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Effects of Long-Term Combined Application of Organic and Mineral Fertilizers on Microbial Biomass, Soil Enzyme Activities and Soil Fertility

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Abstract

Soil health is important for the sustainable development of terrestrial ecosystem. In this paper, we studied the relationship between soil quality and soil microbial properties such as soil microbial biomass and soil enzyme activities in order to illustrate the function of soil microbial properties as bio-indicators of soil health. In this study, microbial biomass C and N contents (Cmic & Nmic), soil enzyme activities, and soil fertility with different fertilizer regimes were carried out based on a 15-year long-term fertilizer experiment in Drab Fluvo-aquic soil in Changping County, Beijing, China. At this site, 7 different treatments were established in 1991. They were in a wheat-maize rotation receiving either no fertilizer (CK), mineral fertilizers (NPK), mineral fertilizers with wheat straw incorporated (NPKW), mineral fertilizers with incremental wheat straw incorporated (NPKW+), mineral fertilizers plus swine manure (NPKM), mineral fertilizers plus incremental swine manure (NPKM+) or mineral fertilizers with maize straw incorporated (NPKS). In different fertilization treatments Cmic changed from 96.49 to 500.12 mg kg^{-1}, and Nmic changed from 35.89 to 101.82 mg kg^{-1}. Compared with CK, the other treatments increased Cmic & Nmic, Cmic/Corg (organic C) ratios, Cmic/Nmic, urease activity, soil organic matter (SOM), soil total nitrogen (STN), and soil total phosphorus (STP). All these properties in treatment with fertilizers input NPKM+ were the highest. Meantime, long-term combined application of mineral fertilizers with organic manure or crop straw could significantly decrease the soil pH in Fluvo-aquic soil (the pH around 8.00 in this experimental soil). Some of soil microbial properties (Cmic/Nmic, urease activity) were positively correlated with soil nutrients. Cmic/Nmic was significantly correlated with SOM and STN contents. The correlation between catalase activity and soil nutrients was not significant. In addition, except of catalase activity, the soil pH in this experiment was negatively correlated with soil microbial properties. In conclusion, soil microbial properties reflect changes of soil quality and thus can be used as bio-indicators of soil health.

Key words: long-term fertilizer experiment, soil microbial biomass, soil enzyme activities, soil fertility

INTRODUCTION

Fertilization is one of the soil and crop management practices which exert a considerable influence on soil quality (Chander *et al.* 1998; Powlson *et al.* 1987; Dilly and Munch 1998). Recent years, some researchers have considered soil microbial properties such as microbial structure, microbial biomass (MB), microbial quantities, and soil enzyme activities as the biological index of soil health (Dilly and Munch 1998; van Bruggen and Semenov 2000; Harris 2003; Schloter *et al.* 2003; Bossio and Fleck 2006).

Soil microbial properties have a strong correlation

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with soil health (Zhou and Ding 2007). Catalase was first considered as the biological index of fertility in 1926 (Zhou 1987). Satefanic *et al.* (1981) proposed that a better biological index of fertility could be derived from the activities of catalase and dehydrogenase. Doran and Parkin (1994) believed that the essential indexes of soil quality should be comprised of soil microbial biomass carbon (Cmic) & nitrogen (Nmic) contents, potentially mineralizable N, soil respiration, Cmic/Corg, etc. While Pankhurst *et al.* (1997) considered that some of soil functional microorganisms, soil microbial community structure and composition, soil enzyme activities were also potential indexes of soil health.

Since the last century, composition of soil microbial and fauna communities have been considered as new criteria from new technologies to investigate the evolution of ecosystem (Anne 2002). Miller and Dick (1995) showed that soil enzyme activity was a sensitive biological indicator of the effects of soil management practices after long-term fertilizer experiments. Ananyeva *et al.* (1999), Goyal *et al.* (1999), and Simek *et al.* (1999) reported the effects of different long-term fertilizer applications on microbial biomass in different soils respectively, and pointed out that soil microbial properties can, on the whole, reflect the evolution of soil quality about 10-22 years.

Little information is available on the relative importance of different long-term fertilization regimes (Sun and Zhao 2003). The objective of this study was to investigate the long-term effects of inorganic fertilizers and organic amendments on the soil microbial biomass carbon (Cmic) & biomass nitrogen (Nmic) contents, enzyme activities, the relationship between them, and soil fertility in agro-ecosystems of China.

