, respectively, increased by 130.9–477.7, 215.1–968.6, 81.6–412.4, and 47.0–183.9% in soil amended with straw (2/4CN+1/4MN+1/4SN, 2/4CN+2/4SN). The activity of N-acetyl-glucosaminidase, L-leucine aminopeptidase, and urease (related to the N cycle) were increased by 58.4–545.2, 0.1–37.8, and 38.8–188.5%, respectively. In contrast, the activity of soil phosphomonoesterase (involved in the P cycle) increased by 8.6–162.9% (Fig. 2). Sulfatase activity in the organic-amended soil was significantly decreased, especially when straw was used. The sulfatase activity fell by 2.1–9.3% compared to 4/4CN, and by 1.2–7.4% compared to pig-manure treated soil (Fig. 2).

Ordination of the fertilization treatments *via* PCA shows that they are primarily related to the first canonical axis (PC1) (PC1=95.1%). The samples were separated into three distinct groups, each possessing a specific range of soil MBC values (Fig. 3-A). The first group included 4/4CN, 3/4CN+1/4MN, and 2/4CN+2/4MN treated soil, i.e., those

generally having lower MBC values of 124.0–171.6 mg kg⁻¹ (Fig. 3-A). The second group included soil treated with 2/4CN+1/4MN+1/4SN which had an intermediate MBC value of 227.0 mg kg⁻¹. The third group, containing 2/4CN+1/4MN+1/4SN treated soil, had a high MBC value of 290.2 mg kg⁻¹. Indeed, RDA confirmed that soil MBC had a statistically significant effect (F=110, P<0.01) on enzyme activity, and that it accounted for 89.5% of the total enzyme activity variation (Fig. 3-B). In addition, DON was also significantly related to enzyme activity (F=2.3, P=0.03) and accounted for 2.8% of the variation in the total enzyme activity (Fig. 3-B).

3.4. Changes in abundance and composition of the microbial communities

Treatments using different organic-amendment substitutes increased the total PLFA content, though to different degrees, with the increases ranging from 7.0 to 66.1% compared with 4/4CN (Fig. 4-A). In particular, the soils treated with 50% N substituted by straw (2/4CN+2/4SN) had significantly higher PLFA content than that in 4/4CN treatment.

The relative abundance of bacteria was significantly higher in the 2/4CN+2/4SN-treated soil than that in 4/4CN-treated soil, whereas there were no differences between pig manure and straw-treated soils. Abundance of fungi did not show a clear trend in response to organic-amended soil, except for a small but significant difference between
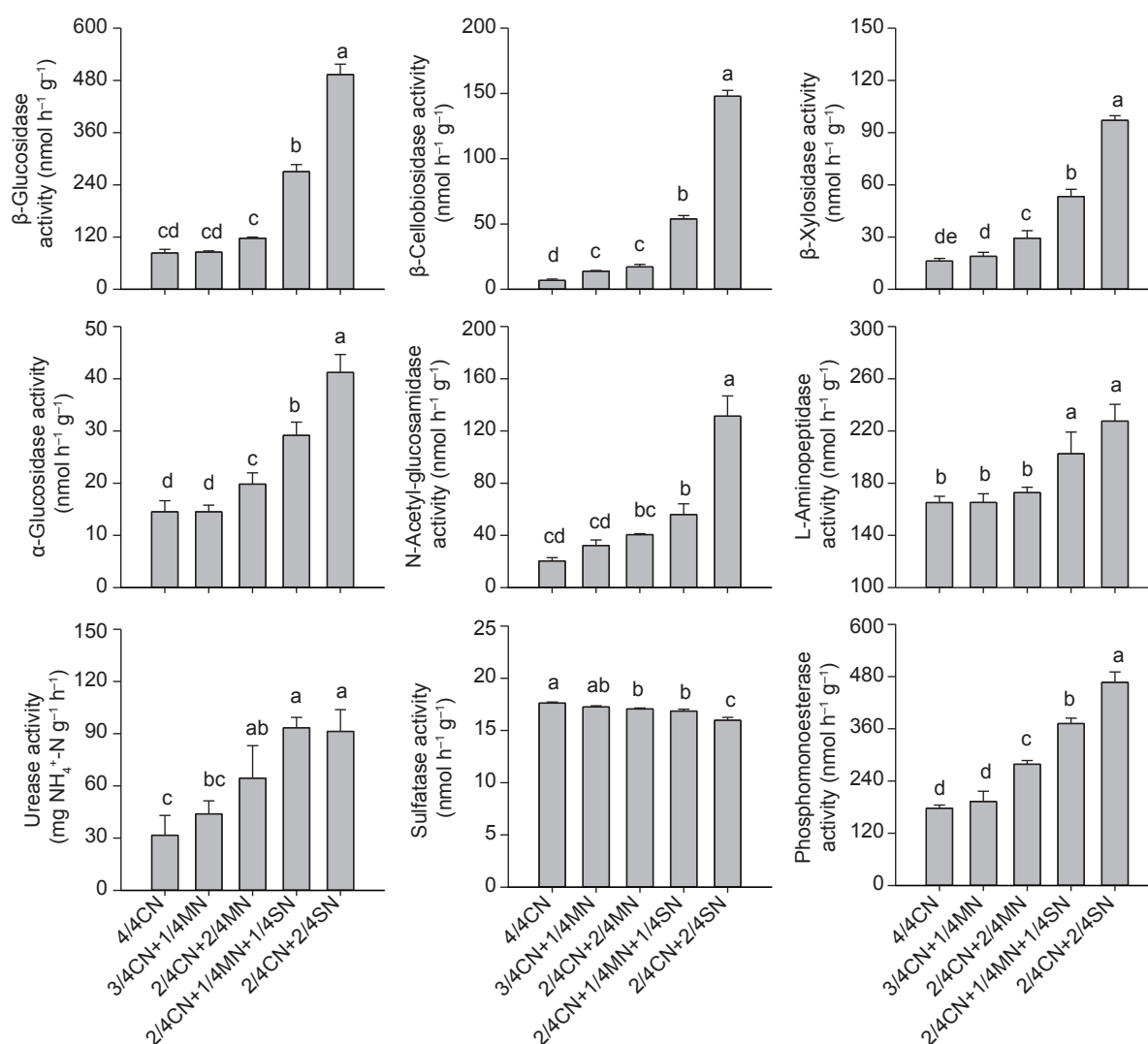
Fig. 2 The response of enzyme activity to different partial organic-amendment replacements in the uprooting stage of the 9th growing season. CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw. Bars indicate standard error, n=3. Different letters indicate significant differences between treatments at P<0.05.

