Advances in Environmental Biology, 2(1): 1-8, 2008
ISSN 1995-0756


This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

# Changes in Soil Microbial Biomass and Bacterial Community in a Long-term Fertilization Experiment During the Growth of Maize

[1,3]Fengping Li, [1]Wenju Liang, [1]Xiaoke Zhang, [1]Yong Jiang and [2]Jingkuan Wang

[1]*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.*
[2]*College of Land and Environment, Shenyang Agricultural University, Shenyang 110161, China*
[3]*Graduate School of the Chinese Academy of Sciences, Beijing 100049, China.*

Fengping Li, Wenju Liang, Xiaoke Zhang, Yong Jiang and Jingkuan WAng,: Changes in Soil Microbial Biomass and Bacterial Community in a Long-term Fertilization Experiment During the Growth of Maize, *Adv. Environ. Biol., 2(1): 1-8, 2008*

## ABSTRACT

The present study was conducted in order to determine the effects of long-term fertilization (*i.e.*, no-fertilizer (CK), N and P fertilizers (NP), manure (M) and manure with N and P fertilizers (MNP)) on the soil microbial biomass C and bacterial community during the growing stages of maize in Northeast China. The dynamics of soil bacterial community was detected via CFU enumeration and DGGE techniques. Principal component analysis (PCA) of DGGE profiles showed significant changes in bacterial diversity that were related to different treatments and growing stages, and significant interaction was also observed between treatments and sampling times for this descriptor. Moreover, two-way ANOVA showed that the changes in microbial biomass C over time were significant, whereas no significant fertilization effects on this parameter were detected throughout the study period except for the seedling stage. Bacterial counts exhibited a significant response to different amendment, while no significant time-related variation along the phenological development of maize. In our study, many factors such as addition of inorganic and organic matter, crop growth stage, and soil chemical conditions were found to affect the soil microbial characteristics. These factors should be considered when using soil microorganisms as a bioindicator of soil quality.

***Key words:*** Bacterial community, soil microbial biomass, long-term fertilization, PCR-DGGE, growing stage of maize

## Introduction

In agricultural soils, microorganisms are known to exert profound influences on the status of soil fertility, in particular on the availability of plant nutrients[21], and play an important role in nitrogen cycling, nitrogen fixation and mineralization processes in all ecosystems[26]. Soil fertilizer amendments and yearly applications can cause changes in the physical, chemical, and biological properties of soils[35]. Clegg *et al.*[9] studied the impact of long-term grassland management regimes with nitrogen-fertilizer application and soil drainage on the microbial community structure and found that nitrogen fertilizer exerted a significant impact on the total bacterial and actinomycete community structures, whereas soil drainage had a significant impact on the actinomycete and pseudomonad communities. Applying organic amendments has been shown to increase soil microbial activity[23], microbial diversity[16,17], and bacterial densities[36]. Gelsomino *et al.*[15] compared organic and conventional agricultural systems through examining their effects on soil microbial biomass, microbial

**Corresponding Author**

Wenju Liang, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.
E-mail: liangwj@iae.ac.cn

activity and substrate utilization and documented an enhancement of microbial biomass in the plots with organic amendments.

Earlier publications reported that the effects of seasonal fluctuations on soil microorganisms are comparable with or larger than those of management practices[2,6]. Temporal changes in soil microbial communities are likely due, in part, to plant root growth as well as environmental conditions, such as soil moisture and temperature[12]. During the growth phase, the plants use main carbon income to produce leaves, which can increase the carbon gain of the plants. During and after plant flowering, the allocation of photosynthates switches to favor reproductive organs[22]. Plant phenology has been determined to influence carbon translocation and the quality of exudates released in the rhizosphere[18]. Bacterial community structure differed in densities, metabolic potentialities, and genetic structure according to maize development stages and root locations[3].

The soil microbial biomass is fundamental to maintaining soil functions because it represents the main source of soil enzymes that regulate transformation processes of elements in soils[5], and it has been suggested as possible indicator of soil environment quality, and is employed in national and international monitoring programs. Season trends in microbial biomass are not well understood[34]. Microbial biomass C is often closely related to organic matter, and soil organic materials is also altered with floristic composition, plant phenology and soil fertility[29,39].