MATERIALS AND METHODS

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 annual average rainfall is 600 mm and the annual average temperature is 11°C. The soil at the test site is a Fluvo-aquic soil. The primary chemical properties of the soil in 1991 were given in Table 1. Seven different treatments were established in a wheat-maize rotation receiving either no fertilizer (CK), mineral fertilizers (NPK), mineral fertilizers with wheat straw incorporated (NPKW), mineral fertilizers with 50 % more wheat straw applied (NPKW+), mineral fertilizers plus swine manure (NPKM), mineral fertilizers plus 50% more swine manure applied (NPKM+) or mineral fertilizers with maize straw incorporated (NPKS). Each treatment was replicated 4 times with plot size of 2 m × 1.5 m in a complete randomized block design. There are 28 plots in the field. The mineral fertilizers are applied twice annually in spring after wheat is harvested and fall after maize is harvested. The organic fertilizers are applied during fall after maize is harvested. All treatments are fertilized with N 160 kg ha^{-1}, P_2O_5 80 kg ha^{-1}, K_2O 60 kg ha^{-1}. And the organic fertilizers were applied W 2.17 t ha^{-1}, W+ 3.3 t ha^{-1}, M 22.5 t ha^{-1}, M + 33 t ha^{-1}, S 2.17 t ha^{-1}. The aboveground crop biomass was removed.

Table 1 Soil chemical properties in 1991 prior to application of fertilizers

SOM (g kg^{-1})	STN (g kg^{-1})	STP (g kg^{-1})	Available N (g kg^{-1})	Available P (g kg^{-1})	pH
12.9	0.482	0.579	6.49	3.77	8.12

Soil sampling and preparation

Soil samples were collected in April 2006 at each plot as described above. In each replicate of the 7 treatments, 5 soil cores were collected from the top 20 cm with an auger and mixed to give a bulk sample. Each soil sample was separated into 2 parts. One part was air-dried and stored at room temperature for determination of chemical properties. The other part was sieved (2 mm mesh) and adjusted to 50 % of its water holding capacity, then was pre-incubated at 25°C for 5 d and stored at 4°C for not more than one week until the start of the experiment.

Physical and chemical analyses

Soil pH was measured in suspensions with a soil to water (w/w) ratio of 1:2.5. Soil water content was determined as weight loss after overnight drying at

105°C. Organic carbon was determined by a colorimetric method with an external heating procedure (Anderson and Ingram 1993) and total nitrogen in soil was measured using the Kjeldahl method (Jackson 1973).

Microbial biomass

Microbial biomass C (Cmic) and biomass N (Nmic) contents were estimated by chloroform fumigation-extraction (Vance *et al.* 1987). One 20-g portion (in dry weight) rewetted to 60% was fumigated for 24 h at 25°C with ethanol-free CHC_{13}. Following fumigant removal, the soil was extracted with 60 mL 0.5 mol L^{-1} K_2SO_4 by 30 min horizontal shaking at 200 r/min and filtered. The non-fumigated portion was extracted similarly at the time fumigation commenced. Organic C in extracts was determined by a dichromate digestion method and unused dichromate titrated against ferrous-ammonium sulphate. Microbial biomass C was calculated as follows: Microbial biomass C = E_C/k_{EC}, where *EC* = Organic C extracted from non-fumigated soils - Organic C extracted from fumigated soils, and k_{EC}=0.38 (Vance *et al.* 1987). The Kjeldahl digestion -distillation-titration method was used to determine the total N in the extracts. Microbial biomass N was calculated as follows: Microbial biomass N = E_N/k_{EN}, where E_N = Total N extracted from fumigated soils - Total N extracted from non-fumigated soils, and k_{EN}= 0.45 (Brookes *et al.* 1985; Jenkinson 1988).

Soil enzyme activities

For the determination of urease activity (Guan 1983), one 5-g (oven-dry) soil sample was incubated in a 100-mL quantificational carafe with 5 mL 10% urease solution and 10 mL citrate buffer (pH 6.7) for 3 h at 38°C. The released ammonium was determined colorimetrically at 578 nm using Indophenol reagent.

