J Soils Sediments (2011) 11:271–280
DOI 10.1007/s11368-010-0308-1

SOILS, SEC 3 • REMEDIATION AND MANAGEMENT OF CONTAMINATED OR DEGRADED LANDS • RESEARCH ARTICLE

Microbial functional diversity, metabolic quotient, and invertase activity of a sandy loam soil as affected by long-term application of organic amendment and mineral fertilizer

Junli Hu · Xiangui Lin · Junhua Wang · Jue Dai · Ruirui Chen · Jiabao Zhang · Ming Hung Wong

Received: 27 July 2010 / Accepted: 3 October 2010 / Published online: 23 October 2010

Abstract
Purpose Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants. Monitoring balanced versus unbalanced fertilization effects on soil microbes could improve our understanding of soil biochemical processes and thus help us to develop sound management strategies. The objective of this study was to investigate the effects of long-term fertilization regimes on soil microbial community functional diversity, metabolic activity, and metabolic quotient and to find out the main factors that influence these parameters.
Materials and methods A long-term fertilization experiment established in a sandy loam soil at northern China has received continuous fertilization treatments for more than 20 years, including control, mineral fertilizers of NK, PK, NP, and NPK, organic amendment (OA), and half organic amendment plus half mineral fertilizer (1/2 OM). Top soil samples (0–15 cm) from four individual plots per treatment were collected for the analysis of chemical properties and microbial parameters. Microbial biomass C was analyzed using the fumigation–extraction method. Invertase activity and basal respiration were determined based on incubation method. Then, the microbial metabolic quotient was calculated as the ratio of basal respiration to microbial biomass C. To this end, microbial functional diversity was evaluated using the community level physiological profile method by Biolog Eco-microplate.
Results and discussion Higher microbial biomass C, invertase activity, and basal respiration, but lower microbial metabolic quotient, were observed in P-fertilized soils, and OA had significantly greater ($P<0.05$) impacts on the biomass, activity, and quotient compared with mineral fertilizers. Both the sole-carbon-source utilization activity and the functional diversity of soil microbial community were significantly increased ($P<0.05$) by balanced fertilization (NPK, OA, or 1/2 OM), and species richness of community and relative abundance of the most common species in the K-deficient (NP) treatment were also significantly increased ($P<0.05$). Principal component analysis and redundancy analysis showed that both organic and mineral fertilizers could affect microbial parameters by increasing soil organic C contents, and P was the key factor to increase soil microbial diversity and soil fertility.
Conclusions Long-term balanced fertilization greatly increased soil microbial biomass, functional diversity, and invertase activity and played an important role in decreasing soil microbial metabolic quotient, while P could be considered as the key factor to control soil microbial diversity as well as soil fertility. With regard to the different effects of OA and mineral fertilizer on soil organic C contents and root exudates, combined application of mineral and organic fertilizers is recommended in the region.

Responsible editor: Jaco Vangronsveld

J. Hu · X. Lin (✉) · J. Wang · J. Dai · R. Chen · J. Zhang
State Key Laboratory of Soil and Sustainable Agriculture,
Institute of Soil Science, Chinese Academy of Sciences,
East Beijing Road 71,
Nanjing 210008, People's Republic of China
e-mail: xglin@issas.ac.cn

J. Hu · X. Lin · J. Wang · J. Dai · R. Chen · M. H. Wong
Joint Open Laboratory of Soil and the Environment,
Hong Kong Baptist University & Institute of Soil Science,
Chinese Academy of Sciences,
East Beijing Road 71,
Nanjing 210008, People's Republic of China

J. Hu · M. H. Wong
Croucher Institute for Environmental Sciences,
Department of Biology, Hong Kong Baptist University,
Kowloon Tong, Hong Kong SAR, People's Republic of China

Keywords Biolog Eco-microplate · Community level physiological profile (CLPP) · Principal component analyses (PCA) · Redundancy analysis (RDA) · Soil basal respiration · Soil microbial biomass C

1 Introduction

Soil microorganisms are essential to the environment due to their role in many reactions, such as in forming soil structure, cycling mineral compounds, decomposing organic materials, promoting/suppressing plant growth, and various soil biological-physical-chemical processes (Harris and Birch 1989; Critter et al. 2004; Wardle and Ghani 1995b). They play crucial roles in biogeochemical cycling and ecosystem functioning (Morin and McGrady-Steed 2004; Bell et al. 2005; Green and Bohannan 2006), and thus influence soil fertility and ecosystem stability directly (Smith et al. 1993). It has long been recognized that appropriate community population, abundant diversity, and high activity of microorganisms are significant factors to maintain the sustainability and productivity of terrestrial ecosystems (Naeem and Li 1997; Bell et al. 2005; Cardinale et al. 2006; Costa et al. 2007). However, soil microorganisms are sensitive to changes in land-use patterns, tillage practices, and management treatments (Steenwerth et al. 2002; Bucher and Lanyon 2005; Ge et al. 2008), for instance, fertilization regime may affect the population, composition, and function of soil microorganisms (Marschner et al. 2003). Therefore, soil microbiological and biochemical properties such as microbial biomass, community composition, metabolic activity, functional diversity, and various enzymatic activities are often measured to provide immediate and accurate information about small changes in soils (Pascual et al. 2000; Chu et al. 2007b; He et al. 2008).