The traditional method used to analyze soil microbial communities has been serial dilution and culturing of samples on various selective media. A major limitation of this method is that only a small portion (lower than 10%) of the soil microbial population can be cultured on laboratory media[1]. Culture-independent molecular techniques used in soil microbial ecology studies have brought new insights into the community structure and dynamics of soil microorganisms in agricultural fields[10,20,33]. In particular, the use of denaturing gradient gel electrophoresis (DGGE) technique to analyze microbial DNA extracted directly from a wide range of habitats has allowed the assessment of microbial diversity, including microbial lineages unknown for pure cultures.

The aim of this study was to determine the long-term fertilization effects on the temporal dynamics of the soil microbiological properties as well as soil abiotic features in a maize field. Both traditional and molecular methods were applied to analyze microbial community during different growing stages of maize in order to assess the potential drifts in the mass, density and diversity of soil microbial communities.

## Materials and methods

### *Experimental design and soil sampling*

This study was undertaken at the experimental field of Shenyang Agriculture University, Northeast China (41°50 N, 121°34 E). The site is located in a continental monsoon zone with a mean annual temperature 7.0–7.9°C, and a mean annual precipitation of 705.4 mm[19]. The test soil is classified as a Hapli-Udic Argosol (Cambisoil, FAO-UNESCO) in Chinese soil taxonomy. Twelve experimental plots (each 69 $m^2$) had been ridge sown with monoculture maize (*Zea mays* L.) in a conventional tillage system since 1987. Four long-term fertilization treatments, *i.e.*, no-fertilizer (CK), N and P fertilizers (NP), manure (M) and manure with N and P fertilizers (MNP) with four replicates were applied. Before maize sowing (April 25) each plot received fertilization with NP (application of N plus P fertilizers, 135 kg N · $ha^{-1}$, 67.5 kg P · $ha^{-1}$), M (application of manure, 135 kg N · $ha^{-1}$), or MNP (combined application of N plus P fertilizers with manure, 135 kg N · $ha^{-1}$, 67.5 kg P · $ha^{-1}$), respectively, except CK. Soil samples from the 0-20 cm layer were collected from each plot with four replications at four growth stages of maize: May 24 (seedling stage), June 26 (jointing stage), July 25 (booting stage) and September 22 (ripening stage), 2006. Each sample, comprised of five soil cores, was placed in an individual plastic bag and taken to the laboratory and sieved (2 mm sieve) in order to remove organic particles and keep at 4°C or -80°C until chemical and biological analyses.

### *Soil chemical analysis*

Soil organic C was measured by TOC 5000 analyzer (Shimadzu, Kyoto), and soil total N was determined by Kjeldahl digestion[25]. Soil available N was converted to $NH_4^+$ under alkaline conditions, collected in a $H_3BO_3$ solution and subsequently determined by titration with standard 0.01 mM $H_2SO_4$ [38]. For measurement of total soil P, soils were digested first by a mixed acid solution of $H_2SO_4$ and $HClO_4$ and then analyzed using the molybdophosphate method. Available soil P was determined by the Olsen method[27]. Soil pH was measured in a 1:2.5 (soil: water) slurry using a glass electrode. Soil moisture was determined gravimetrically by drying at 105°C and weighing.

### *Soil microbial biomass*

Soil microbial biomass was determined using a chloroform fumigation incubation (CFI) assay, according to Vance and Jenkinson[37]. Fumigated and non-fumigated soil samples were extracted with

$K_2SO_4$ and analyzed by a TOC 5050 (Shimadzu, Kyoto). The C obtained from fumigated samples minus that from non-fumigated samples was taken to represent the microbial biomass C flush, and was converted into microbial biomass C using the relationship: microbial $C_{mic}$= 1/0.38 C flush[31].

*Bacterial density analysis*

CFU enumeration of bacteria was carried out as follows: 10 g fresh soil was homogenized in 90 µl sterilized water, ten-fold serial dilutions were performed and 100 µl aliquots were spread onto beef extract agar media. The plates were incubated at 37°C and counted after 2 days[41].

