Journal of Integrative Agriculture 2015, 14(12): 2500–2511

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Microbial community structure and functional metabolic diversity are associated with organic carbon availability in an agricultural soil

LI Juan, LI Yan-ting, YANG Xiang-dong, ZHANG Jian-jun, LIN Zhi-an, ZHAO Bing-qiang

Key Laboratory of Plant Nutrition and Fertilizer, Ministry of Agriculture/Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China

Abstract

Exploration of soil environmental characteristics governing soil microbial community structure and activity may improve our understanding of biogeochemical processes and soil quality. The impact of soil environmental characteristics especially organic carbon availability after 15-yr different organic and inorganic fertilizer inputs on soil bacterial community structure and functional metabolic diversity of soil microbial communities were evaluated in a 15-yr fertilizer experiment in Changping County, Beijing, China. The experiment was a wheat-maize rotation system which was established in 1991 including four different fertilizer treatments. These treatments included: a non-amended control (CK), a commonly used application rate of inorganic fertilizer treatment (NPK); a commonly used application rate of inorganic fertilizer with swine manure incorporated treatment (NPKM), and a commonly used application rate of inorganic fertilizer with maize straw incorporated treatment (NPKS). Denaturing gradient gel electrophoresis (DGGE) of the 16S rRNA gene was used to determine the bacterial community structure and single carbon source utilization profiles were determined to characterize the microbial community functional metabolic diversity of different fertilizer treatments using Biolog Eco plates. The results indicated that long-term fertilized treatments significantly increased soil bacterial community structure compared to CK. The use of inorganic fertilizer with organic amendments incorporated for long term (NPKM, NPKS) significantly promoted soil bacterial structure than the application of inorganic fertilizer only (NPK), and NPKM treatment was the most important driver for increases in the soil microbial community richness (*S*) and structural diversity (*H*). Overall utilization of carbon sources by soil microbial communities (average well color development, AWCD) and microbial substrate utilization diversity and evenness indices (*H'* and *E*) indicated that long-term inorganic fertilizer with organic amendments incorporated (NPKM, NPKS) could significantly stimulate soil microbial metabolic activity and functional diversity relative to CK, while no differences of them were found between NPKS and NPK treatments. Principal component analysis (PCA) based on carbon source utilization profiles also showed significant separation of soil microbial community under long-term fertilization regimes and NPKM treatment was significantly separated from the other three treatments primarily according to the higher microbial utilization of carbohydrates, carboxylic acids, polymers, phenolic compounds, and amino acid, while higher utilization of amines/amides differed soil microbial community in NPKS treatment from those in the other three treatments. Redundancy analysis

Received 21 August, 2015 Accepted 12 November, 2015
LI Juan, Mobile: +86-13581617626, Tel: +86-10-82108664, E-mail: lijuan02@caas.cn; Correspondence ZHAO Bing-qiang, Tel: +86-10-82108658, Fax: +86-10-82108664, E-mail: zhaobingqiang@caas.cn

doi: 10.1016/S2095-3119(15)61229-1

(RDA) indicated that soil organic carbon (SOC) availability, especially soil microbial biomass carbon (Cmic) and Cmic/SOC ratio are the key factors of soil environmental characteristics contributing to the increase of both soil microbial community structure and functional metabolic diversity in the long-term fertilization trial. Our results showed that long-term inorganic fertilizer and swine manure application could significantly improve soil bacterial community structure and soil microbial metabolic activity through the increases in SOC availability, which could provide insights into the sustainable management of China's soil resource.

Keywords: long-term fertilization regimes, organic amendment, soil microbial community structure, microbial functional metabolic activity, carbon substrate utilization

1. Introduction

Organic and inorganic fertilizer amendments not only increase soil nutrient availability to plant, improve soil fertility including soil physical, chemical and biological properties, thus maintain or increase crop yields (Li *et al.* 2008; Gong *et al.* 2009), but also influence soil microorganisms (Chu *et al.* 2007; Liu *et al.* 2007; Yu *et al.* 2015). It has been reported that microbial communities were sensitive to changes of soil environmental conditions or soil nutrient status in long-term organic or inorganic fertilization trials, and soil organic carbon and pH were demonstrated to be major factors to affect soil microbial community composition and activity (Wessén *et al.* 2010; Shen *et al.* 2010; Geisseler *et al.* 2014; Li *et al.* 2015). Changes in microbial structure and activity in turn have important implications for the rates of soil processes. For example, variation in microbial community structure in soils have been observed to influence rates of denitrification, nitrification and nitrogen fixation (Balser and Firestone 2005; Hallin *et al.* 2009; Levy-Booth *et al.* 2014). Therefore, soil microorganisms and the related nutrient cycling processes are essential for long-term sustainability of agricultural systems and are a major component in soil formation and productivity (Vineela *et al.* 2008; Geisseler *et al.* 2014), an improved understanding of the response of soil microbiota to agricultural management practices and exploring the key factors in soil environmental characteristics influencing soil microbial communities can help to identify strategies that maintain and improve soil quality.

The effects of long-term fertilization regimes on soil microorganisms have been given particular attention (Marschner *et al.* 2003; Gu *et al.* 2009; Geisseler *et al.* 2014; Li *et al.* 2014). Application of inorganic fertilizers has been shown to increase the cultivable microbial counts (including bacteria, fungi, and actinomycetes) than no fertilizer application under a wheat-maize cropping system in northern China (Gong *et al.* 2009). Zhong and Cai (2007) reported that balanced inorganic fertilization resulted in higher microbial metabolic diversity than nutrient-deficiency fertilization in soil, which is in agreement with the current report of increased soil microbial function gene structures and abundances under long-term balanced inorganic fertilization in a P-limited paddy soil (Su *et al.* 2015). Meta-analysis of long-term inorganic fertilizer trials revealed that most of studies indicated significant effects on soil microbial community composition (Geisseler *et al.* 2014), and soil microbe was sensitive to N, P and K fertilization in 84% studies with an average length of 8.2 yr (Allison and Martiny 2008). Moreover, higher fungal population was found in a continuous inorganic fertilization trial in India (Vineela *et al.* 2008). However, other studies have reported that long-term inorganic N application reduces or has no effect on soil community structure (Sarathchandra *et al.* 2001; Ogilvie *et al.* 2008; Wu *et al.* 2011) or microbial functional diversity (Kong *et al.* 2008; Li *et al.* 2013; Yu *et al.* 2015). For instance, Kong *et al* (2008) reported that long-term inorganic fertilization would not result in significant changes in the microbial biomass and microbial functional diversity of the black soil in northeast of China.