2/4CN+1/4MN+1/4SN and 4/4CN-treated soil. Compared with the 4/4CN-treated soil, the relative abundances of actinomycetes were remarkably increased in the straw-treated soil and the soils treated with 50% N substituted by pig manure. The ratio of Gram-positive to Gram-negative bacteria and bacteria to actinomycetes was significantly decreased in straw-treated soils (2/4CN+1/4MN+1/4SN and 2/4CN+2/4SN) compared to that in 4/4CN-treated soil. However, there were no significant differences in these ratios between pig manure-treated and 4/4CN-treated soil.

PCA ordination analysis showed that the fertilization treatments could be separated into two distinct groups, each possessing a specific range of soil MBC values (Fig. 5-A). The first group (4/4CN, 3/4CN+1/4MN, and 2/4CN+2/4MN treated soil) generally had lower MBC,

ranging from 124.0 to 171.6 mg kg⁻¹ (Fig. 5-A). The second group (2/4CN+1/4MN+1/4SN and 2/4CN+2/4SN treated soil) had higher MBC, ranging from 227.0 to 290.2 mg kg⁻¹. The difference is significantly related to the change in soil MBC (F=14.3, P<0.01) (Fig. 5-B), which accounted for 52.3% of the total variation in the composition of the microbial community, based on the RDA between microbial community and soil property.