*Soil DNA extraction*

Soil community DNA extraction was undertaken as follow: 1 g soil stored at -80°C was mixed with the extraction buffer (100 mM Tris, 100 mM EDTA and 1.5 M NaCl; pH 8.0) containing lysozyme in centrifuge tubes. The samples were shaken at 180 rpm and 37°C for 2 h. Then, 220 µl SDS (20%) was added to the tubes and the samples were incubated at 65°C for 1 h. After centrifugation at 8,000 rpm, the supernatants were collected. The aqueous phase was extracted with a solution of phenol-chloroform-isoamyl alcohol (25:24:1). Isopropanol was then added to precipitate the DNA and the samples were centrifuged (14,000 rpm) and the DNA pellets suspended in 50 µl TE (pH 8.0).

*Amplification of 16S rDNA*

Amplification of the bacterial 16S rRNA gene sequence was performed using the primer pairs F357 GC/ R518. A GC-rich sequence was added to the 5 end of primer F357 in order to prevent complete melting during the separation in the denaturant gradient. DNA amplification was performed using a 'touchdown' PCR[14] in order to reduce the formation of spurious by-products. During the touchdown PCR the annealing temperature, which was initially set at 10°C above the expected annealing temperature (65°C), was decreased by 2°C every second cycle until a touchdown of 55°C, at which temperature 25 additional cycles were carried out. PCR running conditions were: an initial denaturing step at 94°C for 3 min followed by 35 thermal cycles consisting of 1 min of denaturation at 94°C, 1 min for primer annealing at the appropriate temperature, and 2 min at 72°C for primer extension. Cycling was followed by a final extension step at 72°C for 10 min and cooling to 4°C.

*DGGE analysis*

PCR-amplified 16S rDNA fragments were community fingerprinted using a DCode™ Universal Mutation Detection System (Bio-Rad). PCR products (40 µl) with two replications of each treatment were loaded onto 8% (w/v) acrylamide gels containing a linear chemical gradient ranging from 30% to 70% denaturant (100% denaturant corresponds to 7 M urea plus 40%, v/v, deionized formamide). The electrophoresis was run in a 1×TAE buffer at 60°C and a constant voltage of 180 V for 6 h. After the run the gels were stained with genefinder™ (www.biov.cn) and photographed, scanned, and analyzed with a Molecular Imager FX (Bio-Rad). Banding patterns of DGGE profiles were analyzed by Quantity One 4.2.3 software (Bio-Rad). Community structure based on relative band intensity and position were analyzed by performing principal component analysis[24].

*Statistical analysis*

Data obtained by microbial biomass and bacterial density analyses with four replicates were subjected to statistical (two-way univariate) analysis of variance using the SPSS statistical package. Differences with $P<0.05$ were considered significant. Date obtained by DGGE was interpreted by principal component analysis (PCA) using SPSS. With this method, the number of variables of 16S rDNA bands was reduced to a few axes (PCs), and the first two PCs were subsequently plotted to visualize the results.

**Results and discussions**

*Changes in soil nutrients*

Total organic C values ranged between 0.84% and 0.96 % (Table 1), where only the soil samples collected under the manure (M) treatment were found to be significantly higher than in the other treatments. A similar trend with significant increases was obtained for total N values in soil samples in M and CK plots. However, no significant differences were obtained for Alkali N between treatments. Total P and Olsen P were found to be significantly higher in the M and MNP treatments than in the control and NP treatments. Soil pH showed lower values in NP and MNP plots than in control and M plots (Table 1).

*Changes in soil moisture*

Soil moisture was found to be significantly higher in the seedling stage (range 17.8%~19.5%), and the ripening stage (range 17.6%~19.5%) than in the jointing and booting stages (range 13.4%~15.5%) in all treatments. Significant differences were observed between different treatments or different sampling times, and significant interaction between treatment and time also existed during the study period. A higher soil moisture value of 19.5% was observed at the seedling stage under the MNP treatment, with a similar value observed at the ripening stage under the CK