Catalase activity was determined according to Guan (1983). One 2-g (oven-dry) soil sample was placed in a 125-mL Erlenmeyer flask with 40 mL of distilled water and put on a rotary shaker. To this was added 20 mL of 0.3% H_2O_2 and the slurry was shaken for 20 min. The remaining peroxide was then stabilized by adding 20 mL of 3 mol L^{-1} H_2SO_4, the contents of the flask filtered and a 20 mL aliquot titrated with 0.02 mol L^{-1} K_2MnO_4.

Statistical analysis

All statistical work was done using the DPS ver. 2.00 for Windows. One-way ANOVA was used to analyze means, significant treatment effects were determined using the Duncan's test to least significant difference at the 5% level.

RESULTS

Chemical properties

The long-term addition of organic and inorganic amendments caused significant changes in soil chemical properties (Table 2). Soil pH was the highest in the CK and the lowest in the NPKM+ treatments, with value ranging from 7.56 to 8.25. The amount of soil organic carbon, total N, total P were generally higher for the plots with mineral fertilizers plus swine manure (NPKM+, NPKM), wheat straw (NPKW, NPKW+) and maize straw (NPKS) than for the plots with mineral fertilizers only (NPK) and those without fertilizer (CK). The treatments applied mineral

Table 2 Effects of long-term combined application of organic and mineral fertilizers on soil chemical properties after 15 years of annual fertilizer application (0-20 cm)

Treatments	SOM (g kg^{-1})	STN (g kg^{-1})	STP (g kg^{-1})	C/N (Corg/STN)	pH
CK	15.77 ± 1.06 e	0.55 ± 0.06 e	0.56 ± 0.01d	16.28 ± 2.37 a	8.25 ± 0.06 a
NPK	18.11 ± 1.13 d	0.72 ± 0.06 d	0.71 ± 0.06 c	14.66 ± 1.25 b	8.08 ± 0.08 b
NPKW	19.78 ± 1.35 cd	0.88 ± 0.12 c	0.75 ± 0.04 c	14.07 ± 1.37 bc	7.98 ± 0.04 bc
NPKW+	21.16 ± 0.55 c	0.89 ± 0.07 c	0.79 ± 0.09 c	13.15 ± 1.04 cd	8.03 ± 0.09 b
NPKM	28.74 ± 2.44 b	1.38 ± 0.22 b	1.71 ± 0.14 b	12.09 ± 1.24 d	7.85 ± 0.16 c
NPKM+	35.71 ± 1.84 a	1.66 ± 0.12 a	2.37 ± 0.13 a	11.97 ± 1.08 d	7.56 ± 0.05 d
NPKS	20.39 ± 0.81 d	0.90 ± 0.05 c	0.78 ± 0.03 c	13.30 ± 0.63 bcd	8.01 ± 0.11 b

Average ± standard difference, $P < 0.05$.

fertilizers plus swine manure increased SOM, STN, STP compared to mineral fertilizers plus straw. The lowest rates were found in the CK treatments. And the contents of SOM, STN, STP were enriched by the high rates of manure application, but these values were not significantly different in treatments of NPKW, NPKW + and NPKS. The C/N ratio was the highest in the CK treatments and the lowest after 50% more swine manure applied.

Microbial biomass

Cmic and Nmic contents in these treatments displayed the same trend. The Cmic contents ranged from 96.49 to 500.121 mg kg^{-1}, and the Nmic contents from 35.89 to 101.82 mg kg^{-1}. Microbial biomass (MB) contents were the lowest in the CK treatments and lower in the mineral fertilizers only. And it ws lower in the mineral fertilizers plus straw amended soils than in the mineral fertilizers plus swine manure amended soils. The contents of Cmic and Nmic increased with increasing of application rates of swine manure and straw (Table 3).

Cmic is considered to be more sensitive than total organic carbon to indicate soil changes because it is related to soil microorganisms that are sensitive to soil variations (Liu *et al.* 2003; Wang and Gong 1994). The microbial biomass carbon in most soils represents about 1-4% of total soil organic carbon (Anderson and Domsch 1989). In our study, the microbial biomass carbon as a percentage of soil organic carbon in agricultural soils ranged from 1.04% for the CK soils to 2.94% for the NPKW+ soils. The order of Cmic-to-Corg ratios in all fertilized treatments was NPKW+>NPKM>NPKW>NPKM+>NPKS>NPK>CK.