3.5. Correlations between yield and soil microbial properties

Overall, the trends found in the total PLFA content, composition of the microbial communities (bacteria, actinomycetes, Gram-positive bacteria, Gram-negative
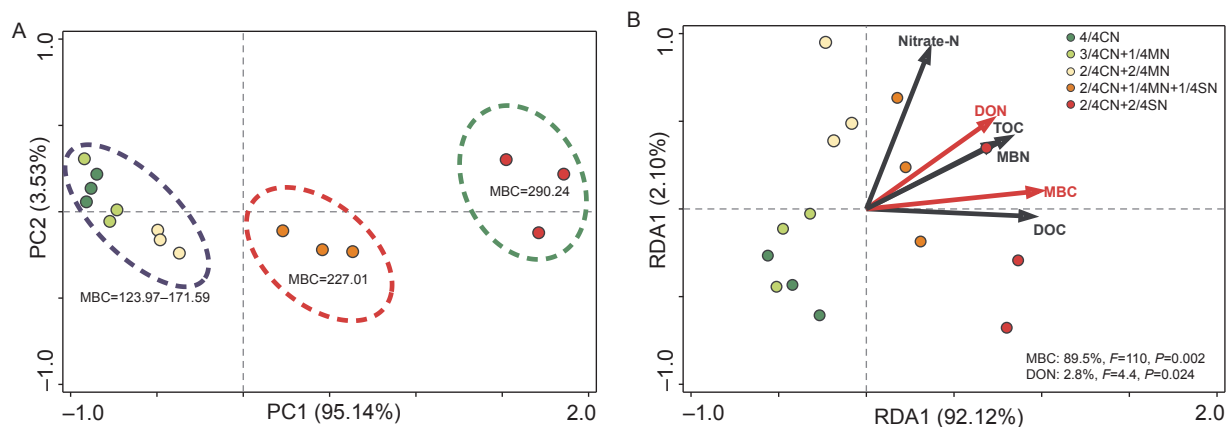
Fig. 3 Results of principal component analysis (PCA) of the enzyme activity in soil subjected to different fertilization treatments (A), and redundancy analysis (RDA) of the correlation between soil parameters and enzyme activity profiles (B). MBC, microbial biomass carbon; nitrate-N, NO_3^--N; DON, dissolved organic nitrogen; TOC, total organic carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon. CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw. The data are from the uprooting stage of the ninth growing season (winter-spring cucumber). Red arrows indicate soil parameters that have a strong and significant impact on enzyme activity ($P<0.05$). The corresponding proportion of the variation accounted for is shown in the lower right corner.
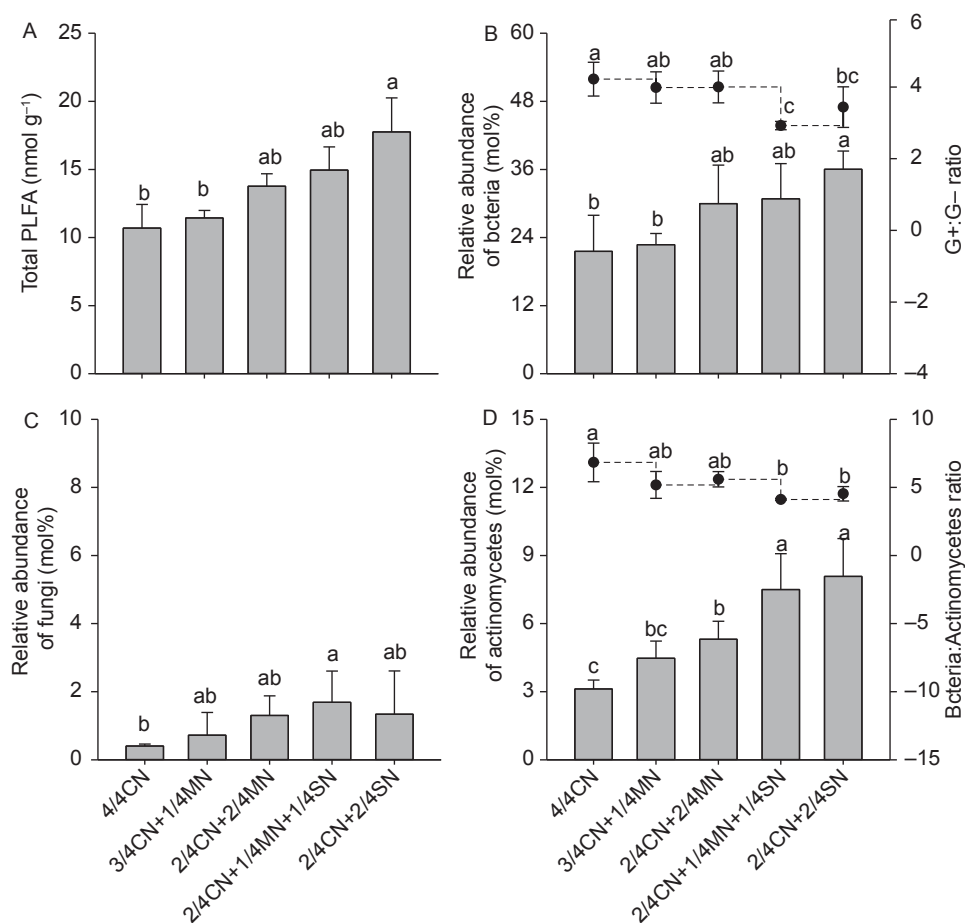