**Table 1:** Soil chemical properties under different fertilization treatments (mean ± SD)

| Treatment | TOC(%) | Total N(%) | Total P (%) | Alkli N( mg/kg) | Olsen P (mg/kg) | pH |
|---|---|---|---|---|---|---|
| CK | 0.84 ± 0.01 a | 0.12 ± 0.02 b | 0.04 ± 0.00 a | 14.1 ± 3.7 a | 15.7 ± 2.0 a | 5.50 ± 0.08 c |
| NP | 0.85 ± 0.03 a | 0.10 ± 0.06 a | 0.03 ± 0.02 a | 18.0 ± 8.6 a | 29.4 ± 2.7 a | 5.01 ± 0.04 a |
| M | 0.96 ± 0.04 b | 0.13 ± 0.02 b | 0.06 ± 0.01 b | 13.6 ± 12.3 a | 83.9 ± 20.7 b | 5.51 ± 0.08 c |
| MNP | 0.84 ± 0.02 a | 0.11 ± 0.01 a | 0.06 ± 0.01 b | 10.7 ± 6.3 a | 66.3 ± 9.6 b | 5.20 ± 0.06 b |

CK: no-fertilizer; NP: N and P fertilizers; M: manure; MNP: manure with N and P fertilizers. Data followed by the diffrent latters in a column are significantaly different ($P < 0.05$)

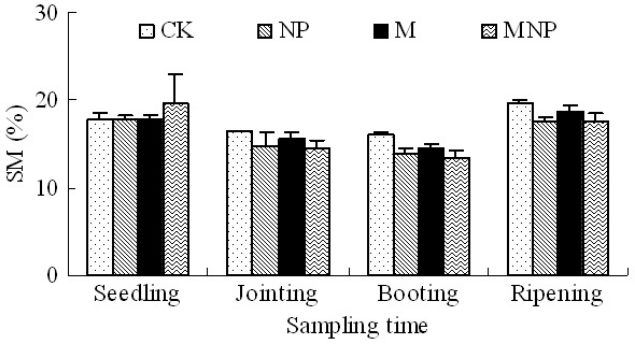


**Fig. 1:** Soil moisture under different treatments during the growing season of maize.

treatment. In both of the above cases the soil moisture levels were found to be significantly higher compared to the other treatments over times, except for soil samples collected under the M treatment at ripening stage (Fig. 1).

*Changes in soil microbial biomass*

Soil microbial biomass C in the soil samples collected during the seedling stage exhibited a significant treatment effect, where the mean value under the MNP treatment reached a maximum value of 334.6 μg C · $g^{-1}$ soil, a pattern that continued to yield a significant difference between the M and NP amendments and the control sampling sites with a minimum value of 125.7 μg C · $g^{-1}$ soil (Fig. 2). No significant amendment effects were observed at the jointing, booting and ripening stages, and the influence of M amendment was found to increase the soil microbial biomass throughout growth of maize. Toward the last two phenological stages, the microbial biomass in the NP and MNP plots decreased between 1.5 and 2-fold (Fig. 2).

*Changes in bacterial density*

The mean soil cultivable bacteria colony reached the highest value of 168 ×$10^5$ CFU/g dry soil and 172 ×$10^5$ CFU · $g^{-1}$ dry soil in the seedling stage with the M and MNP treatments, yielding a significant increase with the two treatments in this stage (Fig. 3). No significant change was found between treatments in the last three (jointing, booting and ripening) stages. The influence of MNP amendment was found to increase soil cultivable bacteria during all stages, while M amendment showed enhancement effects during all stages except for the booting stage (Fig. 3). During the last three stages, soil bacterial counts in the M and MNP plots decreased between 1.4 and 4-fold.

*Changes in bacterial diversity*

The DGGE fingerprints of the 16S rDNA fragments from the bacterial community were reproducible when gels were run on different occasions and with amplicons from different sets of PCRs (data not shown). For all sampling times and treatments, 28 different bands positions were observed with 20–26 bands per PCR-product. Within one treatment, the four sampling times displayed different DGGE-patterns, with a similar trend observed among four treatments within one sampling time. Subsequently, DGGE gels were interpreted using principal component analysis in which data were transformed in two ways, taking into account either the relative intensity or the presence of bands. Using the intensity data, the first two principal components (PC1 and PC2) were sufficient to explain near 60% of the variance. DGGE-profiles of different treatments and times were very diverse over the experiment period as shown by forming open clusters in PCA plots (Fig. 4).