The quality of the amendments, such as C/N ratio, can influence the decomposer community composition. Fungi and bacteria have different C/N ratios. It is reported that the C/N ratio of bacteria is about 5:1 and 10:1 for fungi (Chen and He 1998; Wardle 1998; Lovell *et al.*1995). In this study, bacteria are the major microorganism in different treatments.

Soil enzyme activities

Assays for soil urease have been based on the rates of utilization of added urea or on the rates of formation of carbon dioxide from urea or on the rates of formation of NH_4^+ from urea (Chen 1979). The activity of urease tended to be higher in the NPKM and NPKS treatments compared to the NPK treatments, and was higher in the NPKM treatments than in the NPKS treatments. The urease activity was the lowest in the CK soils. Activity of urease increased with increasing of application rates of manure, and there were no significant differences in the urease activity between the treatments of mineral fertilizers plus wheat straw and of mineral fertilizers plus maize straw (Fig.1).

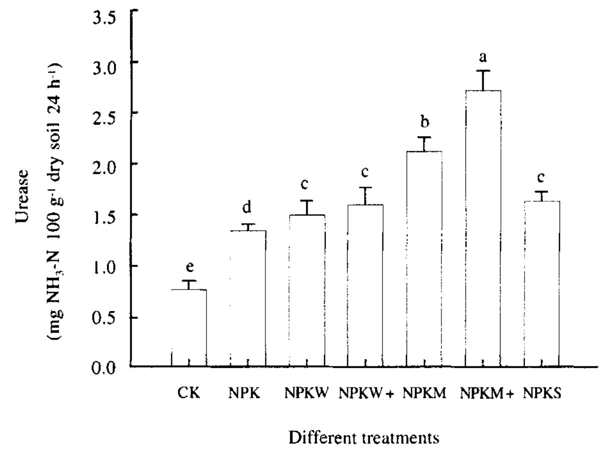

Fig. 1 Effects of long-term combined application of organic and mineral fertilizers on urease activity in the 0-20 cm soil layer.

Table 3 Effects of long-term combined application of organic and mineral fertilizers on Cmic and Nmic in the 0-20 cm soil layer

Treatments	Cmic (mg kg^{-1})	Nmic (mg kg^{-1})	Cmic/Corg (%)	Cmic/Nmic
CK	96.49 ± 21.06 e	35.89 ± 8.27 d	1.04 ± 0.26 d	2.41 ± 0.71 b
NPK	214.12 ± 45.17 d	51.11 ± 4.45 c	1.99 ± 0.45 c	4.25 ± 1.06 a
NPKW	305.18 ± 41.74 c	74.18 ± 6.61 b	2.68 ± 0.45 ab	4.15 ± 0.74 a
NPKW+	364.45 ± 44.56 b	72.02 ± 14.37 b	2.94 ± 0.40 a	5.29 ± 1.46 a
NPKM	451.25 ± 16.98 a	93.81 ± 18.08 a	2.80 ± 0.30 ab	5.14 ± 1.17 a
NPKM+	500.12 ± 77.10 a	101.82 ± 8.17 a	2.60 ± 0.65 ab	5.38 ± 0.98 a
NPKS	281.62 ± 49.24 d	64.09 ± 9.48 b	2.35 ± 0.45 bc	4.54 ± 1.34 a

Average ± standard difference, $P<0.05$.

Catalase activities of soils are based on the rates of release of oxygen from added hydrogen peroxide or on the recoveries of hydrogen peroxide (Wang *et al.* 2006; Chen and Fan 1980). Catalase activity was significantly higher in the NPKM+ treatments than in the other treatments. There were no differences in catalase activity between the other treatments (Fig.2).

Correlation between soil microbial properties and soil chemical properties

Some of soil microbial properties (Cmic contents & Nmic contents, urease activity) were positively correlated with soil chemical properties (SOM, STN, STP). Cmic/Nmic was significantly correlated with SOM and STN contents. The correlation between catalase activity and soil chemical properties (SOM, STN, STP) was not obvious. In addition, with the exception of catalase activity, there was a significantly negative correlation between the other soil microbial properties (Cmic & Nmic contents, Cmic/Corg, Cmic/Nmic, urease activity) and the soil pH value (Table 4).