In general, organic fertilizers, including organic manure, crop residues, green manure, and sewage sludge combined with inorganic fertilizer application for long-term have been revealed to cause shifts in soil microbial community (O'Donnell *et al.* 2001; Marschner *et al.* 2003; Enwall *et al.* 2005; Elfstrand *et al.* 2007; Sradnick *et al.* 2013) by increasing soil total bacterial diversity (Gu *et al.* 2009; Shen *et al.* 2010; Wu *et al.* 2011), altering Gram-positive/Gram-negative bacteria ratios and bacterial/fungal ratios (Marschner *et al.* 2003; Giacometti *et al.* 2013) or increasing soil functional microbial community compositions such as ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and cellulolytic bacteria and fungi (Chu *et al.* 2007; He *et al.* 2007; Wessén *et al.* 2010; Fan *et al.* 2012), and improving soil microbial carbon source utilization profiles (Liu *et al.* 2007; Ge *et al.* 2008; Zhong *et al.* 2010). However, Yu *et al.* (2015) revealed no significant difference of microbial community structure by long-term field fertilization although soil microbial activities

were significantly affected, which means that soil microbial metabolic activity was not consistent with changes of soil microbial community composition in long-term fertilizer trials. And this difference may be due to the impact of changes in environmental characteristics on soil microbial processes (Shen *et al.* 2010). Soil environmental characteristics such as soil water content, temperature, soil organic carbon (SOC), and pH have been reported to affect soil microbial communities significantly (Degens *et al.* 2000; Lipso *et al.* 2000; Drenovsky *et al.* 2004; Shen *et al.* 2010; Geisseler *et al.* 2014). For instance, it is revealed that SOC and pH were positively correlated with soil microbial community composition in long-term fertilization trials (Shen *et al.* 2010; Geisseler *et al.* 2014); soil organic carbon availability, which is defined as dissolved organic carbon (DOC) or soil microbial biomass carbon (Cmic) or the ratio of Cmic/SOC (Sikora *et al.* 1990; Nelson *et al.* 1994), was reported to be major determine for preservation of soil microbial diversity (Lipson *et al.* 2000; Drenovsky *et al.* 2004); Greater differences in microbial community composition and catabolic evenness were demonstrated to have correspondent relationships with greater differences in soil organic carbon fractions (DOC, Cmic, potentially mineralizable C and light fraction organic carbon (LFOC)) (Cookson *et al.* 2005; Cookson *et al.* 2008). In our previous research (Li *et al.* 2008), we investigated that long-term organic and inorganic fertilizer applications could significantly increase soil environmental characteristics such as SOC, total N (TN), total phosphorous (TP), DOC, DON, Cmic, Nmic, and Cmic/SOC, and decrease soil C/N ratio and pH, however, relatively little is known about the precise nature of these changes on soil microbes. In conclusion, our objective is to determine if differences exist in microbial community structure and functional metabolic activities of soil microorganisms after 15 yr of different fertilization regimes in double-cropping wheat-maize rotations, which are widely used in North China Plain (NCP). Their relationships with changes in soil environmental characteristics are also explored to understand how it is regulated or driven by factors such as nutrient inputs in the long-term fertilizer trial.

2. Results

2.1. Effect of long-term fertilization regimes on soil microbial community structure

Here the method of polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) based on 16S rRNA genes was used to investigate the effects of long-term fertilization regimes on soil microbial community structure and diversity. Bacterial DGGE profiles generated from the universal bacterial primers (F357 and R517) revealed differences in the structural composition of the bacterial communities in soil samples (Table 1, Appendix A), and this was due to differences in band positions, band intensities and numbers of bands. Each of the distinguishable bands in the separation pattern represents a bacterial operational taxonomic unit (OTU) (Cocolin *et al.* 2002). Differences in the DGGE profiles revealed that soil bacterial community richness (*S*, the numbers of bands detected by DGGE) were higher in the fertilized soils than that in the CK soil. The NPKM treatment showed the most complex DGGE pattern with 19 visible bands, indicating the presence of a high number of different bacterial taxa, and then followed by NPKS treatment. CK treatment had the lowest number of bands in the DGGE profiles (Table 1). Shannon's diversity index values (*H*) for the whole data set indicated that long-term fertilization regimes significantly affected soil bacterial community diversity which showed similar trend with *S* among different long-term fertilizer treatments.

2.2. Effect of long-term fertilization regimes on functional metabolic diversity of soil microbial community

Average well color development (AWCD) followed a sigmoid curve with incubation time in keeping with the characteristics of microbial growth (Fig. 1). After 24 h of incubation, the values of AWCD for soils with different fertilizer applications increased quickly with the incubation time, and were

Table 1 Effect of long-term fertilizer treatments on indices of soil bacteria structure using denaturing gradient gel electrophoresis (DGGE) bands pattern data and soil microbial functional metabolic diversity indices using Biolog assays data

Treatments[1)]	Soil bacterial structure indices		Soil microbial functional metabolic diversity indices	
	Richness (*S*, detected band)	Shannon's diversity index (*H*)	Shannon's diversity index (*H'*)	Substrate evenness (*E*)
CK	11 (±2) d	2.28 (±0.10) d	2.97 (±0.06) c	0.87 (±0.09) c
NPK	13 (±1) c	2.55 (±0.07) c	3.05 (±0.05) bc	0.89 (±0.09) bc
NPKM	19 (±1) a	2.77 (±0.01) a	3.20 (±0.01) a	0.93 (±0.02) a
NPKS	15 (±1) b	2.68 (±0.07) b	3.10 (±0.05) b	0.90 (±0.08) b

[1)] CK, non-amended control; NPK, commonly used application rate of inorganic fertilizer treatment; NPKM, commonly used application rate of inorganic fertilizer with swine manure incorporated treatment; NPKS, commonly used application rate of inorganic fertilizer with maize straw incorporated treatment.

Long-term means (±SE) followed by the same letter in the same column are not significantly different (P<0.05, Duncan's test). The same as below.

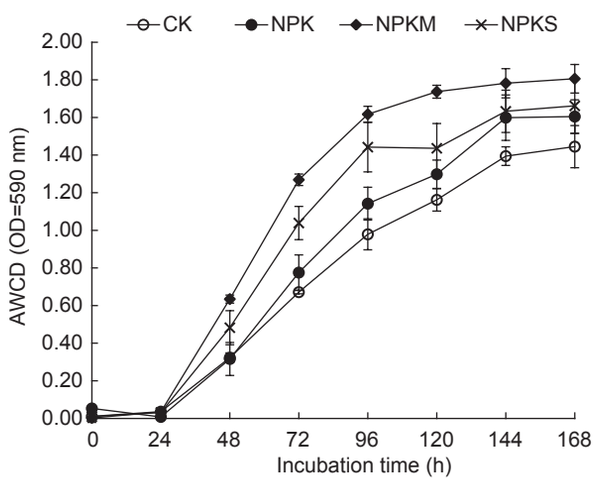

Fig. 1 Average well color development (AWCD) of 31 carbon sources utilization of soil microbial community in different fertilizer treatments in the long-term fertilizer trial using Biolog assays. CK, non-amended control; NPK, commonly used application rate of inorganic fertilizer treatment; NPKM, commonly used application rate of inorganic fertilizer with swine manure incorporated treatment; NPKS, commonly used application rate of inorganic fertilizer with maize straw incorporated treatment. Mean values of four replicates for the different treatments. Error bars represent standard deviation (P<0.05, Duncan's test). The same as below.

