# Microbial community diversity in the profile of an agricultural soil in northern China

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**Abstract**

The soil microorganisms at different depths play an important role in soil formation, ecosystem biogeochemistry, recycling of nutrients, and degradation of waste products. The aims of this study were to observe the microbial diversity in the profile of an agricultural soil in northern China, and to research the correlation between soil microbes and geochemistry. First, the soil geochemistry of the profile was investigated through 25 chemical elements. Secondly, the various physiological groups of microorganisms were studied by traditional culture methods. Thirdly, the functional diversity on sole carbon source utilization (SCSU) was evaluated by the BIOLOG system. Finally, the correlation between the soil microbial diversity and geochemistry was analyzed statistically. The results showed that the amounts and proportions of various physiological groups of microorganisms changed with depth. The bacterial functional diversity on SCSU decreased with increasing depth, but evenness of the substrate utilization increased. Although the microbial metabolic diversity was different at every depth, it could be classified into three main groups by principal component analysis and cluster analysis. The various physiological groups of microorganisms showed remarkable correlation with relevant soil chemical elements. The sensitive microbial indicators of soil health were expected to be screened out from actinomyces or ammonifying bacteria.

**Key words**: BIOLOG; geochemical elements; microbial diversity; soil profile

## Introduction

In recent years, there have been many research about soil microbial diversity, but few focused on the soil surface. The information about the microbial diversity of different soil layers is lacking. The subsurface soil environment, although devoid of sufficient nutrients, oxygen, and other factors, contains abundant soil microorganisms that play an important role in soil formation, ecosystem biogeo-chemistry, recycling of nutrients, and degradation of waste products (Kennedy, 1999; Buckley and Schmidt, 2001; Groudev *et al.*, 2001; Maila and Cloete, 2005). The subsurface microbes are specialized for their environment and are fundamentally distinct from those in surface communities (Fierer *et al.*, 2003; Maila *et al.*, 2005). Their metabolic properties cannot be inferred by studying the microbial communities found at the surface (Ekelund *et al.*, 2001, Fierer *et al.*, 2003). In this case, studying the soil microbial diversity at different depths is essential and has great significance.

In the past decade, several research had attempted to examine the vertical distribution of microbes in soil. At the surface, a large proportion of the microbial biomass (50%) and activity ($CO_2$ evolution) is within the top 0–5

cm of the soil, with reduced numbers at lower depths of 5–10 cm and 10–15 cm in hillside grasslands (Bardgett *et al.*, 1997). BIOLOG-GN analysis has revealed decreased substrate utilization at the lowest depth (0–20 cm), which is coupled with changes in the DNA- and RNA-denaturing gradient gel electrophoresis profiles in upland grassland soil (Griffiths *et al.*, 2003). For culturable bacteria and visible fungi in soil profiles from three Danish forest sites (Ekelund *et al.*, 2001), represented the general tendency in biomass to decrease with increasing depth (1.5–122.5 cm), except for a bacterial peak at 42.5 cm in peat. The number of individual phospholipid fatty acids (PLFAs) detected in soil samples decreased from the soil surface down to 2 m in Santa Barbara depth profiles. PLFA markers showed that Gram positive bacteria and actinomycetes tend to increase in proportional abundance with increasing soil depth, while the abundance of Gram negative bacteria and fungi is the highest at the soil surface and substantially lower at the subsurface (Fierer *et al.*, 2003). In present study, the fact that environmental factors such as available carbon, temperature, and moisture have a potential impact on microbial diversity also were reported.

With technical development, many methods are avalible to measure microbial diversity in soil (Kirk *et al.*, 2004). The traditional culture methods, such as plate counts (PCs)

and the most probable number (MPN) method, can track specific microbes and maximize the recovery of different microbial species. They are usually used to demonstrate the diversity of microorganisms associated with various soil quality parameters, such as disease suppression and organic matter decomposition (Hu and Van Bruggen, 1997; Maloney *et al.*, 1997), soil management pattern (Lawlor *et al.*, 2000), forest ecorestoration, and distribution with depth (Ekelund *et al.*, 2001). The BIOLOG system, based on sole carbon source utilization (SCSU) patterns (Garland and Mills, 1991), can differentiate microbial communities. It has been widely used in assessing the soil microbial community functional diversity in farmland (Ibekwe and Kennedy, 1998; Lupwayi *et al.*, 2001; Larkin, 2003), forest (Rogers and Tate, 2001), and grassland ecosystems (Grayston *et al.*, 2001). In particular, the Ecoplates, which contain a greatly reduced number of substrates compared to the GN plates (31 vs 95), but provide within-plate replication of those substrates, are tailored to ecological applications (Choi and Dobbs, 1999; Lawlor *et al.*, 2000; Classen *et al.*, 2003; Han *et al.*, 2007).