Microbial biomass is a sensitive indicator for changes resulting from agronomic practices and other perturbations of soil ecosystem (Doran 1987; Smith and Paul 1990), and basal respiration is a valuable tool for understanding changes in soil microbial activities (Fernandes et al. 2005; Fließbach et al. 2007). Based on the Odum's theory of ecosystem succession (Odum 1969, 1985), soil microbial metabolic quotient is also widely used as an indicator of ecosystem disturbance and development (Wardle and Ghani 1995a). Afterwards a developed community level physiological profile (CLPP) method using the Biolog system provides a rapid and effective procedure to analyze soil microbial communities (Zak et al. 1994; Zhang and Xu 2008), and subsequently functional diversity of soil microbial community is taken as a more important indicator to assess soil processes and ecological functions (Harris 2003; Nannipieri et al. 2003; He et al. 2009). In addition, soil enzymatic activities, which can be used as another index of microbial functional diversity (Nannipieri et al. 2002), have been suggested as potential sensitive indicators to reveal changes of soil quality due to soil management and to monitor soil microorganism activity related to soil nutrient transformation (Dick 1994; Yang et al. 2008). For example, invertase drives C cycling by catalyzing the hydrolysis of sucrose—thus, testing the activity of soil invertase may be useful for evaluating soil capability of decomposing complex organic compounds into subunits that can be assimilated by microorganisms or plants, in other words, such enzymatic indices would integrate other chemical, physical, and biological characteristics and could monitor the effects of agricultural management on soil's long-term productivity (Frankenberger and Johanson 1983; Mikhailouskaya and Bogdevitch 2009).

Organic and inorganic fertilizers are used primarily to increase nutrient availability to plants (Chu et al. 2007b). Traditional organic fertilizers are by-products of the food and agricultural industries (Hu et al. 2010) and have maintained soil productivity for thousands of years. However, due to the rapid economic development since the 1980s, farmers find mineral fertilizers more affordable, especially as the labor costs of applying organic fertilizers have risen during the same period (Gong et al. 2009). Since farmers are often forced to make decisions about their fertilizer strategy that reflects economic rather than agronomic pressures, more mineral fertilizers and less organic fertilizers are now being used, and unbalanced fertilization is unfortunately widespread at the same time (Chu et al. 2007b). Obviously, the balanced fertilization of major elements (N, P, and K) is essential for the growth of plant above and below ground parts (Hu et al. 2009). In China, because of the concern of soil fertility degradation by replacement of organic fertilizers with inorganic ones, a number of long-term field experiments were set up in agricultural regions at the end of the 1980s to monitor changes in soil fertility with application of either inorganic fertilizers or organic amendments (OAs) alone or a mixed application of both (Cai and Qin 2006).

Huang-Huai-Hai Plain, which is located in low reaches of the Yellow, Huai, and Hai rivers within an area of 350,000 km^2 (Cai and Qin 2006), is one of the most important agricultural regions in China (Yang and Janssen 1997). Results obtained from long-term experiments in this area have already substantially improved our knowledge of changes in soil microbial community structure and metabolic activity with various fertilization practices (Chu et al. 2007a; Chu et al. 2007b; Chu et al. 2008; Ge et al. 2008; Zheng et al. 2009; Wang et al. 2010), as well as in soil productivity and greenhouse gases emissions (Meng et al. 2005; Cai and Qin 2006; Ding et al. 2007). However, uncertainties still remain about the long-term influence of organic and mineral fertilizers on microbial functional diversity, metabolic quotient, and invertase activity in this region. In this study, we selected one long-term field

experiment which was set up in Huang-Huai-Hai Plain in 1989 to determine soil microbial parameters under various treatments. The objectives of this study were to investigate the effects of mineral fertilizer and OA as well as balanced versus nutrient-deficient fertilization on soil microbial biomass, invertase activity, basal respiration, metabolic quotient, and functional diversity and to find out the main factors that influence these parameters.