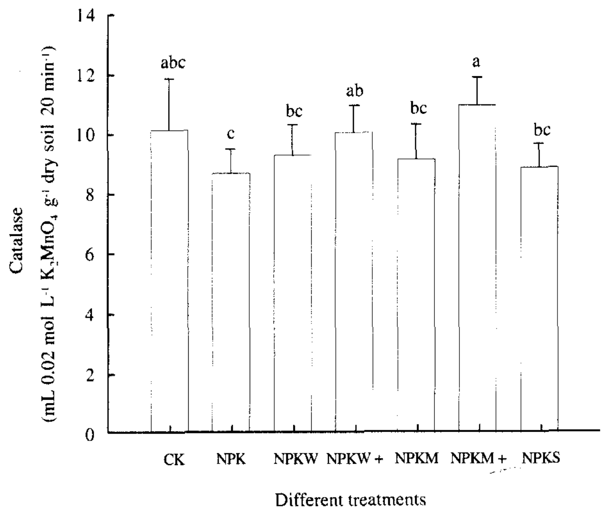

Fig. 2 Effects of long-term combined application of organic and mineral fertilizers on catalase activity in the 0-20 cm soil layer.

DISCUSSION

This study showed that different long-term fertilization regimes affected soil microbial properties and soil chemical properties. When the aboveground crop biomass was removed and not incorporated into the soil, the changes of soil microbial biomass are due to the input of different fertilizers (Zhang and Xiao 2006). In this study, the application of NPKM resulted in higher Corg, STN, STP, Cmic, and Nmic contents compared to NPKS. This finding is contradictory to Zhang *et al.* (2004) who found that microbial biomass in NPKS treatments were significantly higher than in the NPKM treatments. We think that these differences should be ascribed to different soil qualities, crop planting modes, and fertilization regimes. The increase in soil microbial biomass with the application of fertilizers resulted from greater input of root biomass from stronger crop and microorganism growth. Organic management systems and the use of organic residues have been shown to maintain soil organic matter at higher levels than inorganic fertilization (Sneh Goyal *et al.* 1999; Simek *et al.* 1999), and our data confirmed that.

The soil microbial biomass carbon is the active component of the soil organic pool. Ren and Stefano (2000) suggested that Cmic:Corg ratios obtained over long-term treatments also represent C equilibrium in the soil. And the Cmic:Corg ratios can provide an effectively early warning of the improvement or deterioration of soil quality as a result of different management practices (Powlson 1994). Thus, the changes in biomass measured over relatively short periods can indicate the trends in total organic matter content long before these can be detected by chemical

Table 4 Correlative coefficients between soil microbial properties and soil chemical properties

Correlation coefficient (*r*)	SOM	STN	STP	pH
Cmic	0.9078**	0.9378**	0.8471**	-0.9099**
Nmic	0.9172**	0.9463**	0.8660**	-0.9291**
Cmic/Corg	0.5428	0.6075	0.4316	-0.6142**
Cmic/Nmic	0.7129*	0.7566*	0.6215	-0.7498**
Urease	0.9708**	0.9797**	0.9343**	-0.9834**
Catalase	0.5873	0.5215	0.5843	-0.5345

$r_{0.05}$=0.6664, $r_{0.01}$=0.7977, n=7. * $P<0.05$, ** $P<0.01$. * and ** mean significance at 5 and 1% levels, respectively.

analysis.

Wheat straw has a higher C/N ratio compared to swine manure and maize straw. Organic matter with a high C/N ratio is only slowly degraded by microorganism. In the spring of the second year, the treatments of mineral fertilizers plus swine manure, mineral fertilizers plus wheat straw and mineral fertilizers plus maize straw increased microbial biomass compared to NPK and CK treatments. And there was a lower soil Corg in the NPKW+ treatments than in the NPKM (M+) treatments due to the high C/N ratio of the soils. In the NPKW + treatments, the Cmic:Corg ratio was the highest. It may be speculated that, in that period, the wheat straw was rapidly decomposed leading to a relatively low soil Corg and a relatively high microbial biomass.