The purposes of this study were to investigate the variations in microbial diversity in profile of an agricultural soil in northern China and to research the correlation between soil microbes and geochemistry.

# 1 Materials and methods

## 1.1 Profile characterization

The research plot was established in a field site in Yutai, which is one of the biggest green-agricultural-product bases in China and is famous for its rice. The depth soil profile was located on the north nearby South-dam, Dinggang Village (35°03′N, 116°18′E). A hole with an around 2 m$^2$ area was excavated to a depth of 1.5 m (because below 1.5 m depth the profile was penetrated by groundwater). The soil type was fluvo-aquic soil, which has low groundwater level, slight pollution from sewage, and without salination. The site was on the land which mainly crops cotton and maize. The additional species were *Taraxacum mongolicum* Hand.-Mazz., *Cephalanoplos segetum* (Bunge) Kitam., *Setaria viridis* (L.) Beauv., *Eleusine indica* (L.) Gaertn., *Digitaria sanguinalis* (L.) Scop., *Amaranthus tricolor* L., *Euphorbia humifusa* Willd., *Conyza canadensis* (L.) Cronq., *Populus tomentosa* Carr. Near the site, on the dam slope, the dominant species was *Humulus scandens* (Lour.) Merr. Soil pH, organic matter, and moisture were measured with routine analytical methods (Agrochemistry Commission

and Soil Science Society of China, 1983). The properties of the soil profile are shown in Table 1.

## 1.2 Soil sampling

The profile was excavated on 15 August 2005. After excavation, the soil samples were immediately collected from different depths (0–20, 20–40, 40–60, 60–80, 80–100, 100–120, and 120–140 cm, seven layers in all from the top layer to the bottom layer). For each depth, three replicate soil samples were dug out from the deepest layer to the surface layer using a sterilized shovel from a 10-cm-deep horizontal hole. The 10 cm closest to the hole wall was discarded from each sample to ensure that the collected soil was fresh and without effect of excavated hole presence. The samples for microbial analysis were put directly into sterile plastic bags and send to the laboratory, homogenized, sieved to 2 mm, and stored at 4°C. The samples for geochemical analysis were sent to the laboratory and dried in air. All visible root and fresh litter material were removed from the samples prior to sieving.

## 1.3 Soil geochemistry

The contents of geochemical elements, including As, B, Cd, Co, Cr, Cu, F, Hg, Mn, Mo, Ni, P, Pb, V, Zn, Se, N, S, $SiO_2$, $Al_2O_3$, $Fe_2O_3$, MgO, CaO, $Na_2O$, and $K_2O$ were measured by Wuhan Synthetical Analytical Centre of Rock and Minerals, China. According to "Criteria of multi-purpose geochemical exploration (1:250,000)". Samples were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICPS-7510, Shimadzu, Japan) to quantify Co, Mn, Ni, V, $TFe_2O_3$, MgO, and CaO. Using hydride generation-atomic absorption spectroscopy (AFS-230E, Haiguang, China) to quantify As, Hg, and Se. X-ray fluorescence (Spectroscan makc-GV, Spectron, USA) was performed to quantify Cr, Cu, P, Zn, $SiO_2$, $Al_2O_3$, $Na_2O$, and $K_2O$. Using emission spectrometry (WP-1, Modern Rayleigh, China) to quantify B and Pb. Using flame atomic absorption spectrophotometry (AA-6800, Shimadzu, Japan) to quantify Cd. Ion-selective electrode (ISE, Thermo Orin, USA) was performed to quantify F. Mo was quantified with polarography (797 VA Computrace, Metrohm, Switzerland). N and S were analyzed with the distillation method (Li, 1983) and the combustion method (Li, 1983), respectively.

## 1.4 Analysis of various physiological groups of microorganisms

Traditional culture techniques were employed to determine the distribution of eight physiological groups of

**Table 1** Properties of the soil depth profiles

| Depth (cm) | 0–20 | 20–40 | 40–60 | 60–80 | 80–100 | 100–120 | 120–140 |
|---|---|---|---|---|---|---|---|
| Horizons | A | B | BC$_1$ | BC$_1$ | BC$_1$ | C$_2$ | C$_2$ |
| Color | Rose | Rose | Rose | Brown-red | Brown-red | Brown-red | Brown-red |
| Texture | Sand-clay | Sand-clay | Sand-clay | Clay | Clay | Clay | Clay |
| pH | 7.79 | 8.18 | 7.94 | 8.12 | 7.98 | 7.81 | 7.89 |
| Organic matter (%) | 2.09 | 1.69 | 1.39 | 1.02 | 0.93 | 1.15 | 1.06 |
| Moisture (%) | 15.67 | 21.42 | 19.37 | 22.82 | 23.48 | 23.8 | 27.52 |

