Biomass and catabolic diversity of microbial communities with long-term restoration, bare fallow and cropping history in Chinese Mollisols

G.H. Wang[1], J. Jin[1], X.L. Chen[1], J.D. Liu[1], X.B. Liu[1], S.J. Herbert[2]

[1]*Key Laboratory of Black Soil Ecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China*
[2]*Department of Plant, Soil, and Insect Sciences, University of Massachusetts, Amherst, Massachusetts, USA*

ABSTRACT

Microbial biomass and community catabolic diversities at three depths (0–10 cm, 20–30 cm, and 40–50 cm) in Chinese Mollisols as influenced by long-term managements of natural restoration, cropping and bare fallow were investigated. Microbial biomass was estimated from chloroform fumigation-extraction and substrate-induced respiration (SIR), and catabolic diversity was determined by using Biolog® EcoPlate. Experimental results showed that microbial biomass significantly declined with soil depth in the treatments of restoration and cropping, and not in the treatment of bare fallow, where the microbial biomass had a positive relationship with the total soil C content. The inspections into the catabolic capability of the microbial community at the same soil depth showed that the treatment of natural restoration had a relatively stronger metabolic ability than the cropping and bare fallow treatments. Shannon's diversity index, substrate richness and substrate evenness calculated from the Biolog data were higher in the treatments of natural restoration and cropping than the bare fallow treatment with the same soil depth, and with the highest values in the top soil. Principal component analysis indicated that the catabolic profiles not only varied with the soil depth in each treatment, but also differed in the three treatments within the same soil depth. The catabolic profiles of the three treatments were similar to each other in the soil depth of 0–10 cm and distinctly different in the soil depths of 20–30 cm and 40–50 cm. These results suggest that it was microbial biomass rather than community function that was influenced by the different soil management in the topsoil (0–10 cm); in the relative depths, the soil microbial community function was more easily influenced than microbial biomass.

Keywords: microbial community; functional diversity; Biolog®; black soil; soil depth

The productivity of soil system is known to depend greatly upon the structure and functions of soil microbial communities, which regulate and influence many ecosystem processes such as nutrient transformation, litter decomposition, soil structure and plant health (Gallardo and Schlesinger 1994, Kennedy 1999, Zak et al. 2003, Garbeva et al. 2004). Although several researches discovered that soil microbial community structure changed under different soil managements such as crop rotation, tillage, fertilizer, manure and pesticide applications (Sigler and Turco 2002, Crecchio et al. 2004, Spedding et al. 2004, Sun et al. 2004), the link between soil microbial community structure and ecosystem function is poorly understood, because many available approaches for describing microbial communities involve large investments of time and monetary resources, and require a highly specialized expertise (Garland and Mills 1991).

Biolog® system was originally designed to identify bacterial isolates, and was found very useful to characterize soil microbial functional diversity from various environments, including soil, freshwater, sediments, activated sludge and seawater (Garland and Mills 1991, Garland 1996, Guckert et al. 1996, Choi and Dobbs 1999, Selmants et al. 2005). This method based on the multivariate

Supported by the National Science Foundation of China, Projects No. 40541004 and 40671099.

analysis of the utilization pattern of individual carbon substrates generated with commercially available 96-well Biolog microtiter plates, facilitated the classification of microbial community functional diversity. Hence, this technique provides a rapid, relatively inexpensive and highly reproducible method to measure microbial community functional diversity (Classen et al. 2003).

The black soils, classified as Mollisols, in China are primarily distributed in the northeast region of Heilongjiang and Jilin Provinces, and are one of the three largest Mollisol areas in the world (Liu et al. 2003). Although the original black soils are commonly thought to be fertile and productive, with the soil organic matter content between 5 and 8%, a part of these cultivated black soils was degraded over time due to intensive farming practices (Yu and Zhang 2004). In order to maintain or restore the fertility of the black soil, several long-term experiments involving agronomic practices were established in this region and showed that the crop yield and some physico-chemical characteristics of soil were changed with long-term fertilizing treatments (Li et al. 2004, Wang et al. 2004). However, the investigations on the effect of different treatments on soil microbial community and structural or functional diversity in black soils are limited. The objectives of this study were to determine the biomass and the catabolic diversity of soil microbial communities at different depths of a black soil as affected by three long-term treatments, namely natural restoration, bare fallow and cropping.

MATERIAL AND METHODS

Experimental sites. The study was conducted in the Hailun Agro-Ecological Experimental Station (47°26'N, 126°38'E, 240 m above sea level), Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. This research site is in the North Temperate Zone and continental monsoon area (cold and arid in winter, hot and rainy in summer) with the average annual temperature about 1.5°C and a frost-free period of 120–130 days. The annual precipitation is about 500–600 mm with 65% in June, July and August. The original vegetation is meadow grass, but when it turned to farmland, the dominating crops were soybean, maize and wheat. Before the experimental set up, the properties of the soil in the depth 0–20 cm were as follows: total C, 31.3 g/kg; total N, 3.0 g/kg; total P, 0.7 g/kg; total K, 19.6 g/kg; available N, 234.1 mg/kg; available P, 25.78 mg/kg; available K, 190.8 mg/kg; pH 6.80.