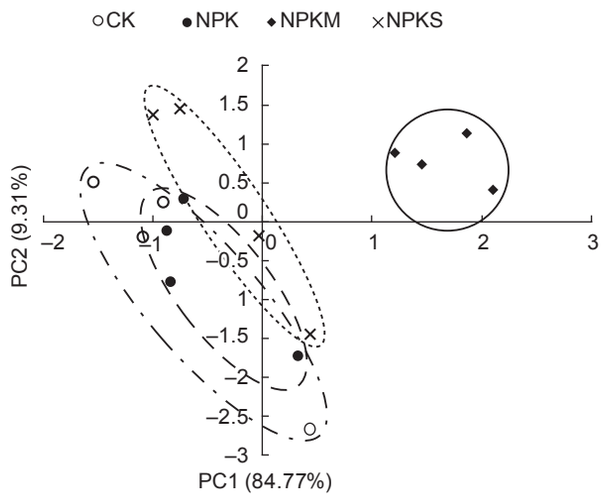

Fig. 2 Principal component analysis (PCA) of the microbial carbon sources utilization profiles using Biolog assays from different fertilizer treatments in the long-term fertilizer trial.

separated from each other among all the treatments. The values of AWCD for NPKM (a commonly used application rate of inorganic fertilizer with swine manure incorporated treatment) treatment were always higher than the others, with values in decreasing order of NPKS (a commonly used application rate of inorganic fertilizer with maize straw incorporated treatment)>NPK (a commonly used application rate of inorganic fertilizer treatment)>CK (a non-amended control treatment).

Shannon's diversity index (H') and evenness (E) calculated using the data from the 72 h incubation readings were listed in Table 1, which indicated that substrate utilization diversity (H') and substrate utilization level (E) of soil microbial community among all treatments showed significantly different. The value of H', ranged from 2.97 to 3.20, was significantly higher in the NPKM treated soils than those in other treatments, and then followed by NPKS treatment with higher H' values compared to CK. However, there was no difference between NPKS and NPK, and no statistical difference was also found between NPK and CK. A smilar trend was observed for the values of E which ranged from 0.87 (CK) to 0.93 (NPKM).

Principal component analysis (PCA) of the optical density values measured at 72 h incubation time was used to analyze the carbon sources utilization ability of soil microbial community in long-term fertilizer treatments (Fig. 2). The first 2 principal components (PCs) accounted for 84.77 and 9.31% of the total variability, respectively. The variability in PC1 was largely influenced by utilization of carbohydrates, carboxylic acids, polymers, phenolic compounds, and amino acid. The PC2 was driven by utilization of amines/amides. Thus approximately 94% of the variability in the carbon source utilization profiles was explained by the utilization of these six common carbon sources. The microbial communities of the four different treatments had distinctive patterns of carbon source utilization based on PCA analysis of the color response data (Fig. 2). Soils in the NPKM treatment had much higher PC scores for PC1 and PC2 than either NPKS, NPK or CK, and this treatment was clearly separated from them. Soils in the NPKS treatment had, on average, higher values for PC2 than in NPK and CK, and were completely separated from the other three treatments. However, there was no clear separation between the NPK and CK treatments.

2.3. Effect of soil environmental characteristics on microbial community structure and functional metabolic diversity under long-term fertilization regimes

Redundancy discriminate analysis (RDA) was performed using soil environmental characteristics (TN, SOC, TP, DOC, DON, pH, C/N, Cmic, Cmic/SOC and Nmic) as explanatory variables and the relative abundance of two indicators (S and H) of soil bacterial community structure as responsible variables to explore the effects of soil environmental characteristics on soil microbial community structure (Fig. 3). The explanatory variables significantly correlated with S and H, respectively (P<0.01) and accounted for 91.76% of total variation (P<0.01). The axes 1 and 2 accounted for

85.42 and 6.34%, respectively. Soil environmental characteristics had positive correlations with microbial community structure indicators (*S* and *H*) except for soil C/N and pH which showed significantly negative correlations with *S* and *H*. The differences of soil microbial community structure indicators (*S* and *H*) among long-term fertilizer treatments were more pronounced. In general, ordination of treatments was related to the first axis (RDA1) and clearly separated with each other (Fig. 3). The structure of microbial community in NPKM treated soil was significantly different from those in soils of other treatments and this difference was mainly due to the changes in soil Cmic (F=57, P<0.01) and SOC (F=4.4, P<0.05), which explained 80.30 and 5.00% of the total variability in the structure of microbial community respectively based on the forward selection model results of RDA analysis. The structure of microbial community in NPKS treated soils also showed significantly positive correlations with Cmic and SOC along axis 1 and had no overlap with other treatments. Whereas the microbial community structure in CK treated soil negatively related to soil Cmic and SOC along axis 1 (Fig. 3).

RDA analysis of exploring the relationship between soil environmental characteristics and microbial metabolic diversity showed that explanatory variables (TN, SOC, TP, DOC, DON, pH, C/N, Cmic, Cmic/SOC and Nmic) accounted for 97.38% of total variation (P<0.01) and the first two axes explained 97.12 and 0.25%, respectively (Fig. 4). All explanatory variables (TN, SOC, TP, DOC, DON, pH, C/N, Cmic, Cmic/SOC and Nmic) were observed to have significant effects on microbial metabolic activity indicators (*E* and *H'*) (P<0.01). Similar with soil microbial community structure, soil environmental characteristics except for soil C/N and pH positively correlated with microbial functional metabolic diversity indicators (*S* and *H*) while soil C/N and pH showed negative trend. The microbial communities of the long-term fertilizer treatments had distinctive patterns