Fig. 4 A comparison of the total phospholipid fatty acid (PLFA; A), the relative abundance of bacteria (histograms) and the G+:G– ratio (dotted line; B), the relative abundance of fungi (C) and the relative abundance of actinomycetes (histograms) and bacteria:actinomycetes ratio (dotted line; D). G+, Gram-positive bacteria; G–, Gram-negative bacteria; CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw. Vertical bars represent the standard error (n=3) and lower case letters indicate significant differences between fertilizer treatments at the $P<0.05$ level.
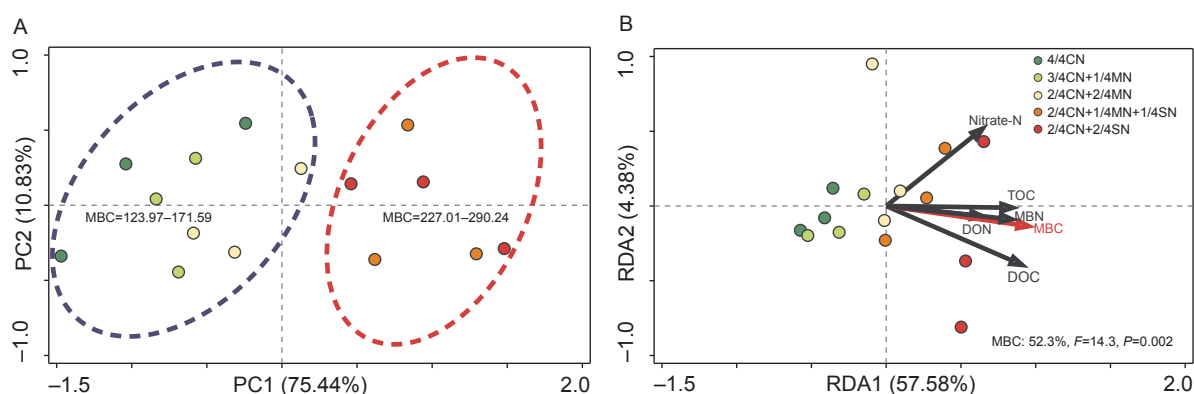
Fig. 5 Results of principal component analysis (PCA) of the microbial community composition in soils receiving different fertilization treatments (A), and redundancy analysis (RDA) of the correlation between soil parameters and microbial community composition (B). MBC, microbial biomass carbon; nitrate-N, NO_3^--N; TOC, total organic carbon; MBN, microbial biomass nitrogen; DON, dissolved organic nitrogen; DOC, dissolved organic carbon. CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw. The data are from the uprooting stage of the ninth growing season (winter-spring cucumber). Red arrows indicate soil parameters that have a strong and significant impact on the microbial community composition ($P<0.05$). The corresponding proportion of the variation accounted for is shown in the lower right corner.

bacteria), and soil enzyme activity showed that these properties generally increased with increasing MBC and MBN (Table 4). A correlation analysis revealed that the activities of most of the enzymes (phosphomonoesterase, β-glucosidase, β-cellobiosidase, N-acetylglucosaminidase, β-xylosidase, α-glucosidase, L-leucine aminopeptidase, and urease) were significantly positively correlated with soil MBC and MBN. However, sulfatase was significantly negatively correlated with MBC and MBN. MBC was significantly positively correlated with the total PLFA content, bacteria, actinomycetes, Gram-positive and Gram-negative bacteria. The analysis also showed that there were significantly positive correlations between enzyme activities (phosphomonoesterase, β-glucosidase, β-cellobiosidase, N-acetylglucosaminidase, β-xylosidase, α-glucosidase, L-leucine aminopeptidase, and urease) and the composition of the microbial communities (bacteria, actinomycetes, Gram-positive bacteria, Gram-negative bacteria). Furthermore, MBC, enzyme activity (phosphomonoesterase, L-leucine aminopeptidase, and urease), and composition of the microbial communities (total PLFA content, bacteria, fungi, actinomycetes, and Gram-negative bacteria) were significantly positively correlated with yield (Table 4).