Three long-term treatments were comprised in this study. Natural restoration and cropping treatments were initiated in 1985, and bare fallow treatment was initiated in 1992. The restoration treatment was a natural fallow plot of 1000 m^2, with no human disturbance involved, and wild grasses growing and evolving naturally. The bare fallow treatment was a small plot of 77 m^2. No fertilizers were applied in this plot, and when grass emerged hand-hoeing practice was used to keep the plot in a bare fallow condition. The cropping treatment was an annual rotational cropping system of maize-soybean-wheat. The fertilizers added in this plot were NPK + farm manure, with N 127.5 kg/ha (no N fertilizer was used when the crop was soybean), P 20 kg/ha, K 60 kg/ha, farm manure 3000 kg/ha. The total area of this treatment was 224 m^2 (Liu et al. 2001).

Soil sampling and analysis. The vegetations of cropping and the natural restoration treatments were soybean and grasses, respectively. Each treatment field was divided into 3 equal parts, which represented 3 replicates. Soil samples were taken randomly in three depths (0–10, 20–30 and 40–50 cm) by using a 2.5-cm-diameter auger on June 23, 2005. Soil cores from four sites were mixed to make one sample. Field-moist soil samples were stored at 4°C for no more than 1 week before biomass determination and Biolog analysis.

Selected physical and chemical characteristics of soil samples are given in Table 1. The soil pH was measured using a glass electrode after equilibrating 10 g of dry soil with 25 ml of deionized water for 30 min. Total soil C and N were determined by Elemental analyzer (VarioEL III, Germany), and the total soil P and K were determined by colorimetric analysis and flame emission spectrometry after soil wet digestion with hydrofluoric acid, respectively (Lu 2000).

Microbial biomass. Total microbial biomass is described by microbial biomass C (MBC) and substrate-induced respiration (SIR) (Anderson and Domsch 1978). The MBC was estimated following the chloroform fumigation-extraction methods, and organic C in the extracts was determined by an automated TOC Analyzer (Shimadzu, TOC-V_{CPH}, Japan) and a K_{EC} of 0.45 was used to convert the difference between organic C extracted with 0.5M K_2SO_4 from chloroform fumigated and unfumigated soil samples to determine MBC (Vance et al. 1987). The SIR was determined on 10 g dry soil mixed with 0.2 g glucose, and moistened with

Table 1. General characteristics of the sampled soil profiles

Soil depth (cm)	Treatment	Total C (g/kg)	Total N (g/kg)	pH	Total C/N
0–10	restoration	31.32 ± 4.72	2.69 ± 0.41	6.10 ± 0.12	11.64 ± 0.02
	cropping	31.96 ± 0.03	2.72 ± 0.04	6.10 ± 0.12	11.42 ± 0.09
	bare fallow	21.08 ± 0.32	1.85 ± 0.05	6.09 ± 0.08	11.77 ± 0.10
20–30	restoration	27.90 ± 1.23	2.35 ± 0.01	6.42 ± 0.01	11.87 ± 0.37
	cropping	28.03 ± 0.62	2.38 ± 0.11	6.42 ± 0.01	12.43 ± 0.50
	bare fallow	26.11 ± 3.76	2.10 ± 0.18	6.39 ± 0.12	11.80 ± 0.19
40–50	restoration	22.11 ± 0.03	1.76 ± 0.08	6.59 ± 0.04	12.60 ± 0.28
	cropping	18.03 ± 0.21	1.48 ± 0.11	6.59 ± 0.04	12.98 ± 0.06
	bare fallow	24.02 ± 3.71	1.85 ± 0.30	6.50 ± 0.01	12.22 ± 0.52

The values are means ± standard error. $N = 3$ for each sampling depth

sterilized distilled H_2O to 40%, then incubated in 500 cm^3 soil jars fitted with rubber septa at 26°C for 2 h, the respired CO_2 was absorbed by 0.1M NaOH, and measured by titration of the NaOH solution with 0.1M HCl with phenolphthalein as the indicator dye.