2 Materials and methods

2.1 Description of the experimental site and soil sampling

The long-term field fertilizer experiment was conducted at Fengqiu Agro-Ecological Experimental Station (35°00′N, 114°24′E) of Chinese Academy of Sciences, Fengqiu County, Henan Province, China. The area had a temperate monsoon climate, with a mean annual rainfall of 615 mm and a mean annual temperature of 13.9°C. The soil, with a sandy loam texture, was derived from alluvial sediments of the Yellow River and classified as aquic inceptisol. The soil contained 4.5 g kg^{-1} of organic C, 0.45 g kg^{-1} of total N, 0.50 g kg^{-1} of total P, and 18.6 g kg^{-1} of total K and had a pH of 8.65 at the beginning of the experiment in September 1989. The crop succession was winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). Wheat was directly sown in October and harvested in early June of the next year. Then, maize was directly sown in June and harvested in late September. Seven treatments with four replicates in completely randomized blocks were established, i.e., 28 plots (9.5×5 m of each). The treatments were as follows: control (no fertilizer); mineral fertilizers NK, PK, NP, and NPK; OA; and half organic amendment plus half mineral fertilizer (1/2 OM). For NPK treatment, N, P, and K were applied in the form of urea (300 kg N ha^{-1} per year), super phosphate (60 kg P ha^{-1} per year), and potassium sulfate (250 kg K ha^{-1} per year), respectively, while no N, P, or K was applied for the PK, NK, and NP treatments, respectively. The OA was a composted mixture of wheat straw, oil cake, and cotton cake in a ratio of 100:40:45, and the OA and 1/2 OM treatments were designed to give the same application rates of N, P, and K as those given in the NPK treatment. All P, K, and OAs were applied as basal fertilizers, whereas urea was added in two applications as both basal and supplementary fertilizers. Detailed information on the experimental design and field management has been described by Meng et al. (2005). Each plot has received the same fertilizer management every year since 1989.

In this study, in order to investigate the effects of long-term different fertilizer treatments and reduce the side effect of the last fertilization on soils, the sampling time was chosen at the wheat jointing stage. On March 31, 2010, soil samples of each plot were collected from 16 points at the depth of 0 to 15 cm, and then mixed and homogenized by sieving through a 2-mm mesh sieve to remove above ground plant materials, roots, and stones. Each soil sample was divided into two portions: fresh soil samples were used for the analysis of microbial parameters, while dried and ground soil samples were used for the analysis of basic chemical properties.

2.2 Soil chemical analysis

Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5. Soil organic C and total N were determined by dichromate oxidation (Mebius 1960) and Kjeldahl digestion (Bremner 1965), respectively. Soil total P and total K were digested by HF–$HClO_4$ (Jackson 1958) and determined by molybdenum blue spectrophotometry and flame photometry, respectively. Soil mineral N was extracted with 2 mol l^{-1} KCl in a 1:4 soil-to-solution ratio for 1 h and then determined by an automated procedure (Skalar SAN^{plus} segmented flow analyzer) (Chu et al. 2007a). Available P in soil was extracted by sodium bicarbonate and determined using the molybdenum blue method (Olsen et al. 1954). Available K in soil was extracted by ammonium acetate and determined by flame photometry (Carson 1980).

2.3 Soil microbial biomass, invertase activity, and basal respiration determination

Soil microbial biomass C was determined using the chloroform fumigation extraction method as described by Vance et al. (1987), and involved analysis of post- and non-fumigation soil extracts (0.5 M K_2SO_4) for total organic C (TOC) was done with a TOC analyzer (Jordan and Beare 1991). Soil invertase activity was analyzed using the constant temperature incubation method as described by Srinivasulu and Rangaswamy (2006), and soil extracts were passed through Whatman No.1 filter paper, and glucose in the filtrate was assayed (Nelson 1944). Soil basal respiration was determined using the sealed incubation-alkali absorption method as described by Gong et al. (1997), and CO_2 evolution was measured by titration of unconsumed 0.1 M NaOH in the CO_2 traps with 0.1 M HCl (CO_3^{2-} was precipitated with Ba^{2+} before titration). To this end, the metabolic quotient was calculated as the ratio of basal respiration to microbial biomass C (Anderson and Domsch 1990).

