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REGULAR ARTICLE

Soil biological activity and their seasonal variations in response to long-term application of organic and inorganic fertilizers

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Abstract The objectives of this study were to explore the effects of long-term and continued application of fertilizers and manures on microbial biomass, soil biological activity and their seasonal variations in surface and subsurface soils in relation to soil fertility. For this, soils were sampled in spring, summer and autumn from Shenyang Long-term Experimental Station, northeastern China. The results showed that soil total nitrogen (N), organic carbon (C), basal respiration, microbial biomass and enzymatic activity increased in manure-amended surface soils, but decreased with soil depth. Long-term application of inorganic fertilizers significantly decreased soil pH value, sucrase activity and microbial biomass C, but increased soil metabolic quotient (qCO_2). However, no significant effect of inorganic fertilizers on soil total N, urease activity and microbial biomass N was observed in comparison with CK0 (neither tillage nor fertilization) and CK (no fertilizers). There was no significant difference between CK0 and CK in soil total N, organic C and microbial activity in surface soil layer (0–20 cm), but these parameters in subsurface soil layer (20–40 cm) were higher in CK than in CK0. Moreover, seasonal changes were observed in terms of soil nutrient contents, enzymatic activity, microbial biomass and soil respiration. There were significant correlations between soil microbial biomass C and N, between organic C and sucrase activity and between total N and urease activity, respectively. It is recommended that combined use of organic manure with inorganic fertilizers should be considered to maintain higher microbial biomass, soil biological activity and soil fertility. Considering considerably high nutrients reserve and microbial activity in subsurface layers of soil and wind-erosion-caused nutrient loss in spring in north China, we also propose that low tillage should be considered to make use of nutrients in soils.

Keywords Enzymatic activity · Microbial biomass · Organic C · Soil fertility · Soil respiration

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Introduction

Soil biological activity mainly including soil enzymatic activity, basal respiration and microbial biomass has been shown to be closely related to both various soil factors including pH values, soil organic matter, soil texture, and modifying factors such as climate, soil moisture and soil temperature regimes (Emmerling et al. 2001; Bastida et al. 2008). Microbial biomass, respiration, metabolic quotient (qCO_2, an index of the respiration activity per biomass unit, CO_2-C/MBC) and soil enzymatic activities can be utilized as indicators for changes in soil quality (Wardle and Ghani 1995; Ros et al. 2003; Bastida et al. 2008). Enzymes are mainly from soil microorganisms, and despite their relatively low amounts, play a crucial role in keeping nutrient cycling in soils such as C, N, P, and S. Nutrient cycling is fundamental not only for primary production but for the long-term functioning of ecosystems as well (Doran and Parkin 1996; Aon et al. 2001). In addition, soil microbial activities including soil respiration, microbial biomass, and soil ATP content have been proved to be powerful indicators of soil quality (Bending et al. 2004; Goberna et al. 2006; Bastida et al. 2008). Soil microbial activity has, for example, been found to be a sensitive indicator of soil agricultural practices, input of fertilizers, organic residues and crop rotation (Emmerling et al. 2001; Bending et al. 2004). Soil respiration, a measure of soil microbial activity, is crucial to estimating biological process in soil ecosystems (Islan and Weil 2000).

The application of balanced amount of fertilizers and manures could increase enzymatic activity and soil respiration (Kanchikerimath and Singh 2001; Tu et al. 2006). Organic fertilizers usually increase soil microbial biomass (Kaur et al. 2005; Liang et al. 2003, 2005), CO_2 evolution, and enzyme activities (Crecchio et al. 2001; Liang et al. 2003). Organic manure-stimulated biological activity and soil microbial biomass has been proven to be positively correlated with improved soil fertility and quality as indicated by higher crop biomass and higher concentration of soil available nutrients and plant nutrient uptake (Mueller et al. 1998; Bending et al. 2004; Liang et al. 2003, 2005; Tu et al. 2006). Furthermore, these biological parameters can be used as sensitive bio-indicators of soil nutrient transformation, biological turnover and bioavailability (Mueller et al. 1998; Liang et al. 2003; Tu et al. 2006). Long-term use or overuse of inorganic fertilizers had relatively less positive effect on soil microbial biomass and activities than organic fertilizers (Hopkins and Shiel 1996; Plaza et al. 2004). Numerous studies showed that microbial biomass could be decreased by application of mineral N fertilizer (Ladd et al. 1994; Hopkins and Shiel 1996; Šimek et al. 1999; Sarathchandra et al. 2001; Bittman et al. 2005), which may be caused by direct toxicity and reduced pH because of ammonium-based fertilizers (Hopkins and Shiel 1996).

It is well known that the dynamics of the soil microbial biomass in a range of agricultural systems is strongly dependent on climate, with only small fluctuations in temperate climates (Wardle 1992; Murphy et al. 2007; Bastida et al. 2008). Organic C and total N were constant and unaffected by rotation phase during the season, but most of the other more labile soil biochemical attributes varied. Much of this temporal variability was associated with changes in soil moisture, temperature and precipitation (Campbell et al. 1999; Feng and Simpson 2009). Murphy et al. (2007) pointed out that temperature and soil moisture content controlled CO_2 production, and the rate of CO_2 production reached a maximum in mid-summer. Chen et al. (2003) found that soil respiration rate was accordant with soil temperature and moisture, and soil respiration was higher in summer and lower in other seasons.

Up to now, much work has been done regarding the effects of organic and inorganic amendments on soil biological activity and/or enzymatic activity (Mueller et al. 1998; Bending et al. 2004; Liang et al. 2003, 2005; Tu et al. 2006; Bastida et al. 2008). However, the majorities of such studies were mainly performed under laboratory incubation conditions where short-term effects were simulated only (Crecchio et al. 2001; Feng and Simpson 2009). Information is still scant on soil biological activities as influenced by long-term application of organic and inorganic amendments, especially seasonal soil biological activities under field conditions. More importantly, relatively less information is available on the interactive effects of soil depth, fertilizer treatment and seasonal change on soil biological activity under long-term field experimental conditions. Such information plays a crucial role in maintaining balanced seasonal nutrient supply and higher soil productivity. In the present study, soil enzymatic activity, basal respiration and microbial biomass related to nutrient (C and N) cycling in both

surface and subsurface layers of soil, and their seasonal variations were examined to study bio-indicators of soil quality and fertility in the soils amended with manures and inorganic fertilizers in a long-term experimental station and to provide some theoretical and practical bases for rational application of fertilizers and sustainable agricultural development.