Biolog method. Catabolic diversity of soil microbial diversity was determined by using Biolog ECO microplate (Biolog, Inc., CA, USA). The ECO plate contains three replicate wells of 31 carbon substrates and a control well containing no carbon substrate. Measurement of substrate metabolism in ECO plate is based on color formation from tetrazolium dye, a redox indicator. The plates were prepared as described in Classen et al. (2003). Briefly, 4 g of soil was extracted with 36 ml of 50mM K_2HPO_4 sterilized buffer adjusted to pH 6.5, and the resulting soil suspensions were shaken for 30 min on a reciprocal shaker. Ten-fold serial dilutions were made and the 10^{-3} dilution was used to inoculate the plates. A 150 μl of solution was inoculated into each well, and all plates were incubated at 25°C for 192 h and optical density at both 590 (color development plus turbidity) and 750 nm (turbidity only) were read every 24 h. The final values used to denote the activity in each well were the 590 nm values minus the 750 nm values after correction for readings in the control well at these wavelengths. Well optical density values that were negative or under 0.06 were set to zero (Classen et al. 2003).

Statistical analysis. Average well color development (AWCD) was calculated as described by Garland and Mills (1991). The final values of each well at 72 h were used to calculate Shannon's diversity index (H), substrate richness (S) and substrate evenness (E) (Schutter and Dick 2001), where $H = -\Sigma(P_i \times \ln P_i)$, and P_i is the proportional optical density value of each well, S is the number of different substrate carbon that were used by the microbial community, and $E = H/\ln S$ (Zak et al. 1994, Lupwayi et al. 1998). For the principal component analysis (PCA), the optical density value of each well at 72 h was divided by the AWCD in order to minimize the influence of inoculum density differences between plates (Garland and Mills 1991, Graham and Haynes 2005). Normalized data were analyzed by the principal component analysis (EXCEL STATISTICS 97, SRI, Tokyo Japan).

RESULTS

Microbial biomass

Total MBC and SIR rates declined significantly ($P < 0.01$) with soil depth in the treatments of natural restoration and cropping (Table 2). For the bare fallow treatment, the total MBC in soil depths of 0–10 cm and 20–30 cm was higher than that of 40–50 cm while no difference was found between the upper two layers; also the SIR rate of the three layers had no remarkable changes within the range of 12.6~14.5 μg CO_2-C/g/h. Both MBC and SIR rates were in the same order of restoration > cropping > bare fallow in the soil depth of 0–10 cm, while no significant differences were found for all three treatments in the soil depth of 20–30 cm. MBC did not vary significantly in soil depth of

Table 2. Microbial biomass as estimated by chloroform fumigation-extraction and substrate-induced respiration methods

Soil depth (cm)	Treatment	$CHCl_3$-extractable biomass C (μg/g)	SIR rate (μg CO_2–C/g/h)
0–10	restoration	1013 ± 75 a	27 ± 2 a
	cropping	610 ± 24 b	23 ± 3 b
	bare fallow	390 ± 15 c	14 ± 4 c
20–30	restoration	538 ± 78 a	13 ± 1 a
	cropping	439 ± 36 ab	15 ± 3 a
	bare fallow	400 ± 27 b	13 ± 2 a
40–50	restoration	278 ± 48 a	7 ± 1 b
	cropping	322 ± 17 a	11 ± 3 a
	bare fallow	302 ± 8 a	15 ± 4 a

The values are means ± standard error. $N = 3$ for each sampling depth. Different letters indicate a significant difference at $P = 0.05$ level among the three treatments in the same soil depth

40–50 cm, but SIR rate varied markedly with the highest value in the bare fallow treatment, and the lowest value in the restoration treatment.

Microbial community substrate utilization profile

The AWCD in the Biolog EcoPlate generally followed the same pattern with incubation time but varied for different soil samples (Figure 1). In general, the soil samples within the same soil depth from restoration treatment had the highest AWCD values and were greater than those from cropping and bare fallow treatments (Figure 1a–c). This implied that the microbial communities in the restoration treatment had a stronger metabolic activity to use the substrates. The AWCD of the same treatment showed that utilization of substrates by microbial communities at different soil depths varied significantly. The order of AWCD values for different soil depths was 20–30 cm > 0–10 cm > 40–50 cm in the treatment of restoration (Figure 1d), and 0–10 cm > 20–30 cm > 40–50 cm

Table 3. Effect of long-term restoration, bare fallow and cropping on soil microbial community catabolic diversity as evaluated by the Shannon's diversity index (H), substrate richness (S) and substrate evenness (E) in the Biolog EcoPlate incubated for 72 h

Soil depth (cm)	Treatment	H	S	E
0–10	restoration	2.81 ± 0.11 a	20.00 ± 2.00 a	0.94 ± 0.01 a
	cropping	2.83 ± 0.12 a	21.00 ± 2.65 a	0.93 ± 0.00 a
	bare fallow	2.64 ± 0.25 a	19.00 ± 4.58 a	0.90 ± 0.01 b
20–30	restoration	2.85 ± 0.14 a	21.33 ± 2.89 a	0.93 ± 0.00 a
	cropping	2.52 ± 0.13 b	15.00 ± 1.73 b	0.93 ± 0.01 a
	bare fallow	2.33 ± 0.39 b	14.00 ± 4.58 b	0.89 ± 0.03 b
40–50	restoration	2.59 ± 0.04 a	16.67 ± 1.15 a	0.92 ± 0.01 a
	cropping	2.55 ± 0.09 a	15.67 ± 1.15 a	0.93 ± 0.03 a
	bare fallow	2.28 ± 0.22 b	12.67 ± 3.21 b	0.91 ± 0.02 a