2.4 Biolog analyses

Functional diversity of soil microbial community was measured with Biolog Eco-microplates (Biolog Inc., Hayward, CA). The method used for the inoculum preparation was adapted from Zak et al. (1994) and Staddon et al. (1998), and 150 μl of soil suspension diluted by 10^{-3} was

added to each well of a microplate. Microplates were incubated at 25°C for 96 h and measured at 590 nm with an Emax precision microplate reader (Biolog Inc., Hayward, CA). The data recorded by Microlog Rel. 4.2 software were expressed as the following four parameters (Garland and Mills 1991; Zak et al. 1994): (1) Average well color development (AWCD) for the metabolic activity of microbial community, (2) Shannon index (H'), (3) Simpson index (D), and (4) McIntosh index (U) for species richness of community; the relative abundance of the most common species in community and the species evenness of community, as respectively sensitive indicators, were calculated as follow: $H' = -\sum P_i \cdot \ln(P_i)$, $D = \frac{1}{\sum (Pi)^2}$, $U = \sqrt{(\sum n_i^2)}$, where n_i is the metabolic activity on each substrate, and P_i is the ratio of n_i to the sum of activities on all substrates.

2.5 Statistical analysis

All results were expressed on an oven-dried soil weight basis (105°C, 24 h). The data were subjected to analysis of variance, and the means and standard deviations for four replicates were calculated. Significant differences of means for all treatments were judged by least significant difference multiple-comparison tests. Principal component analysis (PCA), which is the most commonly used ordination technique for Biolog data analysis, was calculated by SPSS version 13.0 for Windows. Redundancy analysis (RDA), a multivariate direct gradient analysis method that has become widely used in ecology, was calculated by Canoco version 4.5 to elucidate the relationships between soil microbial parameters, soil basic chemical properties, and fertilizer treatments.

3 Results

3.1 Soil pH and nutrient contents

Soil pH and nutrient contents after long-term (20 years) fertilizer management are shown in Table 1. Fertilization significantly decreased ($P<0.05$) soil pH except for the N-deficient (PK) treatment, while significantly increased ($P<0.05$) soil organic C, total N, total P, and available P contents except for the P-deficient (NK) treatment. Since organic fertilizers release nutrients gradually for plant growth, OA did not increase soil mineral N content significantly, while mineral fertilizer treatments significantly increased ($P<0.05$) soil mineral N content except for the N-deficient (PK) treatment. Fertilization significantly increased ($P<0.05$) soil available K content except for the K-deficient (NP) treatment, while it had no significant effects on soil total K content. Compared to balanced fertilization treatments, the P-deficient (NK) treatment resulted in significant increases ($P<0.05$) of mineral N and available K contents.

3.2 Soil microbial biomass, invertase activity, basal respiration, and metabolic quotient

Fertilization greatly increased soil microbial biomass C (Fig. 1a) and invertase activity (Fig. 1b) after long-term application, except for the P-deficient (NK) treatment. Organic fertilizers had a significantly greater impact ($P<0.05$) on both biomass C and invertase activity, compared to mineral fertilizers. Since the soil was characterized by high K content, there were no significant differences in both biomass C and invertase activity between the NPK and the K-deficient (NP) treatments. There were also no significant differences in biomass C between the NPK and the PK treatments, but the latter had a significantly lower impact ($P<0.05$) on invertase activity. In addition, long-term fertilization except for the P-deficient (NK) treatment also significantly increased ($P<0.05$) soil basal respiration (Fig. 2a) and greatly decreased the soil microbial metabolic quotient (Fig. 2b). Specifically, organic fertilizers had a significantly greater impact ($P<0.05$) on microbial metabolic quotient compared with mineral fertilizers.

Table 1 Soil pH and nutrient contents under long-term fertilizer management

Treatment	pH (H_2O)	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Total K (g kg^{-1})	Mineral N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Control	9.1 (0.1)A	3.6 (0.3)E	0.36 (0.03)E	0.53 (0.02)D	19.1 (1.2)A	5.5 (0.7)D	0.67 (0.33)D	75 (11)D
NK	8.8 (0.1)B	3.7 (0.1)E	0.40 (0.00)E	0.55 (0.01)D	19.8 (0.9)A	32.6 (8.4)A	0.51 (0.22)D	349 (23)A
PK	9.1 (0.0)A	4.4 (0.1)D	0.44 (0.02)D	0.87 (0.04)A	20.4 (0.8)A	2.0 (0.4)D	25.71 (3.87)A	305 (29)B
NP	8.8 (0.1)B	5.2 (0.1)C	0.54 (0.03)C	0.79 (0.03)B	19.2 (0.5)A	12.2 (3.0)B	12.07 (3.41)BC	59 (8)D
NPK	8.7 (0.1)B	5.4 (0.0)C	0.57 (0.01)C	0.74 (0.02)C	19.1 (0.6)A	11.3 (1.6)BC	10.10 (1.45)C	166 (17)C
1/2 OM	8.8 (0.1)B	7.1 (0.4)B	0.76 (0.02)B	0.73 (0.02)C	19.2 (1.2)A	10.7 (2.8)BC	14.36 (2.13)B	171 (23)C
OA	8.9 (0.1)B	9.2 (0.6)A	0.97 (0.04)A	0.72 (0.04)C	19.4 (1.2)A	6.7 (1.0)CD	14.17 (1.76)B	167 (23)C