Materials and methods

Experimental conditions and design

The present study was undertaken at the experimental field of Shenyang Agricultural University, northeast China (41°50′N, 121°34′E). The site, founded in 1987, is located in a continental monsoon zone with mean annual temperature of 7.0–7.9°C, and mean annual precipitation of 705.4 mm. The climate in the region is sub-humid with a hot rainy summer and a cold dry winter. The field experiment was conducted in a well-drained field where continuous-maize (*Zea mays* L.) cropping is exercised. The crop growing season started in April and ended in September. The soil is an aquic brown soil (USDA, United States Department of Agriculture) derived from alluvial deposits of the Liao River. In this study, a randomised block design was adopted in the experimental field and six treatments with four replicates each were chosen as follows: (1) neither tillage nor fertilization (CK0); (2) no fertilizers (CK); (3) N fertilizer (NF); (4) combined application of N and P fertilizers (NPF); (5) combined application of organic manure and N and P fertilizers (NPF+OM) and (6) organic manure (OM). The treatment CK0 was specially designed to compare with the treatment CK under the current cultivation system. The content of nutrients for different fertilization treatments was presented in Table 1. Nutrient composition of pig manure was presented as follows: organic C 87 g kg^{-1}, N 10 g kg^{-1} and P 15 g kg^{-1}. The average maize yield for CK, NF, NPF, NPF+OM and OM treatments for 20 years was 3457, 4359, 4926, 5414 and 4413 kg ha^{-1}, respectively (Yu et al. 2003). The physical and chemical properties in the 0–20 cm layer, before the onset of experiment, were as follows: pH in water, 6.39; organic C, 9.05×10^3 mg kg^{-1}; total N, 1.00×10^3 mg kg^{-1}; total P, 0.52×10^3 mg kg^{-1}; total K, 21.56×10^3 mg kg^{-1}; available N, 67.41 mg kg^{-1}; available P, 8.40 mg kg^{-1}; and available K 98.32 mg kg^{-1}. Particle-size fractionation of the soil tested was composed of 1.67% sand, 58.40% silt and 24.91% clay.

Sampling and assay

Soil samples were collected from 0–20 (ploughed layer) and 20–40 cm (plough pan) soil layers of each treatment described above. Soils were sampled at the seeding prophase of maize (April 12, 2006), the jointing stage (June 26, 2006), and the ripening stage before harvesting (September 22, 2006), respectively. Fresh soil samples were passed through a 2-mm sieve and conserved at 4°C in a fridge for the determination of soil basal respiration and microbial biomass. Partial soil samples were air-dried and passed through a 2-mm, 1-mm, 0.149-mm and 0.25-mm sieve under room temperature for determination of soil pH value, enzymes

Table 1 Tillage and fertilizer inputs (kg $ha^{-1}y^{-1}$) of the treatments in soil tested

Fertilizers	N fertilizer	P fertilizer	K fertilizer	Organic manure		
Treatment	N (kg $ha^{-1}y^{-1}$)	P (kg $ha^{-1}y^{-1}$)	K (kg $ha^{-1}y^{-1}$)	N (kg $ha^{-1}y^{-1}$)	P (kg $ha^{-1}y^{-1}$)	C (kg $ha^{-1}y^{-1}$)
CK0	0	0	0	0	0	0
CK	0	0	0	0	0	0
NF	135	0	0	0	0	0
NPF	135	67.5	0	0	0	0
NPF+OM	67.5	67.5	0	67.5	101	587
OM	0	0	0	135	202	1175

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure

activity, total N and organic C, respectively. Soil pH value was measured in soil-water suspensions (1:2.5 *v*/*v*), and soil total N and organic C were determined by Kjeldahl digestion and colorimetrically at 590 nm, respectively (Lu 2000).

Determination of soil organic carbon and total N

Soil samples were air-dried and passed through a 0.25-mm sieve under room temperature. Weighted soil samples (1.000 g) were placed into a 50-mL digested tube together with 5 mL potassium dichromate and 5 mL oil of vitriol. The digested tube was shaken up and then placed in an oven at 100°C for 90 min. After pyrogenation, the digested tube was cooled in cold water, and deionised water was added to make up the test tube to 50 mL. The sample solution was held still for 3 h, and the top limpid solution was collected. Finally, soil organic carbon was determined colorimetrically at 590 nm (U-2800, Japan) within 1 h, and expressed as g C kg soil^{-1} (Lu 2000).

Air-dried and finely sifted soil (1.000 g, passed through a 0.149-mm sieve) was placed into a 50-mL digested tube together with 1.1 g mixed catalyst (K_2SO_4:$CuSO_4 \cdot 5H_2O$:Se = 100:10:1) and 5 mL oil of vitriol. The digested tube covered with a small funnel was shaken and then placed in an electric cooker at 200°C. The temperature did not rise to 300°C until sample solution became offwhite. When the sample solution became offwhite with green, it was resumed to digest for an additional one hour. After digestion, the sample solution was determined by automatic Kjeldahl Analyzer (KDY-9830, China), and soil total N was expressed as g N kg soil^{-1} (Lu 2000).