The values are means ± standard error. $N = 3$ for each sampling depth. Different letters indicate a significant difference at $P = 0.05$ level among the three treatments in the same soil depth

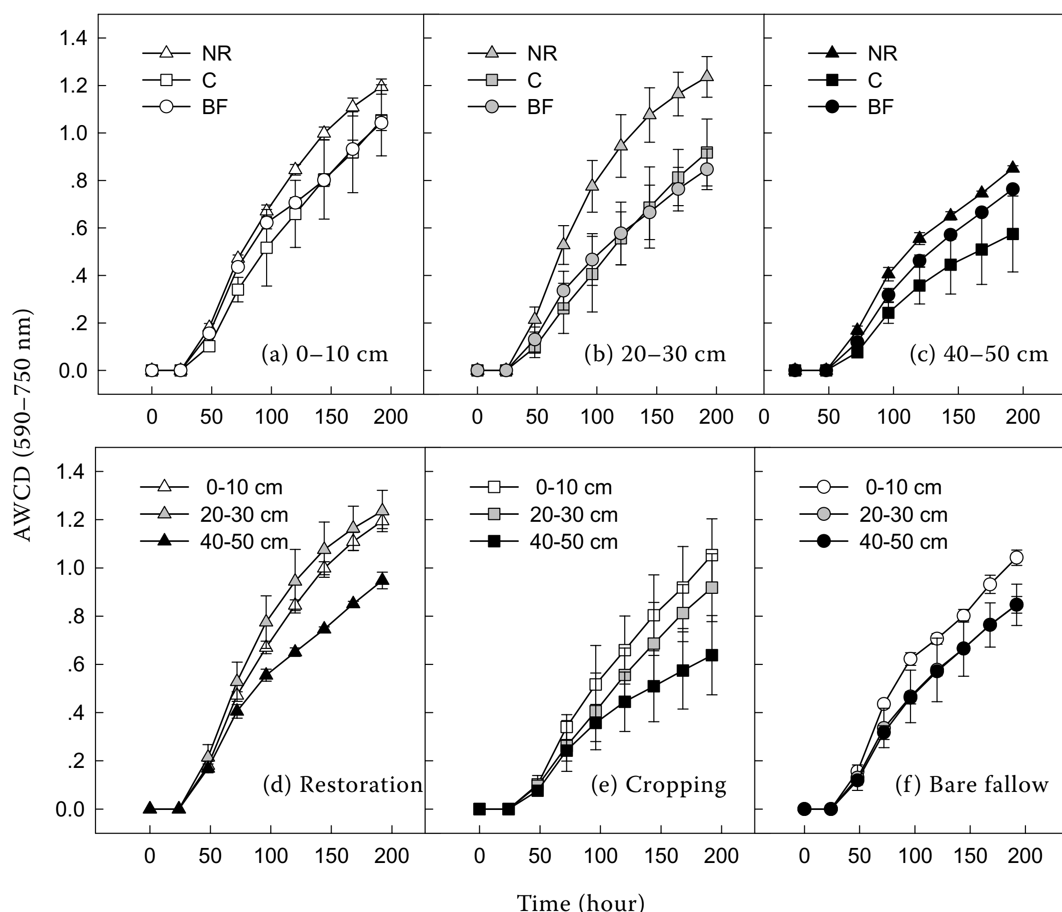

Figure 1. Average well color development (590–750 nm) for the tested soils. NR, C and BF represent the treatment of natural restoration, cropping and bare fallow, respectively

in the treatment of cropping (Figure 1e), but they were the highest in the soil depth of 0–10 cm and similar for both soil depths of 20–30 cm and 40–50 cm in the bare fallow (Figure 1f).

To further compare the catabolic diversity among different treatments, Shannon's diversity index (*H*), substrate richness (*S*) and substrate evenness (*E*) in the incubation time of 72 h are shown in Table 3. In general, diversity, substrate richness and evenness in the treatments of restoration and cropping were higher than those in the treatment with bare fallow in the same soil depth. For the restoration treatment, there were no significant changes in diversity, richness and evenness between the soil depths of 0–10 cm and 20–30 cm, but there was a significant decrease in the soil depth of 40–50 cm. However, for cropping and bare fallow treatments, higher diversity and richness were observed in the top 0–10 cm soil, and no significant difference was found between the 20–30 cm and 40–50 cm soil depths. As for substrate evenness, no greater change was observed among soil samples of the three depths in the treatments of cropping and bare fallow, either.