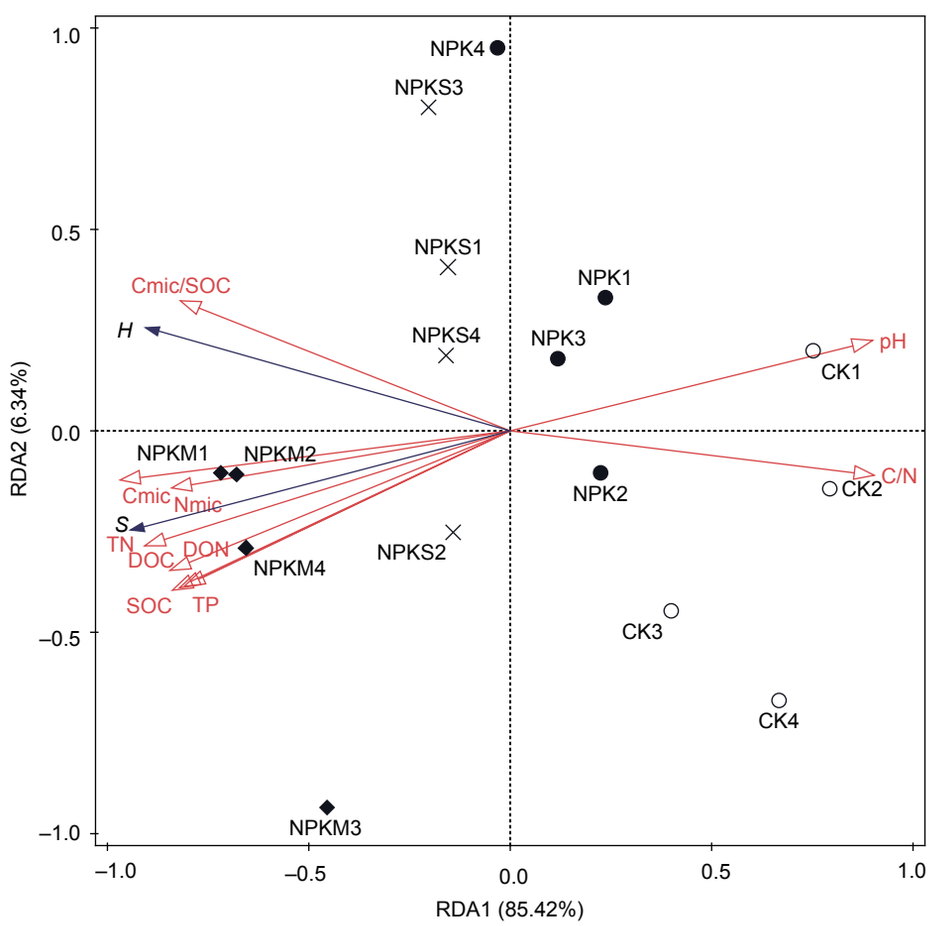

Fig. 3 Redundancy analysis (RDA) of soil microbial community composition (*S* and *H*) constrained by soil environmental characteristics under long-term fertilization regimes from different fertilizer treatments. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorous; C/N, the ratio of SOC to TN; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; pH, soil pH value; Cmic, soil microbial biomass carbon; Nmic, soil microbial biomass nitrogen; Cmic/SOC, the ratio of soil microbial biomass carbon to soil organic carbon. The same as below.

of carbon source utilization and the model of soil Cmic and Cmic/SOC were found to be main factors to affect microbial metabolic activities by forward selection and explained 97.30 and 0.27% of the total variation separately (P<0.01). NPKM treatment had much higher scores along axis 1 than other treatments, and was completely separated from them. There was no significant difference between NPKS and NPK treatments. CK treatment with lower soil Cmic and Cmic/SOC, however, clearly separated with other three treatments (Fig. 4).

3. Discussion

Soil microorganisms play prominent roles in maintaining the long-term fertility of an agroecosystem (Watts *et al.* 2010) and could serve as indicators of evaluating soil quality (Sharma *et al.* 2011). Knowledge about soil microbial community structure and functional metabolic diversity is important for helping us to optimize the fertilization practices and maintain soil productivity and agroecosystem.

Soil microbial community structure were significantly affected by long-term fertilizer application (Table 1), and showed higher values in treatments with inorganic fertilizers plus organic amendments application (NPKM, NPKS) than in treatment with only inorganic fertilizer application (NPK), which is in agreement with other studies that have shown effects on shifts in microbial community structure with long-term addition of different organic and/or synthetic fertilizer amendments (Liu *et al.* 2007; Ge *et al.* 2008; Zhao *et al.* 2014). The increased crop residues and its corresponding impacts on soil environmental status could be expected to increase bacteria community structure in fertilized soils (Sun *et al.* 2015). Organic amendments may provide a greater diversity of potential substrates for microbe growth and reproduction (Liu *et al.* 2009), further, much more crop residues and the improvement of soil environmental characteristics in organic-inorganic fertilized soils were found than those in inorganic fertilized soils due to the added organic amendments (Li *et al.* 2008), which could be acting in concert with increased niche diversity in organic-inorganic

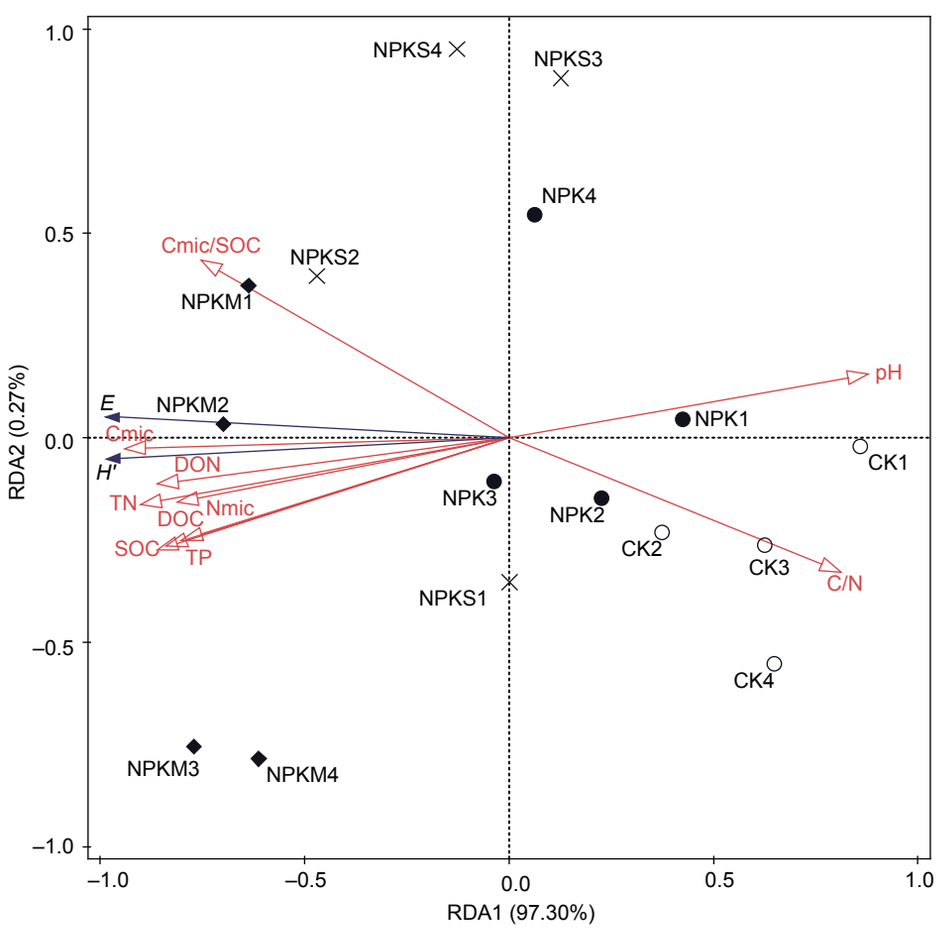

Fig. 4 RDA of soil microbial community functional metabolic diversity (*H'* and *E*) constrained by soil environmental characteristics under long-term fertilization regimes from different fertilizer treatments.

fertilized soil microbial communities to drive diversity differences in this study. This is in accordance with previous report (Sun *et al.* 2015).

It has been reported that long-term fertilization regimes significantly influences soil microbial functional diversity (Zhong and Cai 2007; Sradnick *et al.* 2013; Yu *et al.* 2015). In our report, increased functional metabolic diversity of soil microbial community was also investigated in fertilized soils with the highest value in NPKM treatment, but no statistical difference between NPKS and NPK treatments. This result may be associated with lower inorganization effect of soil status on soil microbial metabolic ability to decompose maize straw just through colder period of the year between sowing and sampling time in NPKS treatment. Furthermore, many previous studies showed decreased microbial functional diversity (Li *et al.* 2013) in the inorganic fertilized soils compared with control, which is in contrast with our report. An explanation for this result is that higher inputs of inorganic N in the form of fertilizer led to enrichment of microbial groups that were well suited to high mineral N environments at the expense of other groups (Li *et al.* 2013).