*Discussion*

Zak and Tilman[40] elucidated the importance of soil organic C, total N, total P, alkaline N and Olsen P, which were found to be higher in manure (M) amendments, with a significant effect on the composition and quantity of the soil microbial community, a trend that was also observed in the present study at the seedling stage of maize. Furthermore, the mixture of M and NP was found to enhance soil microbial populations differently than the NP amendment. Enwall *et al.*[11] reported that

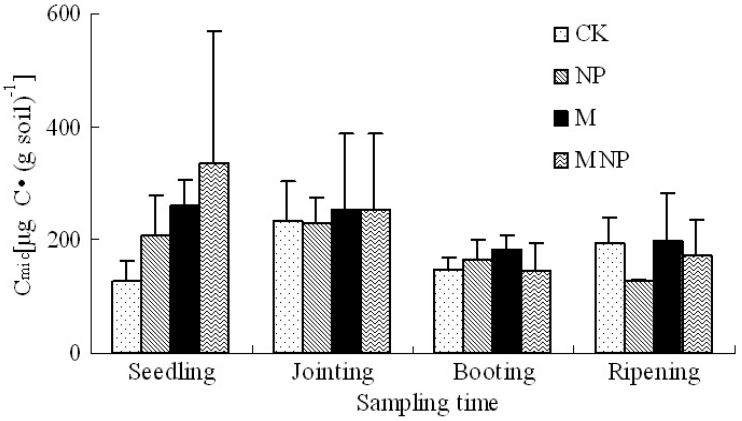


**Fig. 2:** Soil microbial biomass carbon under different treatments during the growing season of maize.

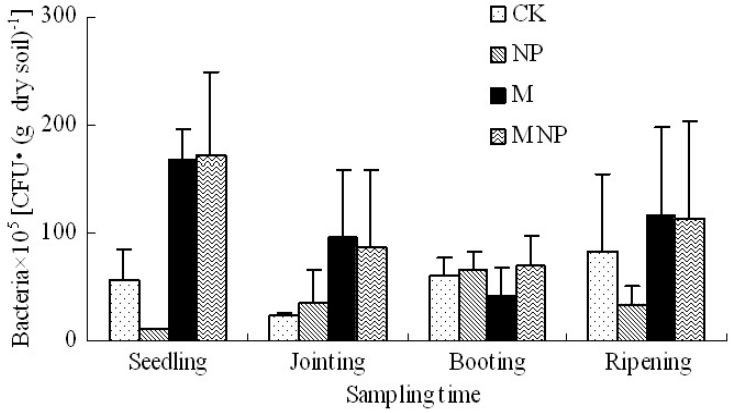


**Fig. 3:** Bacteria CFUs under different treatments during the growing season of maize.

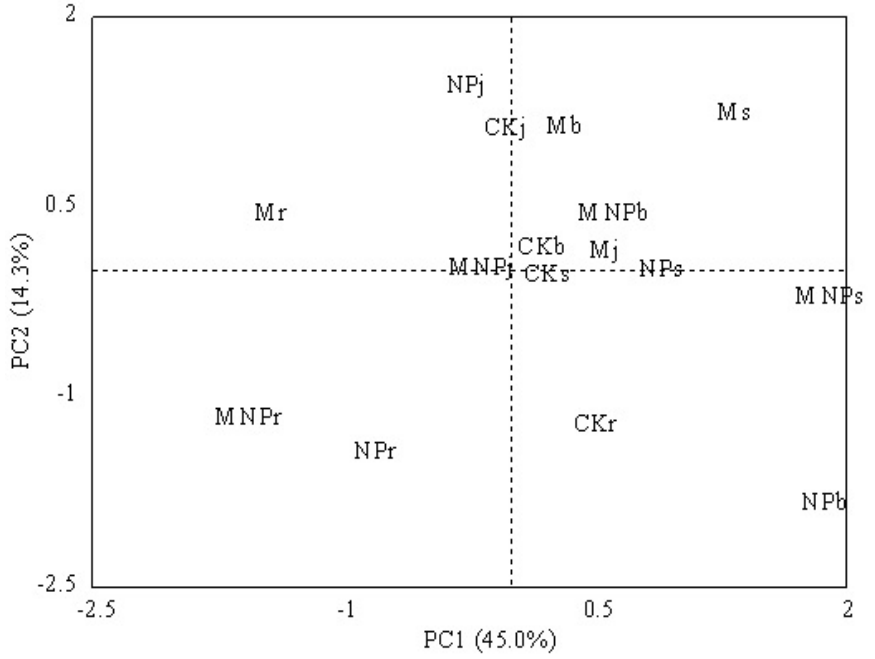


**Fig. 4:** Principal component analysis (PCA) on the DGGE profiles of the bacteria 16S rDNA PCR-products (n=2). The letter s represents the seedling stage; j, the jointing stage; b, the booting stage; r, the ripening stage.

soil pH was found to be an important factor affecting all microbial activities. There were significant changes in soil pH following the different treatments in the present study, and the soil pH was found to be higher in the M plot than in the other plots, suggesting that addition of manure induced a significant increase in soil pH. This agreed with the result reported by Gelsomino and Cacco[14].

Enhancement of microbial biomass after organic amendment has been reported in long-term[13] as well as in short-term microcosm experiments[32]. Our results showed that in a single soil type and over four development steps, with organic or inorganic amendment, microbial biomass C was not sensitive to detect these treatment effects, except during the seedling stage (Fig. 2). During the plant growth cycle soil microbial biomass revealed a remarkably changeable trend, and the bacterial diversity was also affected by the sampling time. Such time-related variation in the microbial biomass and the bacterial community structure has been previously reported[7,34]. In this study, microbial biomass C were affected significantly by the long-term fertilization at the seedling stage, while no significant changes existed between treatments during the last three stages. Ross *et al.