Principal component analysis of Biolog data

To distinguish the effects of the three treatments on soil microbial catabolic diversity, separate PCAs for the three treatments at the same soil depth, and for the three soil depths in the same treatment were performed (Figure 2). The PCA results showed that the carbon substrate utilizing profiles were clearly separated among different treatments (Figure 2a–c) and different soil depths (Figure 2d–f). In the top 0–10 cm soil, the catabolic profiles of the three treatments were similar, but were more concentrative in the restoration treatment (Figure 2a). However, in the depths of 20–30 cm and 40–50 cm, the catabolic profiles of the restoration treatment and bare fallow treatment were separated into two distinct groups, which implied that the soil microbial community functional diversities differed between these two treatments. For the cropping treatment, the catabolic profiles of the three replicates were dispersed, which suggested that individual replicates in this treatment had a relatively different microbial community

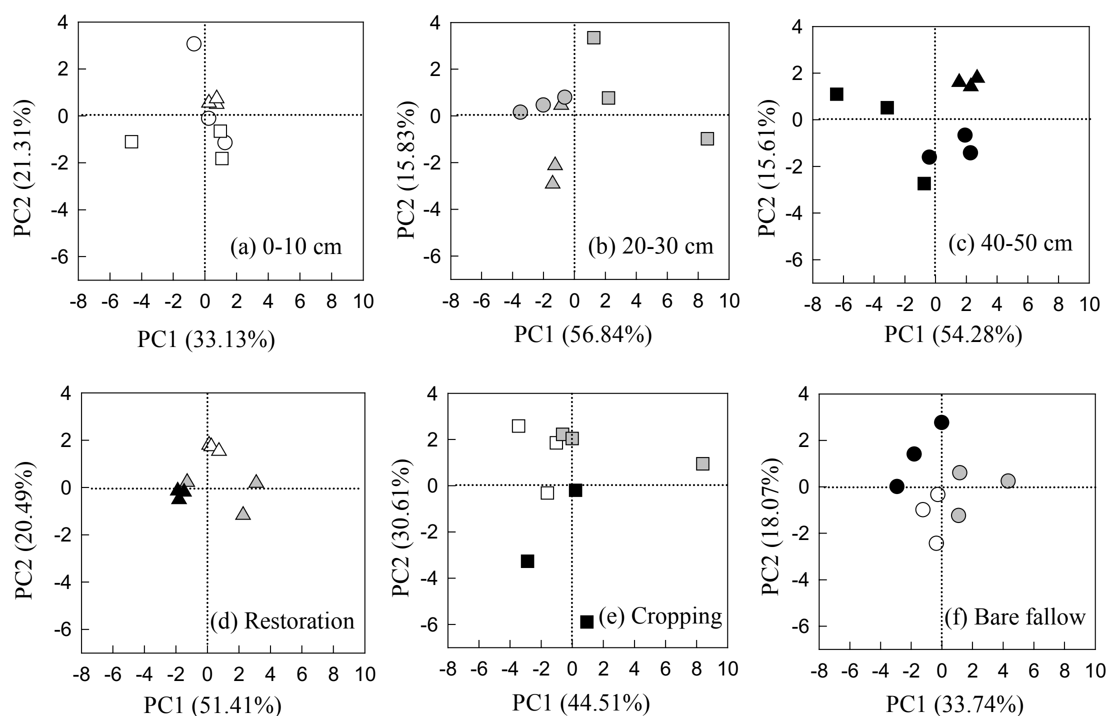

Figure 2. Principal component analysis (PCA) of Biolog Ecoplate data incubated for 72 h from soil samples of different depths in the treatment of restoration, cropping and bare fallow. The symbols of triangle, square and circle represent the treatment of natural restoration (NR), cropping (C) and bare fallow (BF), respectively; and the symbol's colors – none, grey and black – represent the soil samples collected from the depth of 0–10 cm, 20–30 cm and 40–50 cm, respectively

function with those in restoration and bare fallow treatments (Figure 2b, c).

The catabolic profile of soil microbial community in different treatments also varied with the soil depth (Figure 2d–f). In the treatment of restoration, the catabolic profile of one soil sample from the depth of 20–30 cm was dispersed from the other two samples; nevertheless the carbon utilizing profiles of the three different soil layers were separated significantly (Figure 2d). Similar to the restoration treatment, a clear differentiation of catabolic profiles was observed in all three depths in the treatment of bare fallow (Figure 2f). However, in the treatment of cropping, the catabolic profiles of four soil samples for the soil depths of 0–10 cm and 20–30 cm were similar, and distinctly different from the profile of 40–50 cm soil depth (Figure 2e).