Values of pH, organic matter, and moisture are means of three replications.

microorganisms, including aerobic heterotrophic bacteria, fungi, actinomyces, nitrogen-fixing bacteria, nitrifying bacteria, denitrifying bacteria, ammonifying bacteria, and cellulose-degrading bacteria. All media were made up and all microbes were cultivated as described by Dong (1997). Viable total counts of aerobic heterotrophic bacteria, fungi, actinomyces, and nitrogen-fixing bacteria were determined as colony forming units (CFUs) on agar plates with the PC method (Dong, 1997). The media were beef extract peptone medium, Martin's medium, Gause's No. 1 synthetic medium, and Waksman's medium, respectively. Nitrifying bacteria, denitrifying bacteria, ammonifying bacteria, and cellulose-degrading bacteria were counted with the MPN method (Dong, 1997). They were cultivated at 28°C in a constant-temperature incubator. The cultivatioin time was 2, 3, 7, 5, 13, 15, 3, and 15 d, respectively.

### 1.5 SCSU patterns using the BIOLOG system

BIOLOG ECO microplates (BIOLOG, Hayward, USA) were used to evaluate the functional diversity in SCSU. From each soil sample, the bacterial cells were extracted as described by Hitzl et al. (1997). The original samples had a density of $3\times10^3$–$4\times10^4$ cells/well. Each well of the BIOLOG ECO plates was inoculated with 150 μl suspension at 28°C. Absorbance was read at 590 nm every 12 h for 192 h with the BIOLOG Micro Station reader (ML3402, Microlog, USA).

### 1.6 Data analysis

Data analysis of the cultured microbes was performed with correlation matrices among the microbial number and depth, and the content of chemical elements. Methods of analysis of BIOLOG data are shown as following. An average well color development (AWCD) method (Garland and Mills, 1991), i.e.,

$$AWCD = \sum (C - R)/31 \qquad (1)$$

where, $C$ is color production within each well (optical density measurement), $R$ is the absorbance value of the plates control well. Diversity was evaluated by calculating Shannon's diversity index ($H'$) (Zak et al., 1994; Lupwayi et al., 2001):

$$H' = -\sum p_i \ln p_i \qquad (2)$$

where, $p_i$ is ratio of activity (i.e., optical density reading) on the $i$ th substrate to the sum of activites on all substrates.

McIntosh's diversity index ($U$):

$$U = \sqrt{\left(\sum n_i^2\right)} \qquad (3)$$

where,

$$n_i = C_i - R \qquad (4)$$

Gini's evenness index ($G$) (Harch et al., 1997):

$$G = \frac{\sum\limits_{i=1}^{W} \sum\limits_{j=1}^{W} |n_i - n_j|}{2W^2 \bar{x}} \qquad (5)$$

where, $W$ is the total number of the sole-carbon substrates (ECO plates $W$ = 31); $\bar{x}$ = AWCD. Shannon's evenness index ($E$) (Lupwayi et al., 2001) can be described as:

$$E = H/\ln S \qquad (6)$$

where, $S$ is the number of different substrates that were used by the bacterial community. All the values are expressed as mean ± SE. The structure of the bacterial community was characterized by classifying treatments according to their substrate utilization patterns (averaged over replicates) using principal component analysis (PCA) (Choi and Dobbs, 1999). After classification of treatments with PCA, the substrates that were utilized more in some depths than in others were found by correlating principal component scores with optical density readings for individual substrates, and results were presented as factor loadings plots. Above statistical analyses was done using Statistica 6.0 software. The diversity indices and PCA data presented were based on 72 h incubation readings.

## 2 Results

### 2.1 Characteristics of the soil

Table 2 shows that the content of N and P decreased, F increased, and other chemical elements had no obvious correlation with depth. The heavy metals and toxic elements such as Cd, Cr, Cu, Ni, Zn, and As had a higher content at a depth of 60–80 cm than in the surface soil. Under 80 cm all contents exceed the Chinese national grade-one standard values of Environmental Quality Standard for Soils (GB 15618-1995). The level of these six metallic elements was higher in the second layer than in the first, but decreased again in the third layer. In the fourth to sixth layers, the levels increased continuously, with the exception of Cd in the fifth layer, and reached a peak in the sixth layer. In the seventh layer, the levels decreased again, but they were still higher than in the top layer. The levels of these six heavy metals and toxic elements in the sixth layer of soil (100–120 cm) were 1.33, 1.30, 1.50, 1.47, 1.32, and 1.74 times higher than those in the surface soil, respectively.