Swine manure was found the most efficient in raising soil microbial community structure and functional metabolic diversity among all the incorporated organic amendments (swine manure and maize straw), which is confirmed by the recent report that bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure (either swine manure or cattle manure) than wheat straw (Sun *et al.* 2015). Differences in organic matter composition and thus substrate availability are likely to be the main reason for the differences in microbial community observed in the present study such as the increased bacteria categories and the microbial metabolic functional diversities. The temporal dynamics in decomposition depend on the chemical composition of the organic matter that is added to the soil and swine manure has a lower C/N ratio compared to maize straw, which is easily degraded by microorganisms (Lejon *et al.* 2007; Eilers *et al.* 2010) and there have been reported that the decompostion of different types of organic matter stimulated the activity of different special microbial groups (Bastian *et al.* 2009). It has been reported that soil microbial metabolic activity and functional diversity were promoted in long-term inorganic fertilizer plus organic manure plots through the abundance of carbohydrates, carboxylic acids and amino acids which are tested as root exudates released into the soil by crop plants (Zhong *et al.* 2010), which is in consistence with our report. Another primary reason is that the neutral pH and increased C induced by long-term organic manure input will allow more optimal conditions for the growth of soil bacterial community in alkaline soils (Li *et al*. 2008; Orr *et al*. 2011).

Repeated fertilizer applications to soil can change soil microbial community directly or indirectly since they change the soil environmental characteristics such as physical, chemical and biological properties (Beauregard *et al.* 2010; Yu *et al.* 2015). In this study, significant effects of soil environmental characteristics (TN, SOC, TP, DOC, DON, pH, C/N, Cmic, Cmic/SOC and Nmic) induced by 15-yr fertilization regimes on soil microbial community structure (P<0.01) and functional metabolic diversity (P<0.01) were found by using RDA analysis (Figs. 3 and 4). TN, SOC, TP, DOC, DON, Cmic, Cmic/SOC, and Nmic showed positive impacts on soil microbial community structure and functional diversity, which is in agreement with previous report (Li *et al.* 2015), furthermore, soil C/N ratio and pH were found to be negatively related to soil microbial community structure and functional diversity, which is in contrast with other results (Shen *et al.* 2010; Geisser *et al.* 2014). This difference may be due to the values of soil pH because it was demonstrated that soil pH had positive correlation with changes in soil microbial community diversity in acid soils (Shen *et al.* 2010), while showed negative results in alkaline soils (Li *et al.* 2015). Moreover, soil Cmic, Cmic/SOC and SOC which play fundamental roles in soil organic carbon dynamics (Haynes 2005), were observed to be the main predominant factors in changing soil microbial community structure and functional metabolic diversity. This result is confirmed by the previous studies which reported that the main and higher significant effect on soil microbes was related to organic amendments application due to the large amount of available C and labile C fractions suggesting greater efficiency in terms of mineralization per unit soil C (Drenovsky *et al.* 2004; Cookson *et al.* 2005; Vineela *et al.* 2008; Watts *et al.* 2010) which is in concert with increased microbial niche diversity in organic-inorganic fertilized soil with higher to drive diversity differences in our report. The soils with manure application have higher levels of labile organic C (Cmic), soil organic carbon availability (Cmic/SOC) and SOC compared to the soils with maize straw application (Li *et al.* 2008), therefore supporting higher levels of microbial activity. Similar result has been reported that decrease in Cmic reserve in soils reflected the reduction of soil microbial metabolic diversity (Degens *et al.* 2000; Franco *et al.* 2006).

4. Conclusion

Contrasting fertilization management over 15 yr has had profound effects on soil microbial community structure and functional metabolic diversity. Long-term fertilizer application significantly increased soil microbial community structure and functional metabolic diversity with the highest values when inorganic fertilizer and swine manure were

applied. The application of inorganic fertilizer with maize straw incorporated significantly increased soil microbial community structure, however, had no statistically differences in functional metabolic diversity of microbes compared to the application of only inorganic fertilizers, indicating that the application of inorganic fertilizer combined with swine manure was a strategy for farmer to maintain the sustainability of agricultural ecosystems. This research also provide the information of that soil carbon availability, particularly soil Cmic and Cmic/SOC, are the most profound factors to affect the variation of both structure and functional metabolic diversity of soil microbes, and it may improve the agricultural sustainability by better matching fertilization with soil ecosystem through our understanding of the microbial mediated mechanism of soil biochemical processes.

5. Material and methods

5.1. Site description and experimental design

The long-term fertilizer experiment was established in 1991 and is located in Changping County, Beijing, China (40°13′N, 116°14′E). The average annual rainfall is 600 mm and the average annual temperature is 11°C. The soil is a Drab Fluvo-aquic soil with 20.3% sand, 65.0% silt and 14.7% clay. The initial chemical properties of the soil in 1991 are given in Table 2. The site is in a wheat-maize rotation which is a typical cropping system in North China Plain (NCP). The following four fertility treatments were used: a non-amended control treatment (CK), a commonly used application rate of inorganic fertilizer treatment (NPK), a commonly used application rate of inorganic fertilizer with swine manure incorporated treatment (NPKM), and a commonly used application rate of inorganic fertilizer with maize straw incorporated treatment (NPKS). Each treatment was replicated four times with plot size of 2 m×1.5 m in a randomized complete block design. The commonly used application rates of inorganic NPK fertilizers were 160 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 60 kg K_2O ha^{-1} applied twice annually in spring after wheat harvest and fall after maize harvest. Inorganic fertilizers N, P and K are urea, calcium superphosphate and potassium chloride, respectively. Swine manure had 43.87% C, 2.09% N, 3.61% P_2O_5, and 2.01% K_2O and maize straw had 128.25% C, 1.71% N, 0.39% P_2O_5, and 1.81% K_2O in 2005. The organic amendments were applied once during fall with the application rates of 33 t ha^{-1} swine manure (M) and 2.17 t ha^{-1} maize straws (S) before sowing wheat. The field management is according to local farmer's practice. All aboveground crop biomass was removed from the field at harvest.

5.2. Soil sampling and preparation

Soil samples were collected at the jointing stage in wheat in April 2006. In each plot, at least five soil cores were collected from the top 20 cm with an auger and mixed to form a composite sample. The sample was sieved (2 mm mesh) and separated into three parts. The first part was air-dried for the determination of soil chemical properties, the second part was stored at 4°C for not more than 1 wk for microbial carbon utilization analysis and the thrid part was stored at –80°C for molecular analysis.