Determination of soil microbial biomass

Soil moisture of the samples tested was adjusted to 50% field water capacity, pre-incubated at 25°C for 7 days in the dark, and each sample was subdivided into two subsamples (fumigated and non-fumigated treatments) for determining microbial biomass C and N by the fumigation–extraction method. Samples (25 g dry weight) were fumigated with $CHCl_3$ for 24-h at 25°C. After removing the $CHCl_3$, C and N were extracted from the fumigated and non-fumigated samples with 0.5 M K_2SO_4 for 30 min on a shaker (soil : solution at a 1:4 ratio). Organic C was determined using TOC (total organic carbon) analyzer (Multi N/C 3100, Germany) and total N by Kjeldahl digestion method (KDY-9830, China). The difference between C and N extracted from fumigated and non-fumigated samples was converted into microbial biomass C and N by using the K_{EC} and K_{EN} factors with values of 0.38 and 0.45, respectively (Lu 2000).

Determination of soil basal respiration and metabolic quotient (qCO_2)

Soil microbial respiration rate was estimated as the rate of CO_2 evolution according to Xu and Zheng (1986). Moist soils (20 g dry weight equivalent) were incubated in airtight containers at 25°C for 24 h in the dark. Evolved CO_2 was captured with a 0.5 M NaOH solution and titrated using 0.05 M HCl to determine the amount of CO_2 evolved. Soil basal respiration was determined by quantifying the carbon dioxide (CO_2) released in the process of microbial respiration during 24-h-incubation and expressed as mg CO_2-C released kg^{-1} soil h^{-1}. Microbial metabolic quotient (qCO_2) was calculated by dividing respiration rates by microbial biomass C (MBC).

Determination of soil enzymatic activity

Urease activity was determined by the method described by Guan (1986) with minor modification. Two grams of air-dried, finely sifted soil (passed through a 1-mm sieve) was placed into a 50-mL Erlenmeyer flask together with 0.5 mL toluene. Fifteen minutes later, 5 mL 10% urea solution and 10 mL citrate buffer (pH 6.7) were added. The flask was shaken and then placed in an incubator (MEMMERT GmbH + Co. KG, Germany) at 37±0.1°C for 24 h. After incubation, the sample was filtered through a quantitative filter paper. To a 25-mL test tube were added 0.5 mL of the filtrate with 10 mL deionised water, 4 mL sodium phenate solution and 3 mL sodium hypochlorite solution. Twenty minutes later, deionised water was added to make up the test tube to 25 mL. Finally, urease activity was determined colorimetrically at 578 nm (U-2800, Japan) within 1 h, and expressed as mg NH_3-N g soil^{-1} 24-h.

Sucrase activity was determined by the method described by Guan (1986) with modification. Two grams of air-dried, finely sifted soil (passed through a

1-mm sieve) were placed into a 50-mL Erlenmeyer flask together with 15 mL sucrose, 5 mL phosphate buffer (pH 5.5) and five drops of toluene. The flask was shaken and then placed in an incubator (MEMMERT GmbH + Co. KG, Germany) at 37.0±0.1°C for 24 h. After incubation, the sample was filtered through a quantitative filter paper. Then, 0.5 mL of the filtrate and 1.5 mL salicylic acid were taken to a 25-mL test tube, and then, heated for 5 min at 100°C in a water bath. After heating, the test tube was cooled for 3 min with flowing tap water, and subsequently, deionised water was added to make up to 25 mL, and sucrase activity was measured colorimetrically at 508 nm (U-2800, Japan). Sucrase activity was expressed as mg glucose g soil^{-1} 24-h.

Statistical analysis

All data were subjected to three-way analysis of variance using Sigmastat for Windows Version 2.03 (SPSS Inc.), and statistical significance of means of four replicates was judged by the least significant difference (L.S.D.) test at 5% and 1% probability level.

Results

Soil organic C, total N and pH value

Long-term fertilization significantly influenced soil organic C, total N and pH value (Table 2). The soil organic C increased in soils amended with organic manure (OM and NPF+OM treatments), but decreased in soils treated with inorganic fertilizers (NF and NPF treatments), compared with CK0 and CK. There was no significant difference either between NPF+OM and OM or between NF and NPF treatments. Application of inorganic fertilizer significantly decreased soil pH value, while amendment with organic manure showed less effect on soil pH value. Both treatments with inorganic fertilizers and organic manures significantly increased soil total N, with the organic manure treatments being more significantly effective, but the differences between NPF+OM and OM, and between NF and NPF treatments were not statistically significant. Besides, there was no significant difference between CK0 and CK in soil organic C, total N and pH value (Table 2).

Soil organic C and total N contents in all the treatments were significantly higher in soil surface layer (0–20 cm) than in soil subsurface layer (20–40 cm), especially in CK0 treatment (Table 2). However, higher soil pH values were observed in the subsurface layers than in the surface layers. Soil organic C was found to be the highest in the samples collected at the ripening stage of maize, followed at the jointing stage and seeding prophase. However, the highest soil total N content was observed in the samples collected at the seeding prophase, and the other collections showed lower soil total N content. The differences in soil organic C and total N contents between seasons were statistically significant. Besides, significant seasonal changes were also noted in soil pH value (Table 2).

Soil microbial biomass

Soil microbial biomass C and N showed significant variation among different fertilization treatments, soil layers and sampling seasons (Table 3). Organic manure treatments significantly increased ($P<0.05$) soil microbial biomass C and N, while inorganic fertilizers treatments significantly decreased soil biomass C. No significant effect was observed of inorganic fertilizers on soil microbial biomass N, compared with CK0 and CK. The NPF treatment showed higher microbial biomass C and N, compared with NF treatment, but the difference was not statistically significant. The values of microbial biomass C and N were significantly higher in the OM treatments than in the NPF+OM treatments.