*[30] indicated seasonal shifts in microbial biomass have been attributed to inputs of mineralizable N either from plant residues or fertilizer. The present study showed increasing in microbial biomass C in the seedling stage was after an application of 135 kg N · $ha^{-1}$ as nitrogen sources in either organic or inorganic form on April 25, one month prior to sampling. Moreover, two way ANOVA analyses displayed a significantly growing stage effect on microbial biomass among all treatments. No significant interaction existed between treatments and sampling time for this descriptor. This is in agreement with Calbrix *et al.*[8] who found significant changes in microbial carbon biomass that were not related to organic or mineral addition.

Soil bacterial counts were found to shift in response to the fertilization in the four growing stages of maize, and the similar patterns also existed in the bacterial community structure as revealed by the PCA of DGGE fingerprinting. Soil cultivable bacteria were found to be more sensitive in response to the organic amendments on a temporal basis, with a positive response of the bacterial population to M and M+NP (Fig.3). In addition to major plant nutrients such as nitrogen (N) and phosphorus (P), manure contains an array of organic compounds such as carbohydrates, fatty acids and peptides that are substrates for growth of heterotrophic soil microorganisms[28], and application of manure generally increases soil microbial biomass[4]. In our case, there was a significant correlation between microbial biomass C and CFU values (data not shown).

Principal component analysis indicated the DGGE profiles between treatments or times were dissimilar (Fig. 4). This concurred with the data from soil moisture, suggesting soil water contents may be partly responsible of this shift in microbial community structure (Fig. 1). In addition, although not evaluated, it is reasonable to assume that temperature and root growth have contributed in changes of the soil microbial communities. Spedding *et al.*[34] found changes in soil temperature may be one of the reasons that induce the shift in microbial community structure, but other dynamic factors such as root growth or soil fertility level may also have effect on the structure variations. This is in agreement with our data where a stronger plant phonological impact on bacterial community structure was found between different treatments, and a significant interaction was also observed between treatments and sampling times for this descriptor.

**Acknowledgements**

This research was financially supported by the National Key Basic Research Support Foundation of China (No. 2005CB121105) and the National Natural Science Foundation of Liaoning province (No. 20072019).