5.3. Soil chemical and biological properties analyses

Soil chemical and biological properties such as soil organic carbon (SOC), total N (TN), total P (TP), pH, dissolved organic C (DOC), dissolved organic N (DON), microbial biomass C (Cmic), and microbial biomass N (Nmic) were determined by chemical analysis method and have been described earlier (Li *et al.* 2008) (Table 3). SOC were assessed by vitriol acid-potassium dichromate oxidation method, TN by the Kjeldahl method and TP by the $HClO_4$-H_2SO_4 method, and soil pH was measured in a 1:2.5 (soil:water) mixture using the potentiometric method (Lu 2000). Cmic and Nmic contents were assessed using the chloroform-fumigation-extraction method (Vance *et al.* 1987) by shaking with 0.5 mol L^{-1} K_2SO_4 (1:4 soil:K_2SO_4 solution) for 30 min and filtering, non-fumigated soils were extracted for the measurement of DOC and DON.

5.4. DNA extraction and PCR-DGGE analysis

The DNA extraction method used was based on that of Zhou *et al.* (1996) and Moffett *et al.* (2003) with some modifications. 5 g soil sample was added to 13.5 mL of autoclaved extraction buffer (pH 8.0, 100 mmol L^{-1} Tris-HCl, 100 mmol L^{-1} di-sodium EDTA, 100 mmol L^{-1} sodium phosphate, 1.5 mol L^{-1} NaCl, 1% hexadecylmethylammonium bromide (CTAB)). 100 μL of 10 mg mL^{-1} proteinase K was added and the mixture was incubated at 37°C for 30 min by horizon shaking at 150 r min^{-1}. Sodium laurylsulfonate (SDS) was

Table 2 Soil initial chemical properties in 1991 prior to application of fertilizers (0–20 cm)

SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	Available N (NO_3^--N+NH_4^+-N) (mg kg^{-1})	Olsen's P (mg kg^{-1})	Slow-available K (mg kg^{-1})	pH
7.48	0.48	0.58	6.49	3.77	503.67	8.12

SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorous. The same as below.

then added to a final concentration of 2% and each tube was incubated at 65°C in a water bath for 2 h with gentle end-over-end mixing every 15 min. The mixture was then centrifuged at 6 000×g for 5 min and the supernatant was collected. Two or three repeats of centrifugation were performed and the supernatant was combined. The proteins were denatured by the addition of chloroform-isoamyl alcohol and the DNA was precipitated in isopropanol overnight at room temperature. It was then pelleted by centrifugation, washed twice with 5 mL of cold 70% ethanol, dissolved in 100 μL sterile, deionized water and stored at –80°C.

PCR on soil DNA extracts was performed with primers of 16S rRNA gene V3 regions for bacterial groups. The 50 μL PCR mixture contained a primer set (10 pmol each); 10× PCR buffer (100 mmol L^{-1}, included Mg^{2+}), 5 μL; dNTP (10 mmol L^{-1}), 1 μL; DNA template (10–50 ng), 1 μL; TaKaRa Ex*Taq* polymerase, 1.5 U and ultra pure water 40.5 μL. The primers used for PCR were 517R (5′-ATTACCGCGGCTGCTGG-3′) and F357-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGG-GCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) (Muyzer *et al.* 1993). PCR amplification was performed at 94°C for 5 min, followed by 20 touchdown cycles of 94°C for 1 min, annealing at 65°C (with the temperature decreasing 0.5°C each cycle) for 1 min and extension at 72°C for 1 min. This was followed by 15 cycles of 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min with a final single extension at 72°C for 7 min. The size of the PCR product was visualized by electrophoresis in 0.8% agarose gels after ethidium bromide staining.

DGGE analysis was conducted using a DCode system (Bio-Rad Laboratories, Hercules, California). Samples of PCR product (20 μL) were loaded onto 6–12% (w/v) polyacrylamide gels in 1×TAE buffer. The polyacrylamide gels were made with a linear denaturing gradient ranging from 30% denaturant at the top of the gel to 60% denaturant at the bottom. The electrophoresis was run at 220 V and 60°C for 300 min. After electrophoresis, the gels were soaked for 20 min in SYBR Green I nucleic acid gel stain (1:8 000 dilution, Sigma Co., Germany). To get a clear image, the gel was photographed with gel photo system (GelDoc 2000, BioRad, Hercules, CA, USA). Photographs were analyzed with BioRad Quantity One software package.

5.5. Carbon source utilization profiles

Carbon source utilization profiles were used to characterize the soil microbial functional metabolic activity and were determined using the Biolog EcoPlate (Biolog Co., USA) which contains three replicate wells of 31 carbon substrates with the method described by Garland and Mills (1991). Soil, equivalent to 10 g dry soil, was suspended in 90 mL of sterile 0.145 mol L^{-1} NaCl and homogenized in a blender at high speed for 1 min. The solution was serially diluted to 10^{-3}, and 125 μL of the 10^{-3} dilution was inoculated into each well of the plate. Plates were incubated for 7 d at 25°C and well absorbance was measured at 590 nm using a plate reader (Biolog Co., USA) every 24 h. All solutions and equipment were sterilized by autoclaving prior to use. The absorbance values at the start of the incubation were subtracted from the absorbance values of the subsequent readings. In addition, the color development of the control well at each reading was subtracted from the other wells to correct for any respiratory activity due to carbon added with the inoculum. Negative values were set to zero. The average well-color development (AWCD) for each treatment, i.e., the mean absorbance values of all 31 substrates, and for different substrate categories (carbohydrates, carboxylic acids, amines/amides, phenolic compounds, amino acids and polymers) were calculated for every reading. The substrate-utilization profile was analyzed on well-absorbance values at the 72 h reading.

5.6. Calculation and statistical analysis

All statistical work was done using the SPSS 11.5 and Microsoft office Excel 2007 for Windows. One-way ANOVA was used to determine treatment effects and means were compared using Duncan's multiple range Test (P<0.05).

Banding patterns of DGGE profiles were analyzed by the Bio-Rad Quantity One

Table 3 Effects of long-term fertilizer treatments on soil chemical and biological properties after 15 yr of annual fertilizer application (0–20 cm)

Treatments	SOC (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	C/N	DOC (mg kg^{-1})	DON (mg kg^{-1})	pH	Cmic (mg kg^{-1})	Nmic (mg kg^{-1})	Cmic/SOC (%)
CK	9.31 (±0.49) d	0.56 (±0.06) d	0.56 (±0.01)d	16.87 (±1.02) a	29.21 (±2.66) c	7.21 (±0.58) c	8.25 (±0.01) a	96.49 (±21.05) d	36.21 (±6.91) d	1.03 (±0.19) c
NPK	10.43 (±0.35) c	0.72 (±0.03) c	0.71 (±0.04) c	14.46 (±0.21) b	32.34 (±3.25) bc	9.01 (±0.28) c	8.08 (±0.02) b	214.11 (±45.18) c	72.58 (±0.81) b	2.04 (±0.37) b
NPKM	20.98 (±0.52) a	1.65 (±0.02) a	2.349 (±0.06) a	12.69 (±0.19) c	128.16 (±8.23) a	32.81 (±4.27) a	7.56 (±0.05) c	479.60 (±8.68) a	107.51 (±2.98) a	2.29 (±0.04) ab
NPKS	11.48 (±0.19) b	0.92 (±0.05) b	0.79 (±0.01) b	12.49 (±0.39) c	40.18 (±8.30) b	13.41 (±1.57) b	8.02 (±0.07) b	290.79 (±23.04) b	54.48 (±1.89) c	2.53 (±0.17) a

C/N, the ratio of SOC to TN; pH, soil pH value; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; Cmic, soil microbial biomass carbon; Nmic, soil microbial biomass nitrogen; Cmic/SOC, the ratio of soil microbial biomass carbon to soil organic carbon.

software to obtain soil microbial community composition richness (*S*) of different fertility treatments which were the numbers of bands in the lanes by the Diversity Database Fingerprinting software (Bio-Rad Laboratories, Hercules, California) and the number of bands in each lane was limited to no more than 20 (Rademaker *et al.* 1999). Shannon's diversity index (*H*) was calculated by the formula $H=-\sum p_i \ln p_i$, where p_i is the ratio of relative intensity of band *i* compared with the relative intensity of the lane (Orr *et al.* 2011).