The results in Table 3 showed that both microbial biomass C and N were concentrated in the surface layer, and significantly higher than in soil subsurface layer. Besides, significant seasonal change was detected for soil microbial biomass. The soil microbial biomass in the soils collected at the seeding prophase was highest (except biomass C in manure-treated soil at the ripening stage of maize). At the jointing stage, the tested soils showed the smallest microbial biomass values, and higher microbial biomass values were observed at the ripening stage. These seasonal changes in soil microbial biomass were influenced by soil depth and fertilization treatments, as indicated by significant interactions (Table 3). Furthermore, significant correlations existed between soil microbial biomass C and N ($r^2=0.760$, $n=36$, $P<0.01$), between soil organic C and microbial biomass C ($r^2=0.335$, $n=36$, $P<0.05$), and between

Table 2 The effect of long-term fertilization on total N, organic C and pH value in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
Soil total N (g kg soil^{-1})							
0–20 cm	S	1.00 (0.03)	1.04 (0.01)	1.02 (0.01)	1.09 (0.02)	1.13 (0.01)	1.23 (0.03)
	J	0.94 (0.03)	0.94 (0.02)	0.90 (0.02)	0.85 (0.02)	0.89 (0.03)	0.97 (0.03)
	R	0.79 (0.04)	0.78 (0.02)	0.78 (0.02)	0.77 (0.00)	0.86 (0.03)	0.95 (0.04)
20–40 cm	S	0.69 (0.02)	0.93 (0.02)	0.90 (0.02)	0.91 (0.03)	0.98 (0.02)	1.02 (0.02)
	J	0.50 (0.04)	0.71 (0.02)	0.74 (0.03)	0.72 (0.02)	0.76 (0.03)	0.82 (0.04)
	R	0.54 (0.04)	0.67 (0.03)	0.74 (0.01)	0.75 (0.02)	0.81 (0.04)	0.86 (0.03)
Soil organic C (g kg soil^{-1})							
0–20 cm	S	10.21(0.14)	9.56 (0.20)	9.37 (0.14)	10.19(0.21)	10.42(0.21)	11.37(0.17)
	J	11.00 (0.15)	10.46 (0.26)	10.12 (0.28)	10.28 (0.12)	10.36 (0.23)	11.38 (0.29)
	R	12.00 (1.31)	11.80 (0.20)	10.86 (0.31)	11.15 (0.23)	12.70 (0.41)	13.17 (0.54)
20–40 cm	S	6.14 (0.23)	8.40 (0.43)	7.96 (0.19)	8.06 (0.20)	8.38 (0.35)	9.29 (0.13)
	J	7.32 (0.35)	9.19 (0.50)	9.61 (0.21)	9.42 (0.12)	10.07(0.40)	9.96 (0.19)
	R	7.37 (0.45)	10.32 (0.31)	10.74 (0.27)	10.91 (0.22)	11.03 (0.30)	11.63 (0.40)
Soil pH value							
0–20 cm	S	6.47 (0.05)	6.30 (0.55)	5.40 (0.04)	5.45 (0.03)	5.93 (0.06)	6.52 (0.05)
	J	6.69 (0.04)	6.69 (0.03)	5.54 (0.09)	5.77 (0.07)	6.06 (0.04)	6.62 (0.07)
	R	6.68 (0.04)	6.48 (0.55)	5.30 (0.06)	5.00 (0.23)	5.87 (0.03)	6.39 (0.03)
20–40 cm	S	6.11 (0.05)	6.39 (0.05)	5.73 (0.09)	5.81 (0.16)	6.25 (0.02)	6.53 (0.04)
	J	6.41 (0.06)	6.51 (0.07)	6.12 (0.08)	6.19 (0.03)	6.47 (0.07)	6.81 (0.06)
	R	6.31 (0.03)	6.27 (0.03)	6.09 (0.26)	5.91 (0.04)	6.09 (0.04)	6.40 (0.04)

Source of variation	*df*	Soil total N			Soil organic C			Soil pH value		
		$LSD_{0.05}$	$LSD_{0.01}$	*P*	$LSD_{0.05}$	$LSD_{0.01}$	*P*	$LSD_{0.05}$	$LSD_{0.01}$	*P*
Layer	1	0.02	0.02	<0.001	0.21	0.27	<0.001	0.05	0.07	<0.001
Season	2	0.02	0.03	<0.001	0.25	0.34	<0.001	0.07	0.09	<0.001
Treatment	5	0.03	0.04	<0.001	0.36	0.48	<0.001	0.09	0.12	<0.001
L×Se	2	0.03	0.04	<0.001	0.36	0.48	0.007	0.09	0.12	0.31
L×T	5	0.04	0.05	<0.001	0.51	0.67	<0.001	0.13	0.17	<0.001
Se×T	10	0.05	0.06	0.001	0.62	0.82	0.24	0.16	0.21	0.003
L×Se×T	10	0.07	0.09	0.042	0.88	1.17	0.115	0.23	0.30	0.005

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data are means (SE) of four replicates

soil total N and microbial biomass N (r^2=0.659, n=36, P<0.01).

In order to better evaluate the impact of application of fertilizers and manures in the soils, the soil biomass C/N ratio was calculated (Table 4). The results demonstrated that the microbial biomass C/N ratio had no significant variation between treatments, but significant differences were observed between soil layers and among seasons. These changes in soil microbial biomass were influenced by soil layers, seasons and fertilization treatments, as indicated by significant interactions (Table 4).