Standard deviations are given in parentheses. Values within the same column not followed by the same letter differ significantly ($P<0.05$)

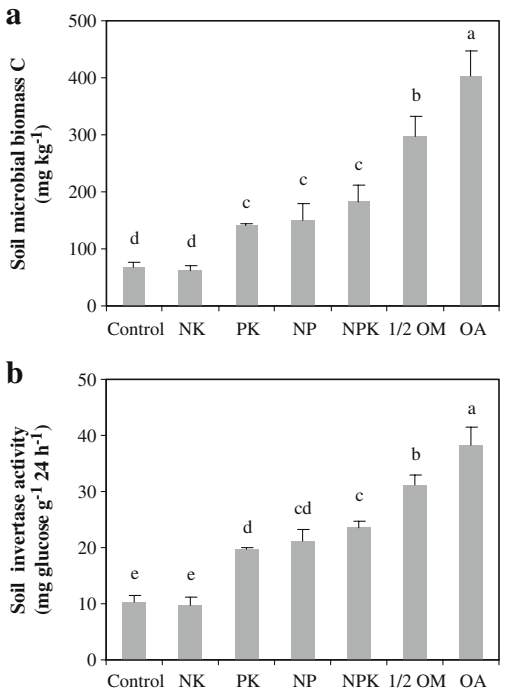

Fig. 1 Soil microbial biomass C (**a**) and invertase activity (**b**) under long-term (20-year) fertilizer management. *OA*, organic amendment; *1/2 OM*, half organic amendment plus half mineral fertilizer; *NPK*, mineral NPK fertilizer; *NP*, mineral NP fertilizer; *PK*, mineral PK fertilizer; *NK*, mineral NK fertilizer; *control*, without fertilization. *Vertical T bars* indicate standard deviations. *Bars* not topped by the *same letter* indicate a significant difference in values ($P<0.05$)

3.3 Soil microbial community metabolic activity and functional diversity

Sole-carbon-source utilization (SCSU) activity and functional diversity of soil microbial community after long-term fertilizer management are shown in Table 2. The AWCD and three functional diversity indices of H', D, and U were all significantly increased ($P<0.05$) by balanced fertilization treatments (NPK, OA, and 1/2 OM) and were not significantly influenced by nutrient-deficient treatments (NK, PK, and NP), except for two significantly increased ($P<0.05$) indices of H' and D in the K-deficient (NP) treatment. Based on the overall PCA for the observed substrate utilization patterns of soil microbial community, the first and second principal components (PC1 and PC2) accounted for 36.3% and 8.9% of the total variance in AWCD, respectively (Fig. 3). Based on the PC1 axis, the SCSU patterns of P-fertilized treatments (PK, NP, NPK, OA, and 1/2 OM) were clearly different from that of the control, and the patterns of two OA-inputted treatments (OA and 1/2 OM) were also well separated from those nutrient-deficient treatments (NK, PK, and NP). These separations might have been mainly associated with increases in utilization of specific carbohydrates, polymers, carboxylic acids, amino acids, amines, and phenolic compounds with inputting OAs or increasing root exudates (Table 3).

3.4 Redundancy analysis of soil microbial parameters, chemical properties, and fertilizer treatments

In the RDA ordination plot (Fig. 4), projecting an object (fertilization treatment) at right angle on a response (microbial) or an explanatory (chemical) variable approximates the value of the object along that variable; the angles between response and explanatory variables or between response variables themselves reflect their correlations, and the relationship between the centroid of a qualitative explanatory variable and a response variable is also found by projecting the centroid at right angle on the variable. Soil microbial biomass C and invertase activity were greatly accelerated by the application of organic fertilizers (OA and 1/2 OM) and were significantly correlated to soil organic C (r=0.993 and 0.972, $P<0.01$) and total N (r= 0.990 and 0.966, $P<0.01$). Soil microbial diversity and metabolic activity were also enhanced by the application of