The normalization of the Biolog values was done by dividing the absorbance values for individual wells by the AWCD for the whole plate, in order to account for differences in inoculum density as suggested by Garland and Mills (1991), and AWCD for each plate was used as an indicator of general microbial activity (Larkin and Honeycutt 2006).

$$AWCD=\frac{\sum_{i=1}^{31}(R_i-R_0)}{31}$$

Where, R_i is color production within the *i*th well (optical density measurement), R_0 is the absorbance value of the plates control well, and Eco plate have 31 substrates. Substrate utilization diversity (Shannon's diversity index, *H'*) were calculated, where $H'=-\sum p_i \ln p_i$, and p_i means proportional color development of the *i*th well over total color development of all wells of a plate, and substrate utilization evenness (the distribution of color development among the substrates, *E*) was calculated as $E=H'/\ln S$, *S* meaning the number of wells with color development, the calculation of *H'* and E were based on 72 h data according to the AWCD curve (Garland and Mills 1991). Differences in the carbon source utilization profiles were analyzed using principal components analysis (PCA). Relationships among samples were obtained by plotting scores of their first two principal components in two dimensions.

Redundancy analysis (RDA) was carried out to elucidate the relationships between soil microbial community structure, functional metabolic diversity and soil environmental characteristics and forward selection model was performed to select the most prominent factor on soil microbial community structure and microbial functional metabolic diversity, respectively, as implemented in Canoco for Windows ver. 4.5.

Acknowledgements

This research was funded by the National Natural Science Foundation of China (NSFC, 31301843) and the National Nonprofit Institute Research Grant of Chinese Academy of Agricultural Sciences (IARRP-202-5). We thank Prof. Julia Mary Cooper, Newcastle University, UK, for improving this manuscript.

Appendix associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

References

Allison S D, Martiny J B H. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 11512–11519.

Balser T C, Firestone M K. 2005. Linking microbial community structure and soil processes in a California annual grassland and mixed-conifer forest. *Biogeochemistry*, **73**, 395–415.

Bastian F, Bouziri L, Nicolardot B, Ranjard L. 2009. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biology and Biochemistry*, **41**, 262–275.

Beauregard M S, Hamel C, St-Arnaud M. 2010. Long-term phosphorus fertilization impacts soil fungal and bacterial diversity but not AM fungal community in alfalfa. *Microbial Ecology*, **59**, 379–389.

Chu H, Fujii T, Morimoto S, Lin X, Yagi K, Hu J, Zhang J. 2007. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. *Applied and Environmental Microbiology*, **73**, 485–491.

Cocolin L, Aggio D, Manzano M, Cantoni C, Comi G. 2002. An application of PCR-DGGE analysis to profile the yeast populations in raw milk. *International Dairy Journal*, **12**, 407–411.

Cookson W R, Abaye D A, Marschner P, Murphy D V, Stockdale E A, Goulding K W. 2005. The contribution of soil organic matter fractions to carbon and nitrogen mineralization and microbial community size and structure. *Soil Biology and Biochemistry*, **37**, 1726–1737.

Cookson W R, Murphy D V, Roper M M. 2008. Characterizing the relationships between soil organic matter components and microbial function and composition along a tillage disturbance gradient. *Soil Biology and Biochemistry*, **40**, 763–777.

Degens B P, Schipper L A, Sparling G P, Vojvodic-Vukovich M. 2000. Decreases in organic C reserves in soils can reduce the metabolic diversity of soil microbial communities. *Soil Biology and Biochemistry*, **32**, 189–196.

Drenovsky R E V D, Graham K J, Scow K M. 2004. Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecology*, **48**, 424–430.

Eilers K G, Lauber C L, Knight R, Fierer N. 2010. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry*, **42**, 896–903.

Elfstrand S, Hedlund K, Martensson A. 2007. Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. *Applied Soil Ecology*, **35**, 610–621.

Enwall K, Philippot L, Hallin S. 2005. Activity and composition of the denitrifying bacterial community respond differently to

long-term fertilization. *Applied Environmental Microbiology*, **71**, 8335–8343

Fan F, Li Z, Wakelin S A, Yu W, Liang Y. 2012. Mineral fertilizer alters cellulolytic community structure and suppresses soil cellobiohydrolase activity in a long-term fertilization experiment. *Soil Biology and Biochemistry*, **55**, 70–77.

Franco W, Frank R, Martin H, Andreas F. 2006. Community structures and substrate utilization of bacteria in soils from organic and conventional farming systems of the DOK long-term field experiment. *Applied Soil Ecology*, **33**, 294–307.

Garland J L, Mills A L. 1991. Classification and characterization of heterotrophic microbial communities on basis of patters of community level sole-carbon-source utilization. *Applied Environmental Microbiology*, **57**, 2351–2359.

Ge Y, Zhang J B, Zhang L M, Yang M, He J Z. 2008. Long-term fertilization regimes affect bacterial community structure and diversity of an agricultural soil in northern China. *Journal of Soils and Sediments*, **8**, 43–50.

Geisseler D, Scow K M. 2014. Long-term effects of mineral fertilizers on soil microorganisms — A review. *Soil Biology and Biochemistry*, **75**, 54–63.

Giacometti C, Demyan M S, Cavani L, Marzadori C, Ciavatta C, Kandeler E. 2013. Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. *Applied Soil Ecology*, **64**, 32–48.

Gong W, Yan X Y, Wang J Y, Hu T X, Gong Y B. 2009. Long-term manure and fertilizer effects on soil organic matter fractions and microbes under a wheat-maize cropping system in northern China. *Geoderma*, **149**, 318–324.

Gu Y, Zhang X, Tu S, Lindström K. 2009. Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping. *European Journal of Soil Biology*, **45**, 239–246.

Hallin S, Jones C M, Schloter M, Philippot L. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME Journal*, **3**, 597–605.

Haynes R J. 2005. Labile organic matter fractions as central components of the quality of agricultural soils: an overview. *Advances in Agronomy*, **85**, 221–268.

He J Z, Shen J P, Zhang L M, Zhu Y G, Zheng Y M, Xu M G, Di H. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology*, **9**, 2364–2374.