The soil microbial biomass carbon is the early indicator of soil organic carbon, soil microbial biomass nitrogen involved in soil nitrogen mineralization. Most of the biochemical reactions involved in the soil N cycle are catalyzed by enzymes, and the primary source of soil enzymes is believed to be microorganism (Wang *et al.* 2005; Li *et al.* 2005). In this study, microbial biomass is strongly correlated to soil organic carbon and STN. It appears that microbial biomass can replace soil organic carbon and STN to evaluate soil quality. The activity of urease involved in N transformations and it was proved that urease activity had a positive correlation with soil nutrients in different fertilizer regimes. The correlation between catalase activity and soil chemical properties (SOM, STN, STP) was not obvious because the activities of catalase were blocked by ion compounds in mineral fertilizers (Zhou 1987). In conclusion, our study proved that soil microbial properties could be the indicator of soil quality in different agricultural managements. Previous studies (Parham and Deng 2000; Deng and Parham 2006; O'Donnell *et al.* 2001) pointed out that enzyme activities and biotransformation processes in soil were closely associated with the pH values in the environment. The changes in microbial and biochemical properties observed in this study were, in part, due to treatment induced by changes in soil pH. This is supported by our data which indicated that most microbiological parameters determined in the present study were significantly negatively correlated with soil pH values except of catalase activities.

CONCLUSION

Soil chemical and biochemical parameters are sensitive indicators of treatment effects on soil processes that dictate nutrient flow in agro-ecosystems. In general, the application of mineral fertilizers plus swine manure or straw increased microbiological activities, microbial biomass C and N contents, and activities of urease, and the microbial properties increased with increasing of application rates of manure and straw.

Some of soil microbial properties (Cmic contents & Nmic contents, urease activity) were positively correlated with soil chemical properties (SOM, STN, STP). There was a significantly negative correlation between the other soil microbial properties (Cmic contents & Nmic contents, Cmic/Corg, Cmic/Nmic, urease activity) and the soil pH value.

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References

Ananyeva N D, Demkina T S, Jones W J, Cabrera M L, Steen W C. 1999. Microbial biomass in soils of Russia under long-term managment practices. *Biology and Fertility of Soils*, **29**, 291-299.

Anderson J M, Ingram J S. 1993. *Tropical Soil Biology and Fertility. A Handbook of Methods*. CAB International, Wallingford, UK.

Anderson T H, Domsch K H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biology and Biochemistry*, **21**, 471-479.

Anne G L (convenor). 2002. Composition of soil microbial and fauna communities: new insight from new technologies. 17th WCSS. Abstracts. Bangkok Thailand. pp. 263-298.

Bossio D A, Fleck J A, Scow K M, Fujii R. 2006. Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biology and Biochemistry*, **38**, 1223-1233.

Brookes P C, Landman A, Pruden G, Jenkinson D S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837-842.

van Bruggen A H C, Semenov A M. 2000. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology*, **15**, 13-24.

Chander K, Goyal S, Nandal D P, Kapoor K K. 1998. Soil organic matter, microbial biomass and enzyme activities in a tropical agroforestry system. *Biology and Fertility of Soils*, **27**, 168-172.

Chen E F. 1979. *Research on the Soil Enzyme and Soil Fertility*. Science Press, Beijing. pp. 54-61. (in Chinese)

Chen G C, He Z L. 1998. Research on the microbial biomass in different red soils use manners. *Chinese Journal of Soil Science*, **29**, 276-278. (in Chinese)

Chen H K, Fan Q S. 1980. *Microbiology*. Agricultural Press, Beijing. (in Chinese)

Deng S P, Parham J A. 2006. Animal manure and anhydrous ammonia amendment alter microbial carbon use efficiency, microbial biomass, and activities of dehydrogenase and amidohydrolases in semiarid agroecosystems. *Applied Soil Ecology*, **33**, 258-268.

Dilly O, Munch J C. 1998. Ratios between estimates of microbial biomass content and microbial activity in soils. *Biology and Fertility of Soils*, **27**, 74-379.

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.

Doran J W, Parkin T B. 1994. Defining and assessing soil quality. In: Doran J W, Coleman D C, Bezdicek D F, eds, *Defining Soil Quality for a Sustainable Environment*. Soil Society of America Spetial Publication (SSSA Spec.Publ). 35. SSSA and ASA. Madison,Wisconsin. pp. 3-21.

Guan S Y. 1986. *Soil Enzyme and Study Method*. Agricultural Press, Beijing. (in Chinese)

Harris J A. 2003. Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science*, **54**, 801-808.

Jackson M L. 1973. *Soil Chemical Analysis*. Prentice Hall Inc., Englewood Cliffs, NJ.

Jenkinson D S. 1988. The determination of microbial biomass carbon and nitrogen in soil. In: *Advances in Nitrogen Cycling in Agricultural Ecosystems*. CAB International, Wallingford. pp. 368-386.