**References**

1. Amann, R.I., W. Ludwig and K.H. Schleifer, 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiological Reviews, 59: 143-169.
2. Bardgett, R.D., R.D. Lovell, P.J. Hobbs, S.C. Jarvis, 1999. Seasonal changes in soil microbial communities along a fertility gradient of temperate grasslands. Soil Biology and Biochemistry, 31: 1021-1030.
3. Baudoin, E., E. Benizri and A. Guckert, 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Applied Soil Ecology, 19: 135-145.
4. Bittman, S., T.A. Forge and C.G. Kowalenko, 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi–year applications of dairy manure slurry and fertilizer. Soil Biology and Biochemistry, 37: 613-623.
5. Böhme, L. and F. Böhme, 2006. Soil microbiological and biochemical properties affected by plant growth and different long-term fertilization. European Journal of Soil Biology, 42: 1-12.

6. Bossio, D.A., K.M. Scow, N. Gunapala, K.J. Graham, 1998. Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecology, 36: 1-12.
7. Buckley, D.H. and T.M. Schmidt, 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology, 5: 441-452.
8. Calbrix, R., S. Barray, O. Chabrerie, L. Fourrie, K. Laval, 2007. Impact of organic amendments on the dynamics of soil microbial biomass and bacterial communities in cultivated land. Applied Soil Ecology, 35: 511-522.
9. Clegg, C.D., R.D.L. Lovell and P.J. Hobbus, 2003. The impact of grassland management regime on the community structure of selected bacterial groups in soil. FEMS Microbiology Ecology, 43: 263-270.
10. Dungan, R.S., A.M. Ibekwe and S.R. Yates, 2003. Effect of propargyl bromide and 1,3-dichloropropene on microbial communities in an organically amended soil. FEMS Microbiology Ecology, 43: 75-87.
11. Enwall, K., K. Nyberg, S. Bertilsson, H. Cederlund, J. Stenstrom, S. Hallin, 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. Soil Biology and Biochemistry, 39: 106-115.
12. Feng, Y., A.C. Motta, D.W. Reeves, C.H. Burmester, E. van Santen, J.A. Osborne, 2003. Soil microbial communities under conventional-till and no-till continuous cotton systems. Soil Biology and Biochemistry, 35: 1693-1703.
13. Garcia-Gil, J.C., C. Plaza, P. Soler-Rovira and A. Polo, 2000. Long-term effects of municipal soil waste compost application on soil enzyme activities and microbial biomass. Soil Biology and Biochemistry, 32: 1907-1913.
14. Gelsomino, A. and G. Cacco, 2004. Compositional shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. Soil Biology and Biochemistry, 38: 91-102.
15. Gelsomino, C.C., A. Ambrosoli, R. Minati, 2004. Ruggiero, Functional and molecular responses of soil microbial communities under differing soil management practice. Soil Biology and Biochemistry, 36: 1873-1883.
16. Girvan, M.S., J. Bullimore, A.S. Ball, J.N. Pretty, A.M. Osborn, 2004. Responses of active bacterial and fungal communities in soil under winter wheat to different fertilizer and pesticide regimens. Applied and Environmental Microbiology, 70: 2692-2701.
17. Grayston, S.J., C.D. Campbell, R.D. Bardgett, J.L. Mawdsley, C.D. Clegg, K. Ritz, B.S. Griffiths, J.S. Rodwell, S.J. Edwards, W.J. Davies, D.J. Elston, P. Millard, 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. Applied Soil Ecology, 25: 63-84.
18. Grayston, S.J., D. Vaughan and D. Jones, 1996. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Applied Soil Ecology, 5: 29-56.
19. Hua, J.F., Y. Jiang and W.J. Liang, 2006. Response of nematodes in a Hapli-Udic Argosol to urea amended with urease and nitrification inhibitors. Pedosphere, 16: 428-434.
20. Ibekwe, A.M., A.C. Kennedy, P.S. Frohne, S.K. Papiernik, C.H. Yang and D.E. Crowley, 2002. Microbial diversity along a transect of agronomic zones. FEMS Microbiology Ecology, 39: 183-191.
21. Kennedy, A.C. and K.L. Smith, 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil, 170: 75-86.
22. Larcher, W., 2003. Ecophysiology and stress physiology of functional groups, In: Physiological Plant Ecology, Science Press, Springer, Berlin, pp: 450.
23. Liu, B. and J.B., 2003. Ristaino, Microbial community structure in soils from organic and conventional agroecosystems. Phytopathology, 96: S. 53.
24. Marschner, P., D. Crowley and C.H. Yang, 2004. Development of specific rhizosphere bacterial communitues in relation to plant species, nutrition and soil type. Plant and Soil, 261: 199-208.
25. Meng, F.X., W. Ou, Q. Li, Y. Jiang, D.Z. Wen, 2006. Vertical distribution and seasonal fluctuation of nematode trophic groups as affected by land use. Pedosphere, 16: 169-176.
26. Neill, C., M.C. Piccolo, C.C. Cerri, P.A. Steudler, J.M. Melillo and M. Brito, 1997. Net nitrogen mineraization and net nitrification reates in soils following deforestation for pasture across the southwestern Brazilian Amazon Basin landscape. Oecologia, 110: 242-252.
27. Nsabimana, D., R.J. Haynes and F.M. Wallis, 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology, 26: 81-92.
28. Paul, J.W. and E.G. Beauchamp, 1989. Effect of carbon constituents in manure on denitrification in soil. Canadian Journal of Soil Science, 69: 49-61.