DISCUSSION

Soil microbial biomass is defined as an indicator of soil fertility and quality, which is easily influenced by soil managements (Pankhurst et al. 1995). Wang et al. (2004) reported that an application of chemical fertilizers (NPK) increased the black soil MBC compared with no fertilization, and reached the same level as the natural restoration in soil layer of 0–20 cm. However, at present research, the highest MBC or SIR rates in the topsoil layer were detected in the restoration treatment; the reason for this difference may be related with different experimental location. The annual temperature of Wang's experimental location is 3.5-4.5°C higher than our research field, which suggests that the decomposition processing of plant debris in our research field is slower, and more organic matters are accumulated in topsoil in the restoration treatment for microbe use. In contrast with the cropping and natural restoration treatments, there was no plant growth in the treatment of bare fallow, which implied no exterior C sources added into the soil system. Additionally, the activity of the top microbial community was stronger than in deep soil (Figure 1f), which implies that more soil C was lost by microbial respiration in topsoil (Table 1), and the lowest MBC was detected in topsoil of the bare fallow treatment.

It is well established that MBC content decreased with increasing soil depth (Taylor et al. 2002, Fierer

et al. 2003, Agnelli et al. 2004). In this study, however, this tendency was only demonstrated in the treatments of restoration and cropping, and not in the bare fallow treatment (Table 2). The reason for this may be related to the soil total C content in the bare fallow treatment which was not changed significantly with depth; the regression results suggest that the microbial biomass estimated by the two methods was well correlated with the total soil C content (Figure 3). This relationship between microbial biomass and soil C content is consistent with other reports (Hu et al. 1999, Peacock et al. 2001).

Although the Biolog method was criticized as little more than a culturing technique (Preston-Mafham et al. 2002), it still remains a rapid and valuable tool in investigating the catabolic diversity of the soil microbial community (Schutter and Dick 2001, Graham and Haynes 2005). Previous researches showed that the difference in AWCD could be explained partly by lower microbial biomass (Garland 1997, Grayston et al. 1998). In this study, however, AWCD values in plates were not correlated with microbial biomass, which is inconsistent with other reports (Bååth et al. 1998, Yao et al. 2003). For example, the microbial biomass of

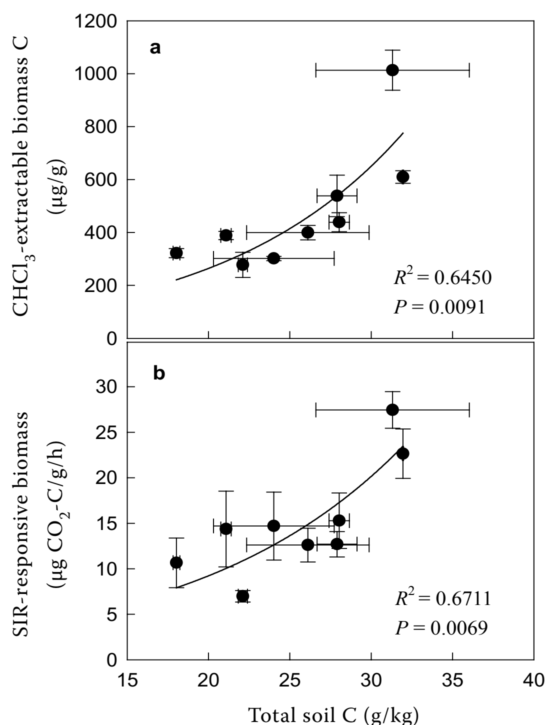

Figure 3. The relationships between total soil C content of black soil and biomass estimated by chloroform fumigation-extraction (a) and SIR (b) for all samples

the 20–30 cm soil depth in the restoration treatment was lower than that of the topsoil, but the AWCD value was still higher during the incubation time. This result implies that the composition of soil microbial community was altered by the long period of different soil management.

Soil microbial community structure is affected by many agricultural practices (Olsson and Alström 2000, Feng et al. 2003, Sun et al. 2004, Dilly et al. 2004). In our study, the microbial biomass of the 0–10 cm soil depth in the bare fallow treatment was significantly lower than that in the treatments of restoration and cropping; the Shannon's diversity index (H) and substrate richness (S) were similar, which suggests that the changes in population and catabolic diversity of microbial community were not correlated (Tables 2 and 3).

Our general hypothesis was that when compared to the relative deeper soil, the topsoil would show significant changes in microbial biomass and catabolic activity; contrary to this, our work indicated that the catabolic profile of the three treatments were similar in the topsoil and different in deeper soil (Figure 2a–c). Blume et al. (2002), using fatty acid methylester (FAME) analysis, observed that the microbial community structure changed seasonally in the subsurface soil, and not in the surface soil. They attributed their results to the higher organic C and microbial biomass in the surface soil, which may enhance the microbial ability to withstand environmental perturbations. Based on their explanations, we presume our results may be caused by the diversity index (H) and substrate richness (S) being lower in deep soil than those in the top soil, especially in the cropping and bare fallow treatments (Table 3), which indicates that the catabolism of microbial community in deeper soil might be easily altered by the long-term soil treatments.