Table 3 The effect of long-term fertilization on microbial biomass N and C in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
Microbial biomass N (mg N kg soil^{-1})							
0–20 cm	S	44.54 (3.37)	47.87 (3.38)	29.06 (2.25)	37.13 (3.01)	48.60 (3.04)	63.32 (4.77)
	J	22.68(2.00)	25.63 (2.06)	23.03 (1.96)	28.29 (1.88)	30.19 (2.46)	36.21 (2.72)
	R	31.50 (5.28)	32.99 (1.98)	26.51 (1.61)	33.04 (2.52)	42.37 (3.21)	46.67 (3.60)
20–40 cm	S	21.38 (1.49)	28.99 (2.33)	26.16 (1.78)	23.67 (1.49)	29.33 (2.24)	31.32 (2.40)
	J	19.09 (1.29)	23.46 (1.75)	21.89 (1.51)	26.87 (2.08)	27.97 (1.63)	33.19 (2.03)
	R	26.81 (2.10)	30.70 (2.27)	25.26 (2.36)	29.70 (2.32)	33.75 (2.92)	36.25 (1.98)
Microbial biomass C (mg C kg soil^{-1})							
0–20 cm	S	146.1 (8.6)	160.2 (10.6)	139.8 (6.6)	146.7 (7.2)	170.0 (9.0)	192.8 (9.6)
	J	116.9 (7.9)	138.4 (7.0)	101.9 (6.9)	100.3 (5.2)	132.9 (8.2)	154.3 (8.5)
	R	107.5 (5.7)	124.3 (9.0)	85.1 (6.5)	99.9 (6.3)	152.2 (6.8)	210.7 (11.5)
20–40 cm	S	122.7 (6.0)	137.5 (6.3)	116.5 (5.5)	130.6 (6.1)	151.0 (7.0)	172.7 (10.3)
	J	74.1 (5.5)	101.8 (5.4)	69.6 (4.5)	89.7 (6.0)	111.2 (6.2)	143.7 (9.4)
	R	77.7 (5.0)	110.4 (6.8)	87.3 (6.1)	104.8 (4.7)	120.3 (8.3)	145.5 (2.7)
Source of variation	*df*	Microbial biomass N			Microbial biomass C		
		$LSD_{0.05}$	$LSD_{0.01}$	*P*	$LSD_{0.05}$	$LSD_{0.01}$	*P*
Layer	1	1.62	2.14	<0.001	4.80	6.36	<0.001
Season	2	1.98	2.62	<0.001	5.88	7.79	<0.001
Treatment	5	2.80	3.71	<0.001	8.32	11.01	<0.001
L×Se	2	2.80	3.71	<0.001	8.32	11.01	0.689
L×T	5	3.97	5.25	<0.001	11.76	15.57	0.034
Se×T	10	4.86	6.43	0.055	14.41	19.07	0.011
L×Se×T	10	6.87	9.09	0.042	20.38	26.97	0.003

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data are means (SE) of four replicates

Soil basal respiration and microbial metabolic quotient

Basal respiration was expressed as mg CO_2-C released kg^{-1} soil h^{-1} (Table 5). The data showed that significant differences in soil respiration were found between soil layers, between treatments and among seasons as well, but no significant interactions among these three factors were found. Soil basal respiration increased in the soils amended with organic manure, but decreased as soil depth increased. Inorganic fertilizers treatments had no significant effect on soil basal respiration, and there was no significant difference either between NPF+OM and OM, or between NF and NPF treatments. The samples collected at the jointing stage showed the highest soil basal respiration, and the lowest basal respiration was observed at the ripening stage.

Microbial metabolic quotient (qCO_2) was calculated by dividing respiration rates by microbial biomass carbon. As shown in Table 6, significant interactions were observed between soil layers, sampling seasons and treatments. Inorganic fertilizers treatments increased soil qCO_2, but organic manures treatments decreased soil qCO_2. The highest qCO_2 values were observed in soils from N-containing fertilizers treatments, differing statistically from the other treatments. By contrast, the smallest qCO_2 values were obtained

Table 4 The effect of long-term fertilization on microbial biomass C/N ratios in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
0–20 cm	S	3.34 (0.32)	3.36 (0.16)	4.93 (0.55)	4.02 (0.35)	2.72 (0.21)	4.01 (0.28)
	J	5.21 (0.31)	5.47 (0.41)	4.61 (0.75)	3.56 (0.10)	4.29 (0.16)	4.44 (0.20)
	R	3.45 (0.17)	3.85 (0.50)	3.20 (0.06)	3.11 (0.39)	4.57 (0.32)	3.67 (0.38)
20–40 cm	S	5.80 (0.39)	4.79 (0.20)	4.51 (0.35)	5.57 (0.37)	4.86 (0.21)	5.91 (0.18)
	J	3.94 (0.40)	4.44 (0.47)	3.23 (0.35)	3.39 (0.29)	4.38 (0.39)	4.00 (0.27)
	R	2.95 (0.30)	3.68 (0.44)	3.57 (0.46)	3.62 (0.40)	4.05 (0.20)	3.62 (0.31)
Source of variation	*df*, LSD values and *P*						
	df		$LSD_{0.05}$		$LSD_{0.01}$	*P*	
Layer	1		0.23		0.30	0.032	
Season	2		0.28		0.37	<0.001	
Treatment	5		0.40		0.53	0.336	
L×Se	2		0.40		0.53	<0.001	
L×T	5		0.56		0.75	0.069	
Se×T	10		0.69		0.91	<0.001	
L×Se×T	10		0.98		1.29	0.02	

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data are means (SE) of four replicates

in the organic manure treatments, which was statistically different from either the treatments receiving no fertilizers or the treatment with inorganic fertilizers alone, and there was no significant difference either between NPF+OM and OM, or between NF and NPF treatments. The higher qCO_2 values were observed in soil subsurface layer and at the jointing stage of maize (Table 6).

Soil enzymatic activity

Both treatments with organic manures and inorganic fertilizers significantly increased soil urease activity, and soil urease activity decreased as soil depth increased. The organic manures treatments showed higher urease activities than inorganic fertilizers treatments. The soil sucrase activity increased in the soils amended with organic manure, but decreased in the soils treated with inorganic fertilizers compared with CK0 and CK, and decreased as soil depth increased. The values of urease and sucrase activity were significantly higher in the OM treatments than in the NPF+OM treatments, and also higher in the NPF treatments than in the NF treatments (Table 7).

In general, the highest sucrase and urease activities were obtained in the two organic manure treatments (OM and NPF+OM). Soil sucrase and urease activities were significantly higher in soil surface layer than in subsurface layer. Compared with the CK, the organic manure treatment (OM) increased sucrase and urease activity by 108.4% and 136.1%, respectively, in the surface layer and by 70.5% and 100% in the subsurface layer. By contrast, inorganic fertilizers treatments significantly decreased soil sucrase activity, respectively, by 61.2% in the surface layer and by 46.4% in the subsurface layer. However, there was no significant effect of inorganic fertilizers treatment on soil urease activity.