29. Quideau, S.A., O.A. Chadwick, A. Benesi, R.C. Graham and M.A. Anderson, 2001. A direct link between forest vegetation type and soil organic matter composition. Geoderma, 104: 41-60.
30. Ross, D.J., T.W. Speir, H.A. Kettles, K.R. Tate and A.D. Mackay, 1995. Soil microbial biomass, C and N mineralization, and enzyme activities in a hill pasture: influence of grazing management. Australian Journal of Soil Research, 33: 943-959.
31. Saggar, S., G.W. Yeates and T.G. Shepherd, 2001. Cultivation effects on soil biological properties, microfauna and organic matter dynamics in Eutric Gleysol and Gleyic Luvisol soils in New Zealand. Soil and Tillage Research, 58: 55-68.
32. Saison, C., V. Degrange, R. Oliver, P. Millard, C. Commeaux, D. Montange and X.L. Roux, 2006. Alteration and resilience of the soil microbial community following compost amendment: effects of compost level and compost-borne microbial community. Environmental Microbiology, 8: 247-257.
33. Smalla, K., G. Wieland, A. Buchner, A. Zock, J. Parzy, S. Kaiser, N. Roskot, H. Heuer and G. Berg, 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology, 67: 4742-4751.
34. Spedding, T.A., C. Hamel, G.R. Mehuys and C.A. Madramootoo, 2004. Soil microbial dynamic in maize-growing soil under different tillage and residue management system. Soil Biology and Biochemistry, 36: 499-512.
35. Tu, C., J.B. Ristaino and S. Hu, 2005. Soil microbial biomass and activity in organic tomato farming systems: effects of organic inputs and surface mulching. Soil Biology and Biochemistry, 37: 1-9.
36. van Bruggen, A.H.C. and A.M. Semenov, 2000. In search of biological indicators for plant health and disease suppression. Applied Soil Ecology, 15: 13-24.
37. Vance, E.D., P.C. Brookes and D.S. Jenkinson, 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.
38. Wang, X.C. and Q. Lu, 2006, Beta-glucosidase activity in paddy soils of the Taihu Lake region, China. Pedosphere, 16: 118-124.
39. Warembourg, F.R. and H.D. Estelrich, 2001. Plant phenology and soil fertility effects on below-ground carbon allocation for an annual (*Bromus madritensis*) and a perennial (*Bromus erectus*) grass species. Soil Biology and Biochemistry, 33: 1291-1303.
40. Zak, D.R., D. Tilman and R.R. Parmenter, 1994. Plant production and soil microorganisms in late-successional ecosystem: a continental scale study. Ecology, 75: 2333-2347.
41. Zhao, B. and S.J. He, 2002. Cultivable technique. In: Microbiological Experiment (In Chinese), Science Press. Beijing, pp: 85-88.