In addition, it is worth mentioning that the microbial community functional diversity in the cropping treatment was similar in soil depths of 0–10 cm and 20–30 cm, and different in 40–50 cm; we suppose that it may be related to the tillage practice in this treatment that disturbed the soil profile and made the microbial community structure homogeneous within the top soil layers.

To conclude, in our research we compared the impact of three long-term black soil treatments on SMB and microbial catabolic diversity by Biolog EcoPlate, and the results indicate that SMB in the top soil layer is more influenced by the three different treatments than in deeper soil layers; however, the soil microbial catabolic profiles were similar in all the treatments in top soil layer and

in deeper soil layers. We thus presume that it was microbial biomass rather than community function that was influenced by different soil management in the topsoil. It is however necessary to perform further experiments to reveal the soil microbial community composition as influenced by the three treatments, by using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) or phospholipid fatty acid (PLFA) methods; for the Biolog MicroPlates showed to primarily select a small part of the total community largely made up of fast-growing microbes, and not the original microbial communities from which the samples were obtained (Smalla et al. 1998, Graham and Haynes 2005).

REFERENCES

Agnelli A., Ascher J., Corti G., Ceccherini M.T., Nannipieri P., Pietramellara G. (2004): Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. Soil Biol. Biochem., *36*: 859–868.

Anderson J.P.E., Domsch K.H. (1978): A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem., *10*: 215–221.

Bååth E., Diaz-Ravina M., Frostegård A., Campbell C.D. (1998): Effect of metal-rich sludge amendments on the soil microbial community. Appl. Environ. Microbiol., *64*: 238–245.

Blume E., Bischoff M., Reichert J.M., Moorman T., Konopka A., Turco R.F. (2002): Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Appl. Soil Ecol., *20*: 171–188.

Choi K.-H., Dobbs F.C. (1999): Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. J. Microbiol. Meth., *36*: 203–213.

Classen A.T., Boyle S.I., Haskins K.E., Overby S.T., Hart S.C. (2003): Community-level physiological profiles of bacteria and fungi: plate type and incubation temperature influences on contrasting soil. FEMS Microbiol. Ecol., *44*: 319–328.

Crecchio C., Curci M., Pizzigallo M.D.R., Ricciuti P., Ruggiero P. (2004): Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. Soil Biol. Biochem., *36*: 1595–1605.

Dilly O., Bloem J., Vos A., Munch J.C. (2004): Bacterial diversity in agricultural soils during litter decomposition. Appl. Environ. Microbiol., *70*: 468–474.

Feng Y., Motta A.C., Reeves D.W., Burmester C.H., vas Santen E., Osborne J.A. (2003): Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biol. Biochem., *35*: 1693–1703.

Fierer N., Schimel J.P., Holden P.A. (2003): Variation in microbial community composition through two soil depth profiles. Soil Biol. Biochem., *35*: 167–176.

Gallardo A., Schlesinger W.H. (1994): Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biol. Biochem., *26*: 1409–1415.

Garbeva P., Veen J.A.V., Elsas J.D.V. (2004): Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu. Rev. Phytopathol., *42*: 243–270.

Garland J.L. (1996): Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. Soil Biol. Biochem., *28*: 2351–2359.

Garland J.L. (1997): Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol., *24*: 289–230.

Garland J.L., Mills A.L. (1991): Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole carbon source utilization. Appl. Environ. Ecol., *57*: 2351–2359.

Graham M.H., Haynes R.J. (2005): Catabolic diversity of soil microbial communities under sugarcane and other land uses estimated by Biolog and substrate-induced respiration methods. Appl. Soil Ecol., *29*: 155–164.

Grayston S.J., Wang S., Campbell C.D., Edwards A.C. (1998): Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol. Biochem., *30*: 369–378.

Guckert J.B., Carr G.J., Johnson T.D., Hamm B.G., Davidson D.H., Kumagai Y. (1996): Community analysis by Biolog: curve integration for statistical analysis of activated sludge microbial habitats. J. Microbiol. Meth., *27*: 183–197.

Hu S.J., Van Bruggen A.H.C., Grünwald N.J. (1999): Dynamics of bacterial populations in relation to carbon availability in a residue amended soil. Appl. Soil Ecol., *13*: 21–30.

Kennedy A.C. (1999): Bacterial diversity in agroecosystems. Agr. Ecosys. Environ., *74*: 65–76.