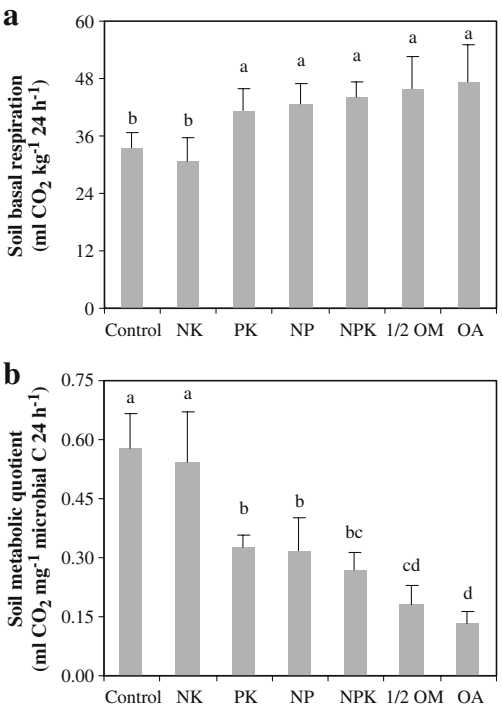

Fig. 2 Soil basal respiration (**a**) and metabolic quotient (**b**) under long-term (20-year) fertilizer management. *OA*, organic amendment; *1/2 OM*, half organic amendment plus half mineral fertilizer; *NPK*, mineral NPK fertilizer; *NP*, mineral NP fertilizer; *PK*, mineral PK fertilizer; *NK*, mineral *NK* fertilizer; *control*, without fertilization. *Vertical T bars* indicate standard deviations. *Bars* not topped by the *same letter* indicate a significant difference in values ($P<0.05$)

Table 2 SCSU activity and functional diversity of soil microbial community under long-term fertilizer management

Treatment	AWCD	Shannon index (*H′*)	Simpson index (*D*)	McIntosh index (*U*)
Control	0.54 (0.09)C	2.95 (0.20)B	16.91 (3.37)C	4.08 (0.35)C
NK	0.54 (0.08)C	2.95 (0.13)B	16.73 (2.27)C	4.11 (0.37)C
PK	0.65 (0.06)BC	3.12 (0.07)AB	19.52 (1.87)BC	4.59 (0.31)BC
NP	0.69 (0.03)BC	3.16 (0.04)A	20.70 (1.03)AB	4.72 (0.24)BC
NPK	0.79 (0.17)AB	3.17 (0.05)A	21.45 (1.75)AB	5.27 (0.89)AB
OA	0.77 (0.08)AB	3.23 (0.05)A	23.00 (1.35)AB	4.99 (0.39)AB
1/2 OM	0.90 (0.14)A	3.26 (0.05)A	23.76 (1.85)A	5.71 (0.68)A

Standard deviations are given in parentheses. Values within the same column not followed by the same letter differ significantly ($P<0.05$)

organic fertilizers or mineral fertilizer P (NPK, NP, and PK), and *H′*, *D*, AWCD, and basal respiration were also significantly correlated to soil organic C ($r=0.824$, 0.870, 0.756, and 0.810, $P<0.05$) and total N ($r=0.820$, 0.870, 0.765, and 0.796, $P<0.05$). However, microbial metabolic quotient was greatly decreased by the application of organic fertilizers or mineral fertilizer P, and was negatively and closely corrected to AWCD ($r=-0.905$, $P<0.01$), *H′* ($r=-0.979$, $P<0.01$), *D* ($r=-0.973$, $P<0.01$), and *U* ($r=-0.868$, $P<0.05$), as well as soil organic C ($r=-0.889$, $P<0.01$) and total N ($r=-0.884$, $P<0.01$).

4 Discussion

4.1 Effects of balanced fertilization of mineral NPK on soil nutrient status and microbial parameters

In our study, long-term balanced application of mineral fertilizer NPK significantly increased microbial biomass, metabolic activity, and basal respiration, while it significantly decreased microbial metabolic quotient. In agreement with metabolic activity, both the SCSU pattern and the functional diversity (*H′*, *D*, and *U*) of NPK were clearly different from those of the control. On one hand, balanced fertilization could meet the nutrient demand of crop growth, and thus promoted soil microbial biomass and metabolic activity through abundant root exudates (Zhong et al. 2010). On the other hand, the increase of soil organic C and nutrients contents would also stimulate microbial activity (Emmerling et al. 2000) and biomass cycling, thus leading to an increase in soil basal respiration (Chander and Brookes 1993; Leita et al. 1995). However, a high respiration rate might indicate either an ecological disorder or a high level of productivity in the ecosystem (Islan and Weil 2000). As a result, the respiration rate per unit of microbial biomass or metabolic quotient has been utilized as a microbial stress indicator and interpreted as "microbial efficiency", since it is a measurement of the energy necessary to maintain metabolic activity in relation to the energy necessary for synthesizing biomass (Bardgett and Saggar 1994). Thus, soils under nutrient-deficient stress would present higher metabolic quotient than non-stressed soils (Fernandes et al. 2005). In addition, it is usually concluded that higher enzymatic activities are associated with higher organic matter content (Srinivasulu and Rangaswamy 2006), and the higher soil invertase activity in the NPK treatment may also be caused by a higher turnover of the microbial biomass (Kandeler et al. 1999).