Kong W D, Zhu Y G, Fu B J, Han X Z, Zhang L, He J Z. 2008. Effect of long-term application of chemical fertilizers on microbial biomass and functional diversity of a black soil. *Pedosphere*, **18**, 801–808.

Larkin R P, Honeycutt C W. 2006. Effects of different 3-year cropping systems on soil microbial communities and Rhizoctonia diseases of potato. *Phytopathology*, **96**, 68–79.

Lejon D P, Sebastia J, Lamy I, Chaussod R, Ranjard L. 2007. Relationships between soil organic status and microbial community density and genetic structure in two agricultural soils submitted to various types of organic management. *Microbial Ecology*, **53**, 650–663.

Levy-Booth D J, Prescott C E, Grayston S J. 2014. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biology and Biochemistry*, **75**, 11–25.

Li C, Yan K, Tang L, Jia Z, Li Y. 2014. Change in deep soil microbial communities due to long-term fertilization. *Soil Biology and Biochemistry*, **75**, 264–272.

Li F, Liu M, Li Z, Jiang C, Han F, Che Y. 2013. Changes in soil microbial biomass and functional diversity with a nitrogen gradient in soil columns. *Applied Soil Ecology*, **64**, 1–6.

Li J, Zhao B Q, Li X Y, Jiang R B, So H B. 2008. Effects of long-term combined application of organic and mineral fertilizers on microbial biomass, soil enzyme activities and soil fertility. *Agricultural Sciences in China*, **7**, 336–343.

Li J, Cooper J M, Lin Z A, Li Y, Yang X, Zhao B. 2015. Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain. *Applied Soil Ecology*, **96**, 75–87.

Lipson D A, Schmidt S K, Monson R K. 2000. Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. *Soil Biology and Biochemistry*, **32**, 441–448.

Liu B, Gumpertz M L, Hu S, Ristaino J B. 2007. Long-term effects of organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight. *Soil Biology and Biochemistry*, **39**, 2302–2316.

Liu M, Hu F, Chen X, Huang Q, Jiao J, Zhang B, Li H. 2009. Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: the influence of quantity, type and application time of organic amendments. *Applied Soil Ecology*, **42**, 166–175.

Lu R K. 2000. *The Analysis Method of Soil Agricultural Chemistry*. Chinese Agricultural Sciences and Technology Press. (in Chinese)

Marschner P, Kandeler E, Marschner B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry*, **35**, 453–461.

Moffett B F, Nicholson F A, Uwakwe N C, Chambers B J, Harris J A, Hill T C J. 2003. Zinc contamination decreases the bacterial diversity of agricultural soil. *FEMS Microbiology Ecology*, **43**, 13–19.

Muyzer G, De Waal E C, Uitterlinden A G. 1993. Profiling of complex microbial populations by denaturing gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiology*, **59**, 695–700.

Nelson P N, Dictor M C, Soulas, G. 1994. Availability of organic carbon in soluble and particle-size fractions from a soil profile. *Soil Biology and Biochemistry*, **26**, 1549–1555.

Ogilvie L A, Hirsch P R, Johnston A W B. 2008. Bacterial

diversity of the broadbalk 'classical' winter wheat experiment in relation to long-term fertilizer inputs. *Microbial Ecology*, **56**, 525–537.

O'Donnell A G, Seasman M, Macrae A, Waite I, Davies J T. 2001. Plants and fertilizers as drivers of change in microbial community structure and function in soils. *Plant and Soil*, **232**, 135–145.

Orr C H, James A, Leifert C, Cooper J M, Cummings S P. 2011. Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. *Applied and Environmental Microbiology*, **77**, 911–919.

Rademaker J L W, Louws F J, Rossbach U, Vinuesa P, De Bruijn F J. 1999. Computer-assisted pattern analysis of molecular fingerprints and database construction. *Molecular Microbial Ecology Manual*, **7**, 1–33.

Sarathchandra S U, Ghani A, Yeates G W, Burch G, Cox N R. 2001. Effect of nitrogen and phosphate fertilisers on microbial and nematode diversity in pasture soils. *Soil Biology and Biochemistry*, **33**, 953–964.

Sharma S K, Ramesh A, Sharma M P, Joshi O P, Govaerts B, Steenwerth K L, Karlen D L. 2011. Microbial community structure and diversity as indicators for evaluating soil quality. In: *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture*. Springer Netherland. pp. 317–358

Shen J P, Zhang L M, Guo J F, Ray J L, He J Z. 2010. Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *Applied Soil Ecology*, **46**, 119–124.

Sikora L J, McCoy J L. 1990. Attempts to determine available carbon in soils. *Biology and Fertility of Soils*, **9**, 19–24.

Su J Q, Ding L J, Xue K, Yao H Y, Quensen J, Bai S J, Wei W X, Wu J S, Zhou J Z, Tiedje J M, Zhu Y G. 2015. Long-term balanced fertilization increases the soil microbial functional diversity in a phosphorus-limited paddy soil. *Molecular Ecology*, **24**, 136–150.

Sun R, Zhang X X, Guo X, Wang D, Chu H. 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry*, **88**, 9–18.

Sradnick A, Murugan R, Oltmanns M, Raupp J, Joergensen R G. 2013. Changes in functional diversity of the soil microbial community in a heterogeneous sandy soil after long-term fertilization with cattle manure and mineral fertilizer. *Applied Soil Ecology,* **63**, 23–28.

Vance E D, Brookes P C, Jenkinson D S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Vineela C, Wani S P, Srinivasarao C, Padmaja B, Vittal K P R. 2008. Microbial properties of soils as affected by cropping and nutrient management practices in several long-term manurial experiments in the semi-arid tropics of India. *Applied Soil Ecology*, **40**, 165–173.

Watts D B, Torbert H A, Feng Y, Prior S A. 2010. Soil microbial community dynamics as influenced by composted dairy manure, soil properties, and landscape position. *Soil Science*, **175**, 474–486.

Wessén E, Nyberg K, Jansson J K, Hallin S. 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. *Applied Soil Ecology*, **45**, 193-200.

Wu M, Qin H, Chen Z, Wu J, Wei W. 2011. Effect of long-term fertilization on bacterial composition in rice paddy soil. *Biology and Fertility of Soils*, **47**, 397–405.

Yu C, Hu X M, Deng W, Li Y, Xiong C, Ye C H, Han G M, Li, X. 2015. Changes in soil microbial community structure and functional diversity in the rhizosphere surrounding mulberry subjected to long-term fertilization. *Applied Soil Ecology*, **86**, 30–40.

Zhao J, Ni T, Li Y, Xiong W, Ran W, Shen B, Shen Q R, Zhang R F. 2014. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. *PLOS ONE*, **9**, e85301. doi: 10.1371/journal.pone.0085301

Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q, Shen W. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant and Soil*, **326**, 511–522.

Zhong W H, Cai Z C. 2007. Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. *Applied Soil Ecology*, **36**, 84–91.

Zhou J, Bruns M A, Tiedje J M. 1996. DNA recovery from soils of diverse composition. *Applied Environmental Microbiology*, **62**, 316–322.

(Managing editor WANG Ning)