Li D P, Wu Z J, Chen L J. 2005. Influence of fertilizing modes of organic agriculture on the soil microbial activities. *Chinese Journal of Eco-Agriculture*, **13**, 99-101. (in Chinese)

Liu M Q, Hu F, He Y Q, Li H X. 2003. Seasonal dynamics of soil microbial and its microbial biomass and its significance to indicate soil quality under different vegetations restored on degraded red soils. *Acta Pedologica Sinica*, **40**, 937-944. (in Chinese)

Lovell R D, Jarvis S C, Bardgett R D. 1995. Soil microbial biomass and activity in long-term grassland: effects of management changes. *Soil Biology and Biochemistry*, **27**, 969-975.

Miller M, Dick R P. 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. *Soil Biology and Biochemistry*, **27**, 1161-1166.

Parham J A, Deng S P. 2000. Detection,quantification and characterization of β-glucosamnidase activity in soil. *Soil Biology and Biochemistry*, **32**, 1183-1190.

Parkhust C E. 1997. *Bioindicators of Soil Health*. CAB International, Oxon, United Kingdom.

Powlson D S. 1994. The soil microbial biomass: before, beyond and back. In: Ritz K, *et al.* eds, *Beyond the Biomass*. Wiley, Chichester. pp. 3-20.

Powlson D S, Brookes P C, Christensen B T. 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry*, **19**, 159-164.

Ren T Z, Stefano G. 2000. Soil bio-indicators in sustainable agriculture. *Scientia Agricultura Sinica*, **33**, 68-75. (in Chinese)

Satefanic F, Eliade G, Chirnoganu I. 1981. Researches concerning a biological index of soil fertility. In: *5th Symposium on Soil Biology*. Romanian National Society of Soil Science, Bucharest. pp. 35-45.

Schloter M, Dilly O, Munch J C. 2003. Indicators for evaluating soil quality. *Agriculture, Ecosystems and Environment*, **98**, 255-262.

Simek M, Hopkins D W, Kaláík J, Picek T, Santruckova H, Stana J, Trávnik K. 1999. Biological and chemical properties of arable soils affected by long-term organic and inorganic fertilizer applications. *Biology and Fertility of Soils*, **29**, 300-308.

Goyal S, Chander K, Mundra M C, Kapoor K K. 1999. Influence of inorganic fertilizers and organic amendments on soil o rganic matter and soil microbial properties under tropical conditions. *Biology and Fertility of Soils*, **29**, 196-200.

Sun R L, Zhao B Q. 2003. Effects of long-term fertilization on soil its role in adjusting-controlling enzyme activities and soil fertility. *Plant Nutrition and Fertilizer Science*, **9**, 406-410. (in Chinese)

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.

Wang D M, Wang C Z, Han X R. 2006. Effects of long-term application of fertilizers on some enzymatic activities in Brunisolic soil. *Chinese Journal of Soil Science*, **37**, 263-267. (in Chinese)

Wang H Y, Gong Y B, Gong W. 2005. Review on the relationships of soil fertility with soil micro organism and soil enzyme activity in the forest stand. *Sichuan Forestry Exploration and Design*, **3**, 9-14. (in Chinese)

Wang X J, Gong Z T. 1994. Assessment and prediction of soil changes under different land use patterns at a small area level in red soil hilly region. *Acta Pedologica Sinica*, **35**, 135-139. (in Chinese)

Wardle D A.1998. Controls of temporal variability of the soil microbial biomass: A global synthesis. *Soil Biology and Biochemistry*, **30**, 1627-1637.

Zhou L K. 1987. *Soil Enzymology*. Science Press, Beijing. (in Chinese).

Zhou L X, Ding M M. 2007. Soil microbial characteristics as bioindicators of soil health. *Biodiversity Science*, **15**, 162-171. (in Chinese).

Zhang H Y, Xiao Y H. 2006. Microbial biomass as an indicator for evaluation of soil fertility properties. *Chinese Journal of Soil Science*, **37**, 422-425. (in Chinese)

Zhang P J, Li L Q, Pan G X, Zhang J W. 2004. Influence of long-term fertilizer management on top soil microbial biomass and genetic diversity of a paddy soil from the Tai Lake region, China. *Acta Ecologica Sinica*, **24**, 2818-2824. (in Chinese)

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