Furthermore, significant seasonal changes were detected in soil enzymatic activities (Table 7). The highest urease and sucrase activity values were observed at the seeding prophase and ripening stage of maize, respectively, and for the other collections, the soil enzymatic activities were relatively lower. Besides,

Table 5 The effect of long-term fertilization on basal respiration in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
0–20 cm	S	10.56 (0.22)	11.07 (0.31)	9.86 (0.44)	7.97 (0.47)	10.86 (0.57)	10.23 (0.50)
	J	10.80 (0.39)	10.49 (0.26)	11.69 (0.12)	11.31 (0.46)	11.56 (0.61)	12.76 (0.42)
	R	8.88 (0.30)	9.84 (0.65)	9.59 (0.16)	9.20 (0.45)	9.44 (0.37)	9.97 (0.45)
20–40 cm	S	9.36 (0.37)	9.83 (0.26)	8.60 (0.62)	8.10 (0.35)	8.66 (0.22)	9.55 (0.07)
	J	9.80 (0.56)	10.24 (0.40)	10.36 (0.18)	11.25 (0.32)	9.54 (0.24)	11.88 (0.43)
	R	7.99 (0.48)	7.86 (0.48)	7.86 (0.63)	7.22 (0.27)	8.31 (0.37)	7.80 (0.16)
Source of variation	*df*, LSD values and *P*						
	df		$LSD_{0.05}$		$LSD_{0.01}$	*P*	
Layer	1		0.27		0.35	<0.001	
Season	2		0.33		0.43	<0.001	
Treatment	5		0.46		0.61	<0.001	
L×Se	2		0.46		0.61	0.076	
L×T	5		0.66		0.87	0.249	
Se×T	10		0.80		1.06	<0.001	
L×Se×T	10		1.14		1.51	0.186	

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data are means (SE) of four replicates

the sucrase and urease activity values followed the same trend as the values obtained from microbial biomass C and N, respectively. There were significant correlations between soil microbial biomass C and sucrase activity (r^2=0.605, n=36, P<0.01), and between soil microbial biomass N and urease activity (r^2=0.663, n=36, P<0.01). And significant correlations were also noted between organic C and sucrase activity (r^2=0.761, n=36, P<0.01), and between total N and urease activity (r^2=0.684, n=36, P<0.01).

Discussion

Soil total N, organic C and pH and their seasonal variations in response to long-term fertilization

In the present study, application of organic manure increased soil organic C and total N content, and balanced soil pH value (Table 2, also see Mueller et al. 1998; Liang et al. 2003, 2005; Tu et al. 2006). Amendment with inorganic fertilizer significantly increased soil total N, but decreased soil organic C and pH value (Table 2), which can be attributable to the repeated use of mineral fertilizers. The application of mineral fertilizers could increase soil N and P content, but could decrease soil pH value and sucrase activity. On the other hand, soil organic C presented an increased trend with plant growth (Table 2). These results might be attributed mainly to the increasing amounts of plant residues and rhizodepositions with crop growth (Toal et al. 2000, Liang et al. 2005). As expected, soil total N decreased progressively with plant growth with the highest soil N content observed in the samples collected at the seeding prophase (Table 2). Soil total N was highest at the seeding prophase because of the input of plenty of manure and fertilizers. However, soil N was absorbed by plants progressively with the plant growth, and in the meantime, nitrate leaching loss and ammonia volatilization, especially caused by strong wind-erosion in spring in north China, happened ceaselessly, As a consequence, soil total N decreased and tended to be lowest at the ripening stage (Table 2). This is

Table 6 The effect of long-term fertilization on metabolic quotient (qCO_2) in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
0–20 cm	S	7.31 (0.50)	7.01 (0.55)	7.06 (0.06)	5.45 (0.24)	6.41 (0.30)	5.34 (0.30)
	J	9.40 (0.80)	7.65 (0.48)	11.61 (0.69)	11.30 (0.15)	8.78 (0.67)	8.30 (0.28)
	R	8.33 (0.48)	8.08 (0.83)	11.48 (1.00)	9.36 (0.93)	6.26 (0.49)	4.77 (0.32)
20–40 cm	S	7.68 (0.47)	7.18 (0.33)	7.42 (0.58)	6.26 (0.51)	5.76 (0.22)	5.58 (0.31)
	J	13.40 (1.12)	10.17 (0.83)	15.07 (0.96)	12.71 (0.95)	8.67 (0.56)	8.32 (0.35)
	R	10.56 (1.41)	7.27 (0.85)	9.04 (0.61)	6.95 (0.49)	6.98 (0.45)	5.36 (0.17)
Source of variation	*df* , LSD values and *P*						
	df		$LSD_{0.05}$		$LSD_{0.01}$	*P*	
Layer	1		0.42		0.56	0.007	
Season	2		0.52		0.68	<0.001	
Treatment	5		0.73		0.97	<0.001	
L×Se	2		0.73		0.97	<0.001	
L×T	5		1.03		1.37	0.03	
Se×T	10		1.26		1.67	<0.001	
L×Se×T	10		1.79		2.37	0.003	

CK0 neither tillage nor fertilization, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data are means (SE) of four replicates

coincident with the recent report (Shi et al. 2004) which showed that soil total N, NO_3-N and NH_4-N content were significantly higher in May than in July and September, but no significant seasonal change of soil organic C content was observed. In addition, it is interesting to note that soil total N and organic C content in subsurface layers of soil were also at a considerably high level though they were significantly lower than in surface soils (Table 2). This huge nutrient reserve in subsurface soils can be an additional source of nutrient supply for maize, a deep-rooting crop planted in this area. Accordingly, we recommend that low tillage should be taken to prevent from nutrient (and water) loss caused by strong wind-erosion in spring in north China to make use of nutrients (and water) in surface and subsurface soils.