Compared with the NPK-balanced treatment, the K- or N-deficient (NP and PK) treatment had a trend towards smaller but not significantly different effects on soil microbial parameters, except for a significantly decreased invertase activity in PK; however, the P-deficient (NK) treatment significantly decreased microbial biomass, functional diversity, metabolic activity, basal respiration, and invertase activity, and the SCSU pattern was also clearly different from that of NPK or NP. This reveals the complex interaction of optimal availability of nutrients to microbial growth and was consistent with soil organic C content, as well as crop yield in the following order: NPK > NP > PK > NK (Wang et al. 2010). Microorganisms under

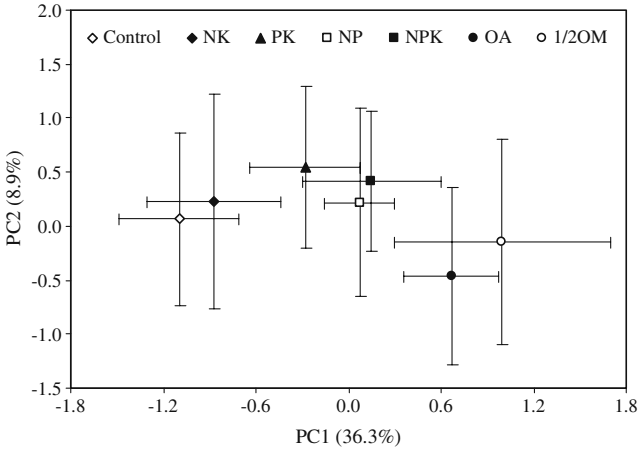

Fig. 3 PCA of SCSU profiling of soil microbial community under long-term (20-year) fertilizer management. PC1 and PC2 accounted for 36.3% and 8.9% of the variance, respectively. *OA*, organic amendment; *1/2 OM*, half organic amendment plus half mineral fertilizer; *NPK*, mineral NPK fertilizer; *NP*, mineral NP fertilizer; *PK*, mineral PK fertilizer; *NK*, mineral NK fertilizer; *control*, without fertilization

Table 3 Substrates with high Pearson's correlation coefficients (>0.6) for PC1 in the PCA of substrate utilization patterns of soil microbial community under long-term fertilizer management

Carbon type	Substrate name	*r*	Carbon type	Substrate name	*r*
Amines	Phenylethylamine	0.703	Amino acids	L-phenylalanine	0.739
	Glycyl-L-glutamic acid	0.690		L-threonine	0.632
Carbohydrates	Glucose-1-phosphate	0.828	Carboxylic acids	γ-Hydroxybutyric acid	0.717
	α-D-lactose	0.827		Pyruvic acid methyl ester	0.659
	i-Erythritol	0.759		Itaconic acid	0.617
	D-cellobiose	0.746	Phenolic compounds	4-Hydroxy benzoic acid	0.799
	D-galactonic acid-γ-lactone	0.686	Polymers	α-Cyclodextrin	0.881
	N-acetyl-D-glucosamine	0.650		Glycogen	0.739
	β-methyl-D-glucoside	0.608		Tween-80	0.707

balanced fertilizer treatments had higher efficiency of C utilization or higher efficient metabolism, and the decrease in efficient metabolism under nutrient-deficient treatments was chiefly due to the unavailability of P, followed by N and K (Zheng et al. 2009). Specifically, soil microorganisms in the control and NK treatments were under stress to cope with the deficiency of at least one necessary nutrient (P) for their proper metabolic activity, and thus led to higher CO_2 emission (see Fig. 2b) and higher heat dissipation (Zheng et al. 2009). Therefore, these results demonstrated the importance of balanced fertilization, as well as the role of P in promoting biomass, diversity, and activity of soil microorganisms. In other words, the application of P could be considered as the key factor to control soil fertility and microbial diversity in the region of study.