4. Discussion

Many studies on grain cropland and vegetable fields have shown that the use of organic amendments, e.g., manure, compost, straw, can help to increase crop yield and improve soil quality (Bowles *et al.* 2014; Agegnehu *et al.* 2016). In this study, however, we found that crops grown in 4/4CN-

treated soil produces the best cucumber and tomato yields in the first three growing seasons. This is mainly because the topsoil was removed when the greenhouse was newly built, and the organic amendments release nutrients more slowly compared to chemical fertilizer (Tian *et al.* 1992). In our study, the highest yield was achieved using the straw-substituted treatments after three consecutive fertilization management seasons. Thus, successive applications of the organic amendments were required to effectively improve cucumber and tomato yield and soil quality in the new greenhouse. Productivity in agricultural ecosystems relates to the size and activity of the microbial biomass (Anand *et al.* 2015). Soil MBC was found to be significantly correlated with yield in our study. This result confirms previous findings that there is a close relationship between crop yield and microbial biomass in the soil, under both greenhouse (Chen *et al.* 2000) and open field conditions (Anand *et al.* 2015).

We also found that cucumber and tomato yields were significantly correlated with soil enzymes associated with N (L-leucine aminopeptidase and urease) and P cycling (phosphomonoesterase). This may be because large amounts of organic C are brought into the soil by the manure and straw and because a portion of the N and P absorbed from the soil by microbes and plants will lead to microbes regulating extracellular enzyme production to acquire the limited nutrients (Allison *et al.* 2007; Bowles *et al.* 2014). Interestingly, indicator PLFAs for Gram-negative bacteria, fungi, and actinomycetes were also significantly and positively correlated with yield. Dai *et al.* (2013) previously reported that peanut (*Arachis hypogaea* Linn.) growth and yield are promoted by an increase in Gram-negative bacteria. A higher proportion of Gram-negative bacteria

Table 4 Correlations between the soil microbial biomass carbon (MBC) and nitrogen (MBN), enzyme activity, microbial community composition, and yield for different partial organic-amendment replacements in the uprooting stage of the 9th growing season (winter-spring cucumber)[1]

	MBC	MBN	Pho	Sul	βG	CBH	NAG	βX	αG	LAP	U	Total PLFA	Bcteria	Fungi	Actinomycetes	G+	G−	Yield
MBC	1																	
MBN	0.76**	1																
Pho	0.97**	0.78**	1															
Sul	−0.86**	−0.62*	−0.81**	1														
βG	0.95**	0.69**	0.93**	−0.88**	1													
CBH	0.93**	0.66**	0.89**	−0.89**	0.99**	1												
NAG	0.89**	0.59**	0.83**	−0.95**	0.93**	0.95**	1											
βX	0.96**	0.71**	0.93**	−0.93**	0.98**	0.98**	0.95**	1										
αG	0.92**	0.69**	0.90**	−0.93**	0.93**	0.92**	0.93**	0.97**	1									
LAP	0.84**	0.47	0.83**	−0.87**	0.85**	0.82**	0.88**	0.88**	0.92**	1								
U	0.75**	0.63**	0.87**	−0.59**	0.71**	0.62**	0.57**	0.70**	0.71**	0.73**	1							
Total PLFA	0.78**	0.69**	0.79**	−0.52*	0.68**	0.68**	0.64**	0.69**	0.68**	0.58**	0.61*	1						
Bcteria	0.81**	0.71**	0.76**	−0.54*	0.66**	0.65**	0.64**	0.69**	0.67**	0.53*	0.58*	0.87**	1					
Fungi	0.45	0.48	0.53*	−0.13	0.29	0.25	0.17	0.29	0.26	0.21	0.53	0.74**	0.55*	1				
Actinomycetes	0.87**	0.77**	0.89**	−0.62*	0.75**	0.72**	0.68**	0.76**	0.75**	0.67**	0.76**	0.92**	0.85**	0.76**	1			
G+	0.83**	0.76**	0.80**	−0.61*	0.72**	0.71**	0.69**	0.74**	0.71**	0.55**	0.60**	0.82**	0.98**	0.48	0.83**	1		
G−	0.88**	0.75**	0.85**	−0.61*	0.74**	0.71**	0.70**	0.75**	0.75**	0.68**	0.70**	0.87**	0.90**	0.62*	0.94**	0.88**	1	
Yield	0.53*	0.38	0.63*	−0.42	0.39	0.35	0.37	0.44	0.50	0.59	0.80**	0.52	0.46	0.60	0.67**	0.43	0.57	1