Soil microbial biomass C and N and their seasonal variations in response to long-term fertilization

In the present study, long-term application of organic manure significantly increased ($P<0.05$) the biomass C and N (Table 3, also see Kaur et al. 2005; Chu et al. 2007), but inorganic fertilizers significantly decreased soil biomass C and showed less effect on soil microbial biomass N, compared with CK and CK0. This phenomenon may be due to the direct toxicity and reduced pH caused by ammonium-based fertilizers (Table 2, also see Hopkins and Shiel 1996). Soil pH could significantly affect microbial biomass. The microbial biomass changes, as a function of soil pH, appeared to follow a normal distribution with the original soil pH value at the apex and as pH increased or decreased, microbial biomass declined (Chen et al. 2004). Besides, higher microbial biomass observed in the CK0 treatment is believed to be attributed to abundant energy undisturbed for microorganism in surface soil layer under the no-tillage condition. By contrast, soil microbial biomass in subsurface soil layer was significantly higher in CK0 than in any other treatment, which is in agreement with the previous reports by Aslam et al. (1999) and Xu et al. (2002).

Soil microbial biomass C and N represented obvious seasonal changes (Table 3), depending partly

Table 7 The effect of long-term fertilization on urease and sucrase activities in soils collected at the seeding prophase, jointing stage and ripening stage of maize

Soil layer	Season	Treatment					
		CK0	CK	NF	NPF	NPF+OM	OM
Urease activities (mg NH_3-N g $soil^{-1}$ d^{-1})							
0–20 cm	S	0.60 (0.03)	0.65 (0.01)	0.69 (0.02)	0.69 (0.01)	0.74 (0.02)	0.79 (0.02)
	J	0.40 (0.02)	0.36 (0.02)	0.46 (0.01)	0.42 (0.01)	0.49 (0.01)	0.63 (0.03)
	R	0.56 (0.04)	0.49 (0.01)	0.58 (0.00)	0.51 (0.02)	0.65 (0.04)	0.85 (0.04)
20–40 cm	S	0.62 (0.02)	0.65 (0.00)	0.62 (0.02)	0.65 (0.02)	0.69 (0.02)	0.70 (0.01)
	J	0.41 (0.00)	0.35 (0.01)	0.40 (0.01)	0.38 (0.01)	0.43 (0.01)	0.54 (0.03)
	R	0.42 (0.02)	0.44 (0.01)	0.48 (0.01)	0.45 (0.00)	0.53 (0.03)	0.67 (0.02)
Sucrase activities (mg glucose g $soil^{-1}$ d^{-1})							
0–20 cm	S	19.96 (0.56)	22.31 (1.07)	16.20 (0.91)	16.62 (0.70)	18.80 (1.10)	25.01 (0.64)
	J	19.65 (1.12)	17.80 (0.50)	12.04 (0.97	15.26 (0.52)	18.90 (0.64)	24.97 (1.05)
	R	27.55 (1.15)	30.93 (1.54)	18.36 (0.83)	24.38 (0.51)	30.14 (1.31)	37.14 (1.03)
20–40 cm	S	12.01 (1.42)	19.64 (0.75)	15.22 (0.68)	16.15 (0.72)	22.52 (0.55)	25.42 (0.48)
	J	6.43 (0.23)	17.56 (0.95)	12.05 (0.84)	14.81 (0.97)	15.52 (0.26)	20.19 (1.03)
	R	11.80 (0.59)	22.39 (1.36)	17.76 (1.21)	23.67 (0.80)	28.90 (0.66)	30.02 (0.95)
Source of variation	*df*	Urease activities			Sucrase activities		
		$LSD_{0.05}$	$LSD_{0.01}$	*P*	$LSD_{0.05}$	$LSD_{0.01}$	*P*
Layer	1	0.01	0.02	<0.001	0.60	0.79	<0.001
Season	2	0.02	0.02	<0.001	0.73	0.97	<0.001
Treatment	5	0.02	0.03	<0.001	1.04	1.37	<0.001
L×Se	2	0.02	0.03	<0.001	1.04	1.37	<0.001
L×T	5	0.03	0.04	<0.001	1.47	1.94	<0.001
Se×T	10	0.04	0.05	<0.001	1.79	2.38	<0.001
L×Se×T	10	0.05	0.07	0.363	2.54	3.36	<0.001

CK0 neither tillage nor fertilizers, *CK* no fertilizers, *NF* application of urea, *NPF* combined application of urea and ammonium dibasic phosphate, *NPF+OM* pig manure with urea and ammonium dibasic phosphate, *OM* pig manure. *S* seeding prophase, *J* jointing stage, *R* ripening stage, *L* soil layer (Layer), *Se* Season, *T* Treatment

Data is mean (SE) of four replicates

upon soil temperature and soil moisture regimes as well as organic and inorganic inputs (Kandeler et al. 1999; Emmerling et al. 2001; Feng and Simpson 2009). In the present study, we measured soil moisture contents in samples collected in spring (April), summer (June) and autumn (September). The corresponding soil water contents were 17.22%, 20.77% and 15.83% in surface layer (0–20 cm), and 16.64%, 18.56%, 16.02% in subsurface layer (20–40 cm), respectively. Moreover, the minimal/maximal atmosphere temperature regimes at the three sampling time points were 2/10°C, 19/29°C and 10/28°C, respectively. As shown in Table 3, the highest biomass C and N were observed at the seeding prophase (except biomass C in manure-amended soil at the ripening stage). This phenomenon could be partly attributed to the fact that abundant energy accumulated by deadwood and defoliation during the autumn was not consumed considerably by soil microorganism during the whole cold winter. Besides, organic manures containing large numbers of variable and dead-living microorganisms, and huge quantities of readily utilizable energy sources and inorganic fertilizers rich in available nutrients were applied annually to soil at the seeding prophase before sampling (April 12) (Table 1), thereby stimulating microorganisms to absorb nutrients and resulting in higher soil microbial biomass (also see Ladd et al.