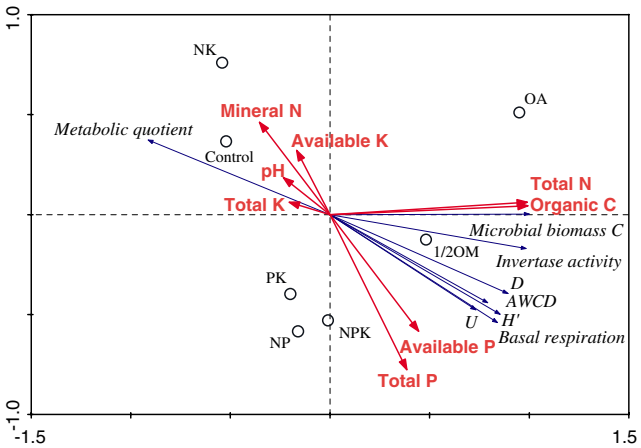

Fig. 4 RDA of soil microbiological properties with soil chemical variables under long-term (20-year) fertilizer management. *OA*, organic amendment; *1/2 OM*, half organic amendment plus half mineral fertilizer; *NPK*, mineral NPK fertilizer; *NP*, mineral NP fertilizer; *PK*, mineral PK fertilizer; *NK*, mineral NK fertilizer; *control*, without fertilization; *AWCD*, average well color development; *H'*, Shannon index; *D*, Simpson index; *U*, McIntosh index

4.2 Effects of organic amendment on soil microbial diversity, metabolic quotient, and invertase activity

Compared to the NPK treatment, OA had greater impacts on soil microbial biomass, metabolic quotient, and invertase activity. It is commonly known that C is a key factor governing soil microorganism growth (Grayston et al. 1998), and OAs are essential for improving soil organic C content which lead to great shifts of C utilization pattern (Zhong et al. 2010), since they act as a source of C and other nutrients, which favor microbial diversity and activity, and improve soil structure as well as organic C (Albiach et al. 2000). The increase in microbial functional diversity and soil invertase activity may be explained by an increase in C availability as a consequence of amendment incorporation (Gomez et al. 2006), and the decrease in metabolic quotient may also be caused by the improved soil condition. Esperschütz et al. (2007) also found that OA was the main factor causing soil microbial community differentiation. However, the combined application of organic and mineral fertilizers revealed the strongest influence on functional diversity indices based upon the Biolog techniques adopted here, while application of organic or mineral fertilizers alone revealed influences at an intermediate level, which might be explained by the multitude of factors that may act in different ways, such as direct C sources and root exudates.

Since nutrient release from OAs is dependent on temperature (Ellert and Bettany 1992), Cai and Qin (2006) reported crop yield in the OA treatment seemed to be more dependent on climate than that in the NPK treatment, and thus on average over 17 years, both wheat and maize yields were significantly higher in NPK than in OA (Wang et al. 2010). It follows that OA had less effects on crop growth and root exudates, which have important functions in promoting the growth of soil microorganisms (Zhong et al. 2010). Our results indicated that microbial communities in balanced fertilized soils might have much higher substrate utilization of specific C or other com-

pounds after long-term continuous management. From the listed substrates with high correlation coefficients for PC1 (see Table 3), 4-hydroxy benzoic acid, D-galactonic acid-γ-lactone, and L-threonine are tested as root exudates. In other words, a relatively higher microbial metabolic activity and functional diversity under the 1/2 OM treatment was also sustained by the abundant root exudates released into the soil by crop plants. Therefore, a compromise between root exudates and direct C sources which is the combined application of inorganic fertilizers and OAs, such as the treatment of 1/2 OM, is recommended in the region of study. This research would also provide valuable data on the effects of fertilizer management on soil microbial diversity and relationships between biodiversity and crop yields, and it may improve land-use sustainability by allowing farmers to better match mineral and organic fertilizers with crop demand in arable soils.

5 Conclusions

Long-term fertilization had significant effects on soil microbial functional diversity, metabolic quotient, and invertase activity. Most of these microbial parameters were mainly correlated with soil organic C and total N, indicating that fertilization could affect microbial parameters indirectly by increasing the contents of these critical nutrients. Organic amendments could affect microbial parameters in different ways from mineral fertilizers and could play a greater role in decreasing soil microbial metabolic quotient, but the application of P could be considered as the key factor to control soil microbial diversity as well as soil fertility in this region. Our results provided a better understanding of the importance of OA plus balanced fertilization with N, P, and K in promoting soil microbial functional diversity and thus enhancing crop growth and production.

Acknowledgements We wish to acknowledge Shengwu Qin, Xiuli Xin, Linyun Zhou, Jinfang Wang, and Jian Liu for their excellent field management and support on soil sampling. We would like to thank Jing Li and Bo Zhu for their great assistance in soil collection and sample analysis. We are also grateful to Dr. Haiyan Chu, Dr. Rui Yin, and Dr. Huayong Zhang, as well as two anonymous reviewers, for their valuable comments and useful suggestions on manuscript revision and English editing. This work was supported by the National Basic Research Program (2011CB100505) and the National Natural Science Foundation (no. 40801090) of China, and the Knowledge Innovation Programs (Kzcx2-yw-408, Kscx1-yw-09-05) of Chinese Academy of Sciences.

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