Li D.P., Wu Z.J., Chen L.J., Zhu P., Ren J., Liang C.H. (2004): Dynamics of phosphates activity and influencing factors in black soil under long-term fertilization. Plant Nutr. Fertil. Sci., *10*: 550–553. (In Chinese)

Liu H.X., Wang D.L., Wang S.Y., Meng K., Han X.Z., Zhang L., Shen S.M. (2001): Changes of crop yields

and soil fertility under long-term application of fertilizer and recycled nutrients in manure on a black soil. Chinese J. Appl. Ecol., *12*: 43–46. (In Chinese)

Liu X.B., Han X.Z., Song C.Y., Herbert S. J., Xing B. (2003): Soil organic carbon dynamics in black soil of China under different agricultural management systems. Commun. Soil Sci. Plant Anal., *34*: 973–984.

Lu R.K. (2000): Analysis Methods of Soil Agricultural Chemistry. Beijing, China, Agricultural Science and Technology Press: 231–232. (In Chinese)

Lupwayi N.Z., Rice W.A., Clayton G.W. (1998): Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. Soil Biol. Biochem., *30*: 1733–1741.

Olsson S., Alström S. (2000): Characterisation of bacteria in soils under barley monoculture and crop rotation. Soil Biol. Biochem., *32*: 1443–1451.

Pankhurst C.E., Hawke B.G., McDonald H.J., Buckerfield J.C., Michellsen P., O'Brien K.A., Gupta V.V.S.R., Doube B.M. (1995): Evaluation of soil biological properties as potential bioindicators of soil health. Aust. J. Exp. Agr., *35*: 1015–1028.

Peacock A.D., Mullen M.D., Ringelberg D.B., Tyler D.D., Hedrick D.B., Gale P.M., White D.C. (2001): Soil microbial community responses to dairy manure or ammonium nitrate applications. Soil Biol. Biochem., *33*: 1011–1019.

Preston-Mafham J., Boddy L., Randerson P.F. (2002): Analysis of microbial community functional diversity using sole-carbon-source utilization profiles-a critique. FEMS Microbiol. Ecol., *42*: 1–14.

Schutter M., Dick R. (2001): Shifts in substrate utilization potential and structure of soil microbial communities in response to carbon substrates. Soil Biol. Biochem., *33*: 1481–1491.

Selmants P.C., Hart S.C., Boyle S.I., Stark J.M. (2005): Red alder (*Alnus rubra*) alters community-level soil microbial function in conifer forests of the Pacific Northwest, USA. Soil Biol. Biochem., *37*: 1860–1868.

Sigler W.V., Turco R.F. (2002): The impact of chlorothalonil application on soil bacterial and fungal populations as assessed by denaturing gradient gel electrophoresis. Appl. Soil Ecol., *21*: 107–118.

Smalla K., Wachtendorf U., Heuer H., Liu W.-T., Forney L. (1998): Analysis of Biolog GN substrate utilization patterns by microbial communities. Appl. Environ. Microbiol., *64*: 1220–1225.

Spedding T.A., Hamel C., Mehugs G.R., Madramootoo C.A. (2004): Soil microbial dynamics maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem., *36*: 499–512.

Sun H.Y., Deng S.P., Raun W.R. (2004): Bacterial community structure and diversity in a century-old manure-treated agroecosystem. Appl. Environ. Microbiol., *70*: 5868–5874.

Taylor J.P., Wilson B., Mills M.S., Burns R.G. (2002): Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques. Soil Biol. Biochem., *34*: 387–401.

Vance E.D., Brooks P.C., Jenkinson D.S. (1987): An extraction method for measuring microbial biomass C. Soil Biol. Biochem., *19*: 703–707.

Wang J., Xie H.T., Zhang X.D. (2004): Effect of fertilization on soil microbial biologic carbon in black soil. Chinese J. Eco-Agr., *12*: 118–120. (In Chinese)

Yao H., Xu J., Huang C. (2003): Substrate utilization pattern, biomass and activity of microbial communities in a sequence of heavy metal-polluted paddy soil. Geoderma, *115*: 139–148.

Yu L., Zhang B. (2004): The degradation situation of black soil in China and its prevention and countermeasures. J. Arid Land Res. Environ., *18*: 99–103.

Zak D.R., Holmes W.E., White D.C., Peacock A.D., Tilman D. (2003): Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology, *84*: 2042–2050.

Zak J.C., Willig M.R., Moorhead D.L., Wildman H.G. (1994): Functional diversity of microbial communities: a quantitative approach. Soil Biol. Biochem., *26*: 1101–1108.

Received on October 26, 2006

Corresponding author:

Prof. Guanghua Wang, Ph.D., Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, 138 Haping Road, Harbin, 150081 PR China
phone: 86 451 8660 2745, fax: 86 451 8660 3736, e-mail: guanghuawang@hotmail.com