### 2.2 Analysis of various physiological groups of microorganisms

Table 3 shows the numbers of various physiological groups of microorganisms in different depths. The number of bacteria, fungi, and actinomyces tended to decrease with increasing depth. In the three upper layers, the microbial numbers substantially decreased with depth keeping in the same quantitative level, but in the fourth layer, the numbers decreased sharply. In a depth of 120–140 cm, the numbers of bacteria, fungi, and actinomyces were 11.3%, 5.4%, and 4.1% of those at the surface. In each soil depth, the number of fungi was always less than that of bacteria or actinomyces. The proportion of bacteria, fungi, and actinomyces changed with depth. For example, the ratio of bacteria to actinomyces in the profile was 0.9, 0.7, 0.6, 0.9, 0.8, 1.4, and 2.4 from the first layer to the seventh layer.

**Table 2** Elements in soil at different depths

| Depth (cm) | 0–20 | 20–40 | 40–60 | 60–80 | 80–100 | 100–120 | 120–140 |
|---|---|---|---|---|---|---|---|
| As (μg/g) | 12.4 | 17.7* | 12.4* | 20.8* | 20.7* | 21.6* | 19.1* |
| B (μg/g) | 52.5 | 59.2 | 43.6 | 71.2 | 53.6 | 109.7 | 80.7 |
| Cd (μg/g) | 0.218* | 0.267* | 0.200* | 0.262* | 0.247* | 0.289* | 0.234* |
| Co (μg/g) | 14.0 | 18.3 | 13.4 | 18.5 | 19.6 | 19.9 | 17.6 |
| Cr (μg/g) | 75.1 | 87.1 | 73.5 | 93.3* | 97.7* | 97.8* | 89.1 |
| Cu (μg/g) | 29.5 | 39.0* | 27.1 | 37.5* | 41.9* | 44.2* | 36.9* |
| F (μg/g) | 558 | 695 | 607 | 756 | 779 | 822 | 745 |
| Hg (μg/g) | 0.026 | 0.030 | 0.024 | 0.035 | 0.035 | 0.037 | 0.028 |
| Mn (μg/g) | 744 | 957 | 722 | 1,029 | 1,105 | 1,092 | 989 |
| Mo (μg/g) | 0.78 | 0.93 | 0.74 | 0.93 | 1.11 | 1.17 | 0.95 |
| Ni (μg/g) | 34.7 | 43.9* | 33.6 | 46.7* | 48.7* | 50.9* | 42.4* |
| P (μg/g) | 896 | 678 | 712 | 599 | 560 | 589 | 577 |
| Pb (μg/g) | 25.6 | 30.2 | 25.9 | 31.2 | 34.4 | 33.2 | 30.7 |
| V (μg/g) | 88.4 | 113.9 | 92.5 | 112.9 | 122.6 | 125.5 | 113 |
| Zn (μg/g) | 80.8 | 99.4 | 74 | 92.3 | 102.8* | 106.7* | 94.2 |
| Se (μg/g) | 0.20 | 0.20 | 0.16 | 0.20 | 0.20 | 0.19 | 0.17 |
| N (%) | 0.143 | 0.092 | 0.087 | 0.078 | 0.076 | 0.073 | 0.065 |
| S (%) | 0.028 | 0.023 | 0.027 | 0.038 | 0.04 | 0.047 | 0.033 |
| SiO$_2$ (%) | 51.11 | 46.54 | 52.07 | 44.84 | 43.54 | 43.17 | 48.17 |
| Al$_2$O$_3$ (%) | 13.27 | 14.48 | 13.26 | 14.74 | 14.98 | 15.14 | 14.23 |
| TFe$_2$O$_3$ (%) | 4.95 | 6.28 | 4.93 | 6.87 | 7.23 | 7.29 | 6.61 |
| MgO (%) | 2.64 | 3.01 | 2.67 | 3.16 | 3.18 | 3.21 | 2.94 |
| CaO (%) | 7.99 | 9.51 | 7.9 | 10.32 | 10.7 | 10.76 | 9.41 |
| Na$_2$O (%) | 1.22 | 0.89 | 1.27 | 0.84 | 0.77 | 0.77 | 1.02 |
| K$_2$O (%) | 2.62 | 2.85 | 2.58 | 2.87 | 2.93 | 2.96 | 2.75 |

* Element contents exceed the Chinese national grade-one standard value of Environmental Quality Standard for Soils (GB 15618-1995). All values are mean of three replications.