1994; Kandeler et al. 1999). At the jointing stage, the smallest microbial biomass C and N were observed (Table 3). This can be attributable to the accelerated microbial activity and the depletion of nutrients caused by higher soil temperature and water content in summer season and by plant uptake. The higher microbial biomass values observed at the ripening stage might be a consequence of both the increased nutrients storage by microorganisms that were stimulated by high amounts of plant residues and root secretions, and the decreased soil temperature and moisture in autumn season.

The microbial biomass C/N ratio had no significant variation either between treatments or between layers (Table 4). According to Wardle (1992), a high microbial biomass C/N ratio could be an indication of stressful conditions. The variation in soil microbial biomass C/N ratio did not present a direct relation with sampling season, nor with the applied fertilizers and manures (Table 4). This observed variation suggested the occurrence of succession in the microbial community according to their greater or smaller specificity for the decomposition of different organic matter forms (also see Silvana et al. 2005).

Soil basal respiration and metabolic quotient (qCO_2) and their seasonal variations in response to long-term fertilization

Soil basal respiration increased in the soils treated with organic manure, but decreased as soil depth increased (Table 5). These facts might be related to a greater amount of organic materials available at the soil surface (Tables 1 and 2). The observed data can be explained by an increase in the contents of soil organic matter and nutrients, which would stimulate microbial activity (Emmerling et al. 2001) and also microbial biomass cycling, thus leading to an increase in basal respiration (Leita et al. 1999). Besides, N-containing fertilizers increased soil respiration and decreased soil pH value at the jointing stage of maize, suggesting that lower pH conditions possibly stimulated soil microbial activity in the tested soil. Furthermore, a high respiration rate might indicate either an ecological disorder, or a high level of productivity in the ecosystem (Islan and Weil 2000). The respiration rate per unit of microbial biomass or metabolic quotient is a variable of easier interpretation. The qCO_2 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 (Silvana et al. 2005). Thus, soils under stress would present higher qCO_2 values than non-stressed soils. Our results shown in Table 6 are coincident with the finding of Hopkins and Shiel (1996) who also reported that the N-contained-fertilizer-treated soil had higher qCO_2. Besides, the qCO_2 was negatively correlated with the microbial biomass C content ($r^2=-0.821$, $n=36$, $P<0.01$). Thus, it is clear that repeated use of mineral fertilizers, especially N-containing fertilizers, will lead to a decrease in soil pH value, microbial biomass and microbial activity but an increase in metabolic quotient. This is not good for maintenance of soil fertility and sustainability of agriculture.

Temperature and moisture are two key factors that control soil respiration rate (Emmerling et al. 2001; Chen et al. 2003; Feng and Simpson 2009). It was reported that the change of soil respiration rate was accordant with soil temperature and moisture, and the seasonal change trend of respiration rate indicated that soil respiration was higher in summer and lower in other seasons (Chen et al. 2003). As shown in Table 6, soil respiration and qCO_2 values were higher in summer (June) than in spring (April) and autumn (September). Clearly, this difference can be attributable to the accelerated microbial activity caused by high soil temperature and high water content regimes in summer (see above). However, lower temperature and water content in other seasons depressed microbial activity and accordingly decreased soil respiration.

Soil enzymatic activities and their seasonal variations in response to long-term fertilization

In the present study, organic manures significantly increased soil urease and sucrase activities (Table 7), which are in agreement with the previous studies under incubation conditions or using pot experiments (Crecchio et al. 2001; Liang et al. 2003, 2005). By contrast, using inorganic fertilizers increased soil urease activity but decreased soil sucrase activity compared with CK0 and CK. And interestingly, no significant difference in urease or sucrase activity was observed between CK0 and CK. This seems to suggest that sucrase activity was highly sensitive to the inhibitory effects associated with large amount of

fertilizers added. Besides, the enzymatic activity decreased as soil depth increased, and this could be related to a greater amount of organic material available at the surface soil (Ladd et al. 1994).

Soil enzymatic activities showed obvious seasonal changes at different soil sampling time points. Xiong et al. (2004) reported that the highest soil urease activity value was observed in April, and then decreased with crop growth. They also found that soil sucrase activity increased from spring to autumn, and reached maximum in October. In the present study, soil urease and sucrase activities showed general consistent trend with the findings by Xiong et al. (2004). Besides, the sucrase and urease activity values followed the same trend as the values obtained for microbial biomass C and N, respectively (Table 3, see also Zhou and Ding 2007). There were significant correlations between soil microbial biomass C and sucrase activity (r^2=0.605, n=36, P<0.01), and between soil microbial biomass N and urease activity (r^2=0.663, n=36, P<0.01). Similar results were also reported by Albiach et al. (2000) and Silvana et al. (2005).

In conclusion, soil biological activity varied significantly, depending on different fertilization treatments, sampling seasons and soil depths. Long-term application of organic manure significantly increased soil microbial biomass, basal respiration and soil enzymatic activity, but decreased soil microbial qCO_2. However, inorganic fertilizers increased soil microbial biomass N, basal respiration and urease activity, but decreased soil microbial biomass C, sucrase activity and soil pH value. The increase in the microbial activities following application of inorganic fertilizers was attributed not to increased microbial biomass but to increased soil respiration. There were significant correlations between soil microbial biomass C and N, between organic C and sucrase activity and between total N and urease activity as well. The soil pH value greatly influenced soil sucrase activity and microbial biomass C content. Soil microbial biomass, basal respiration and soil enzymatic activity were higher in surface soil than in subsurface soil, and showed obvious seasonal changes. Considering that agricultural use of inorganic fertilizers unavoidably decreases microbial activity and intensifies soil acidification to some extent in the tested soil, we recommend that combined use of organic manure with inorganic fertilizers should be considered based on the balance between crop demand and soil supply of available nutrients. In addition, considering considerably high nutrients reserve and microbial activity in subsurface layers of soil, we propose that low tillage should be considered to prevent from nutrient and water loss caused by wind-erosion in spring and to attain high nutrient use efficiency.

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