[1] Pho, phosphomonoesterase; Sul, sulfatase; βG, β-glucosidase; CBH, cellobiohydrolase; NAG, N-acetylglucosaminidase; βX, β-xylosidase; αG, α-glucosidase; LAP, L-leucine aminopeptidase; U, urease; G+, Gram-positive bacteria; G−, Gram-negative bacteria.
*, $P<0.05$; **, $P<0.01$.

in the soil is usually interpreted as a shift from oligotrophic to more copiotrophic conditions (Borga et al. 1994; Saetre and Bååth 2000). Additionally, actinobacteria can produce a wide variety of antimicrobial metabolites (Basilio et al. 2003), which are beneficial to crop growth. These results indicate that manipulating these MBC-mediated microbial properties may be an important method of improving the yield in the greenhouse-vegetable field. Improved soil biological function has been revealed by noting general trends pointing to positive effects on soil enzyme activities and soil respiration in vegetables grown in plastic tunnels (Bonanomi et al. 2014). This suggests that soil MBC is a good indicator to assess the change in biological activity of greenhouse-vegetable soil caused by alternatives to inorganic fertilizers.

Several studies have shown that the composition of the soil microbial community is changed by organic amendments and that these changes are related to the soil C content (Lazcano et al. 2013; Willekens et al. 2014). Interestingly, we found that the composition of the microbial community was more strongly affected by straw than pig manure. This may be because the input of new organic matter significantly stimulated the growth of microorganisms in the soil (Shi et al. 2015), and because straw provided more organic C than the pig manure (Table 5). It has also been reported that straw may improve the physical properties of the soil by providing nutrients to directly promote microbial growth and enhance the SOC pool (Lu et al. 2015). It is generally recognized that soil MBC is used to indicate the size of the microbial community (Bastida et al. 2008). However, enhancement of the soil microbial biomass usually occurs through specific groups of microbial communities (e.g., bacteria, actinomycetes) (Dong et al. 2014). The results acquired in this study show that the relative abundances of bacteria, Gram-positive bacteria, Gram-negative bacteria, and actinomycetes were significantly and positively correlated with soil MBC. We also found that the soil microbial community composition (except for fungi) is significantly correlated with all of the soil enzyme activities involved in the C, N, P, and S cycles, indicating a close relationship between microbial community composition and soil enzyme activity (Allison et al. 2007; Burns et al. 2013).

Table 5 Total organic carbon (TOC), dissolved organic carbon (DOC) and nitrogen (DON), and soil nitrate-nitrogen (NO_3^--N) for different partial organic-amendment replacements in the uprooting stage of the 9th growing season (winter-spring cucumber)

Fertilizer treatments[1]	TOC (g kg^{-1})	DOC (mg kg^{-1})	DON (mg kg^{-1})	NO_3^--N (mg kg^{-1})
4/4CN	7.57±0.44 d	50.2±2.1 c	24.5±0.4 c	68.1±10.1 c
3/4CN+1/4MN	9.55±0.10 c	58.3±6.8 c	29.1±0.4 b	81.8±13.2 bc
2/4CN+2/4MN	12.86±0.30 b	68.7±9.3 bc	32.8±1.4 a	111.3±1.1 a
2/4CN+1/4MN+1/4SN	13.30±0.42 b	90.9±5.2 b	34.0±1.5 a	100.9±21.0 ab
2/4CN+2/4SN	14.43±0.52 a	123.4±9.1 a	34.7±2.9 a	90.8±17.6 ab

[1] CN, nitrogen in chemical fertilizer; MN, nitrogen in pig manure; SN, nitrogen in corn straw.
Data are the mean±SE, *n*=3. Different letters indicate significant differences among treatments at the *P*<0.05 level.

5. Conclusion

This study demonstrates the changes occurring in soil microbial biomass, enzyme activity, microbial community composition, and cucumber and tomato yield as a result of organic amendment substitution in greenhouse production. The development of indicators of soil ecological functions in the greenhouse vegetable soil may help farmers to evaluate and discover an optimal fertilization management strategy to improve soil quality and increase vegetable yield. We have further presented evidence that MBC-induced changes in soil enzyme activity and microbial community composition might be an important mechanism by which vegetable yield may be improved. In particular, straw-substituted treatments can support high and sustainable yields in greenhouse-vegetable production systems. In summary, considering all of the effects of different proportions of manure and/or straw substitution on microbial characteristics and vegetable yield (and practical feasibility), combined application of chemical fertilizer, manure, and straw (that is, the combination 2/4CN+1/4MN+1/4SN) appears to be a superior fertilization pattern to use for high, sustainable yields in greenhouse-vegetable production systems.

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