**Table 3** Total numbers of various physiological groups of microorganisms at different depth ($\times 10^5$/g dry soil)

| Depth (cm) | 0–20 | 20–40 | 40–60 | 60–80 | 80–100 | 100–120 | 120–140 |
|---|---|---|---|---|---|---|---|
| Aerobic heterotrophic bacteria | 403.2 (6.8) | 156.5 (3.3) | 112.9 (2.9) | 74.5 (4.2) | 26.1 (3.3) | 118.1 (4.3) | 45.5 (1.6) |
| Fungi | 0.44 (0.01) | 0.31 (0.04) | 0.28 (0.04) | 0.02 (0.00) | 0.01 (0.00) | 0.04 (0.00) | 0.02 (0.00) |
| Actinomyces | 462.5 (9.7) | 216.3 (4.2) | 188.2 (4.3) | 81.4 (1.5) | 31.4 (3.3) | 81.8 (1.2) | 18.9 (3.3) |
| Nitrogen-fixing bacteria | 21.0 (2.2) | 19.5 (2.8) | 12.6 (0.8) | 8.6 (0.2) | 6.1 (0.5) | 6.4 (2.1) | 1.8 (0.5) |
| Nitrifying bacteria | 0.03 (0.00) | 0.06 (0.00) | 0.01 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| Denitrifying bacteria | 2.99 (0.02) | 2.02 (0.01) | 3.10 (0.01) | 0.58 (0.00) | 5.88 (0.01) | 1.21 (0.00) | 1.29 (0.01) |
| Ammonifying bacteria | 533.6 (5.3) | 31.8 (2.1) | 372.1 (3.2) | 97.2 (8.7) | 58.8 (3.2) | 32.8 (1.9) | 158.7 (2.3) |
| Cellulose-degrading bacteria | 0.03 (0.00) | 0.06 (0.00) | 0.03 (0.00) | 0.12 (0.00) | 0.00 (0.00) | 0.21 (0.01) | 0.03 (0.00) |

Values are expressed as mean (SE).

The number of nitrogen-fixing bacteria decreased with depth. In a depth of 120–140 cm, the number of nitrogen-fixing bacteria was 8.6% of the number at the surface. For nitrifying, ammonifying, and cellulose-degrading bacteria, the general tendency in number represented to decrease, but fluctuation for denitrifying bacteria. Among these five bacterial communities having specific physiological functions, the ammonifying bacteria were preponderant in every soil layer.

### 2.3 Substrate utilization analysis

BIOLOG ECO plates were used to compare the relative capacity for substrate utilization in different soil depths. Fig.1 shows that the amount of carbon-substrate utilization declined substantially with depth. The greatest carbon source utilization was observed in 0–20 cm depth, which showed utilization of (82.8±3.9)% of the 31 carbon sources. Communities showing the lowest substrate utilization capacity were seen in the 120–140 cm samples, which utilized only (12.9±4.9)% carbon sources. The intermediary substrate utilization of the remaining samples
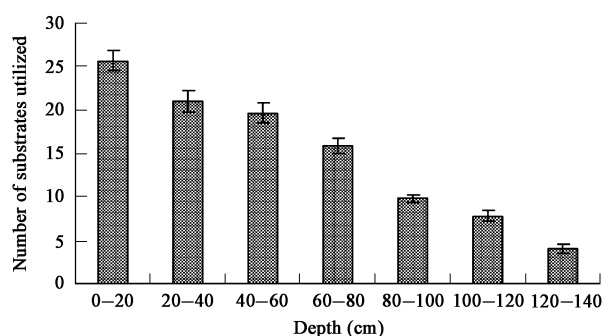


**Fig. 1** Number of substrates utilized in different soil depths.

from top to bottom exhibited (67.7±7.4)%, (63.4± 9.2)%, (51.1±3.8)%, (31.7± 7.9)%, and (25.3±5.1)%, respectively.

The microbes in different depths had different AWCD values, and the AWCD curves declined with increasing depth (Fig.2). AWCD of the three upper depth levels increased rapidly in the first 48 h and reached a maximum after 192 h, but in the deeper four depths, AWCD values
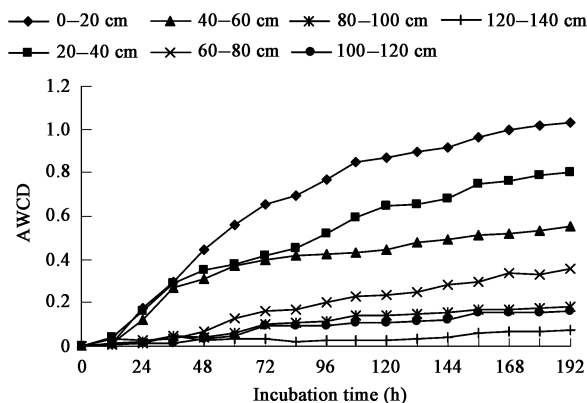
**Fig. 2** AWCD values at different soil depths.

increased lightly. Increasing depths delayed the onset of AWCD increase, reduced the rate of AWCD increase, and decreased the maximum AWCD.

Table 4 shows that the diversity and evenness indices for soil microbial communities changed with depth. Shannon's and McIntosh's diversities indice declined with increasing depth, but Gini's and Shannon's evenness indices increased gradually.

PCA (Fig.3a) and cluster analysis (Fig.3b) showed that the microbes in different depths could be classified into three main groups. The first group included 0–20, 20–40, 40–60, and 60–80 cm samples, and the second group included 80–100 and 100–120 cm samples. The 120–140 cm samples were separate. The first principal component axis (PC1) explained 37.3% of the variance in the data, while the second principal component axis (PC2) explained 21.4%. Factor 1 (i.e., right to left, in Fig.3a) showed that the bacterial community structure in the 120–140 cm depth was different from the community structures in the other. The cluster tree (Fig.3b) showed that there was no difference in linkage distance between the 0–20 and 20–40 cm samples, and the distance among the other samples changed with depth. The factor loadings plot (Fig.3c) showed that the substrates that were utilized more by bacteria in a depth of 120–140 cm than in other depths included $L$-phenylalanine (C4), itaconic acid (F3), Tween 80 (D1), $L$-threonine (E4), $L$-asparagine (B4), Tween 40 (C1), α-cyclodextrin (E1), $i$-erythritol (C2), while the substrates that were utilized more in other depths included $D$-mannitol (D2), glucose-1-phosphate (G2), $D$-malic acid (H3), $L$-arginine (A4), 4-hydroxybenzoic acid (D3), β-methyl-$D$-glucoside (A2), α-$D$-lactose (H1), $D,L$-α-glycerol phosphate (H2), putrescine (H4). Factor 2 (i.e., top to bottom in Fig.3a) separated by the upper six depths. Fig.3c showed that the substrates $N$-acetyl-$D$-glucosamine (E2), glycyl-$L$-glutamic acid (F4), β-methyl-$D$-glucoside (A2), phenylethylamine (G4), $D$-cellobiose (G1), β-methyl-$D$-glucoside (A3), α-ketobutyric acid (G3) were among those utilized more in the upper four depths than in 80–100 and 100–120 cm, while substrates like 4-hydroxybenzoic acid (D3), putrescine (H4), 2-hydroxybenzoic acid (C3), $D,L$-α-glycerol phosphate (H2), $L$-arginine (A4) were utilized more in 80–100 and 100–120 cm depths.
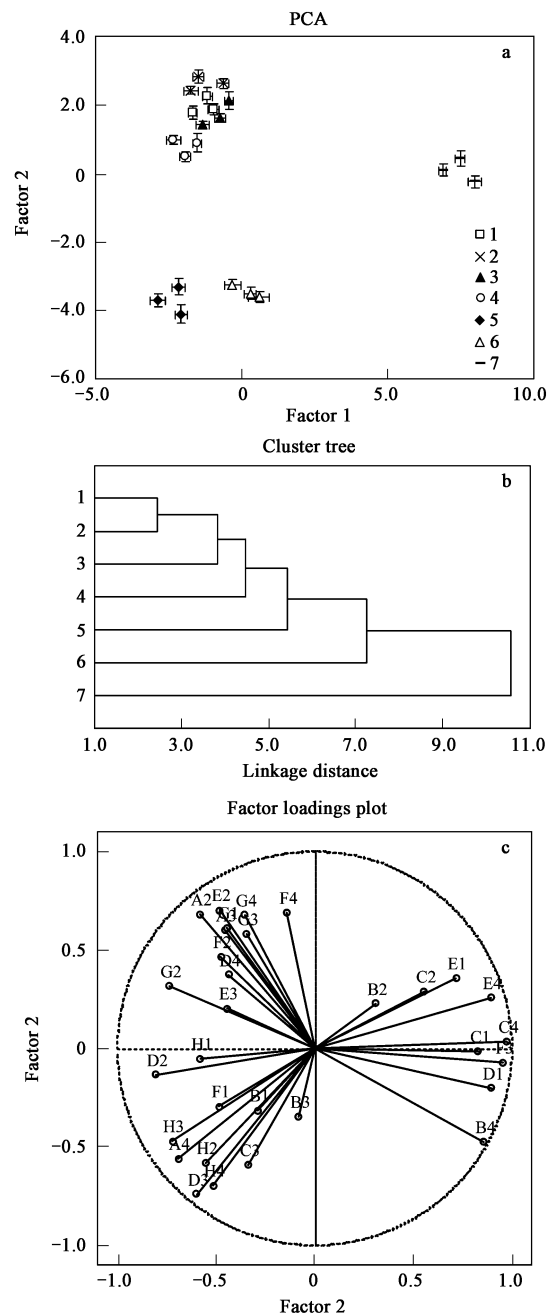


**Fig. 3** Classification of treatments by PCA (a), cluster analysis (b), and the factor loadings plot in PCA (c) at different depths. Factor 1 accounted for 37.3%, and Factor 2 for 21.4% of the variance. (1) 0–20 cm; (2) 20–40 cm; (3) 40–60 cm; (4) 60–80 cm; (5) 80–100 cm; (6) 100–120 cm; (7) 120–140 cm.

## 3 Discussion

Soil microbes play fundamental roles in various biogeochemical cycles (BGCs) and are responsible for the cycling of organic compounds (Kirk *et al.*, 2004). Our research found that the numbers of aerobic heterotrophic bacteria ($r = -0.80$, $P < 0.05$), fungi ($r = -0.89$, $P < 0.01$), actinomyces ($r = -0.87$, $P < 0.05$), nitrogen-fixing bacteria ($r = -0.97$, $P < 0.001$) showed remarkable correlation with depth. The numbers of bacteria, fungi, actinomyces, and nitrogen-fixing bacteria decreased with depth, which implied that the relevant BGC functions were weakened.

**Table 4** Diversity and evenness indices for soil microbial communities in different depths

| Depth (cm) | Shannon's diversity index ($H'$) | McIntosh's diversity index ($U$) | Gini's evenness ($G$) | Shannon's evenness index ($E$) |
|---|---|---|---|---|
| 0–20 | 3.12±0.03 | 5.89±0.48 | 0.42±0.02 | 0.98±0.01 |
| 20–40 | 2.92±0.08 | 4.64±0.17 | 0.53±0.03 | 1.00±0.01 |
| 40–60 | 2.74±0.22 | 3.68±0.72 | 0.60±0.08 | 1.01±0.03 |
| 60–80 | 2.89±0.03 | 1.91±0.27 | 0.59±0.01 | 1.14±0.02 |
| 80–100 | 2.37±0.28 | 1.40±0.50 | 0.72±0.07 | 1.64±0.49 |
| 100–120 | 2.22±0.23 | 1.30±0.29 | 0.76±0.05 | 1.18±0.13 |
| 120–140 | 1.25±0.21 | 0.46±0.18 | 0.90±0.02 | 1.38±0.09 |

Values are expressed as mean ± SE.

A number of factors may concomitantly cause the differentiation of soil microbial communities, but resource availability is the primary control on microbial community composition within the soil profiles (Fierer *et al.*, 2003). In our study the content of organic matter, N, and P decreased with depth, which are essential factors in the formation of many nutrients. From the data, we also found that the proportions of different microbial communities changed with depth. They showed that the composition and structure of soil microbial communities changed to adapt to the special environment. It is in agreement with others that subsurface microbial communities are distinct in composition from surface communities (Fritze *et al.*, 2000; Blume *et al.*, 2002; Ekelund *et al.*, 2001; Taylor *et al.*, 2002). All studies imply microbial communities contained in deeper soil are specialized for their environment, and are fundamentally distinct from surface communities. The result of the vertical distribution of fungi was similar to Fierer *et al.* (2003), using PLFAs as biomarkers. This showed that the abundance of fungi was the highest on the soil surface and substantially deeper on the subsurface. Our results are also similar to Ekelund *et al.* (2001), using a microscopy indirect enumeration method, showed that the number of fungi decreased with increasing depth in a profile from a dry beech forest situated on mor. However, our results with actinomycetes were dissimilar from Fierer *et al.* (2003), which the proportional abundance of actinomycetes should increase with soil depth. This could be due to the fact that the different soil profiles have distinct physical and chemical characteristics. On the other hand, the different research methods usually lead to varied results. The dilution plating methods we used bias towards culturable and fast growing individuals in the profile. PLFA analysis is a culture-independent method which based on the variability of fatty acids present in cell membranes (Hill *et al.*, 2000).

AWCD reflects the total microbial activity. Shannon index and Gini coefficient show the substrate richness and evenness (Harch *et al.*, 1997). McIntosh's diversity index, which is based on Euclidean distance, also reveals bacterial functional diversity for SCSU. In this study the number of carbon substrates utilized and the AWCD curves declined substantially with depth. They showed that the total microbial activity decreased from the surface to the subsurface soil. Shannon's and McIntosh's indices declined with increasing depth, but Gini's and Shannon's evenness increased gradually. Correlation analysis showed that McIntosh's diversity index and Gini's coefficient demonstrated remarkable correlations with depth ($r = -0.97$, $P < 0.001$). All of these showed that the bacterial functional diversity for SCSU decreased with increasing depth, but evenness of the substrate utilization increased. The results were similar to Griffiths *et al.* (2003), who reported that a decreased capacity for substrate utilization down the soil profile in an upland grassland soil. Although the microbial metabolic diversity for SCSU patterns was different in every depth, it can be classified into three main groups, by PCA and cluster analysis. The variation tendencies of Shannon's and McIntosh's diversity indices, and of Gini's and Shannon's evenness indices, with depth were coincident (Table 4). The BIOLOG method reflects the microbial functional diversity based on SCSU patterns, but can not detect the community structural diversity based on fatty acids or gene fragments. In recent years, an increasing numbers of studies using molecular strategies to research the soil microbial structural diversity in genetic profiling (Maila *et al.*, 2005; Hu *et al.*, 2007) are present. Therefore,, in the future work, the combination of polyphasic methods is required to obtain accurate information on the microbial community characteristics.

Analysis of the correlations between the levels of 25 chemical elements and depth (Table 5) showed that F, N, and P had remarkable correlations, but other elements. The content of F had remarkable positive correlation to depth ($P < 0.05$), but the content of N and P had a significant negative correlation with depth ($P < 0.05$). Our data showed that the heavy metals and toxic elements were easily to be enriched in deep soil layers. The concentration of the heavy metals in the subsurface soil influences the security of the groundwater directly. Therefore, to control heavy metal pollution in the subsurface soil is important. Correlation matrices between cultured microbes and chemical elements (Table 5) showed that bacteria, fungi, and actinomyces had significant negative correlations with As, Co, Cr, F, Mn, Ni, Pb, V, $Al_2O_3$, $TFe_2O_3$, MgO, and CaO, and remarkable positive correlation with N and P ($P < 0.05$). It showed that the contents of As, Co, Cr, F, Mn, Ni, Pb, V, $Al_2O_3$, $TFe_2O_3$, MgO, and CaO in this soil profile suppressed the growth of the aerobic heterotrophic bacteria, fungi, and actinomyces, and as the levels increased, the suppression became more serious. However, the content of N and P promoted the growth of aerobic heterotrophic bacteria, fungi, and actinomyces. The content of N and P, which are essential elements in the formation of many nutrients, decreased with increasing depth. This leads to a decrease in the level of nutrients

**Table 5**　Spearman correlations between soil microbial diversity and 25 elements in the profile

| | Aerobic-heterotrophic bacteria | Fungi | Actinomyces | Nitrogen-fixing bacteria | Cellulose-degrading bacteria | Ammonifying acteria | Nitrifying bacteria | Denitrifying bacteria | $U$ | $G$ | Depth |
|---|---|---|---|---|---|---|---|---|---|---|---|
| As | −0.85 | −0.88 | −0.90 | −0.68 | −0.22 | −0.76 | −0.43 | −0.19 | −0.79 | 0.62 | 0.71 |
| B | −0.39 | −0.57 | −0.59 | −0.53 | 0.29 | −0.40 | −0.38 | −0.58 | −0.59 | 0.58 | 0.67 |
| Cd | −0.49 | −0.50 | −0.60 | −0.23 | 0.13 | −0.59 | −0.02 | −0.37 | −0.38 | 0.25 | 0.35 |
| Co | −0.79 | −0.76 | −0.87 | −0.53 | −0.21 | −0.74 | −0.25 | −0.09 | −0.66 | 0.52 | 0.60 |
| Cr | −0.81 | −0.85 | −0.89 | −0.64 | −0.20 | −0.72 | −0.42 | −0.05 | −0.75 | 0.58 | 0.68 |
| Cu | −0.71 | −0.70 | −0.85 | −0.49 | −0.13 | −0.69 | −0.21 | −0.06 | −0.62 | 0.51 | 0.59 |
| F | −0.87 | −0.91 | −0.92 | −0.75 | −0.28 | −0.82 | −0.50 | −0.16 | −0.85 | 0.72 | 0.80 |
| Hg | −0.62 | −0.72 | −0.68 | −0.46 | 0.03 | −0.58 | −0.40 | −0.08 | −0.57 | 0.33 | 0.48 |
| Mn | −0.84 | −0.84 | −0.91 | −0.64 | −0.25 | −0.74 | −0.39 | −0.05 | −0.75 | 0.60 | 0.69 |
| Mo | −0.68 | −0.74 | −0.84 | −0.59 | −0.10 | −0.62 | −0.38 | 0.06 | −0.68 | 0.59 | 0.67 |
| Ni | −0.76 | −0.78 | −0.83 | −0.55 | −0.13 | −0.72 | −0.34 | −0.10 | −0.67 | 0.50 | 0.60 |
| P | 0.99 | 0.94 | 0.92 | 0.85 | 0.62 | 0.92 | 0.54 | 0.08 | 0.91 | −0.81 | −0.84 |
| Pb | −0.85 | −0.84 | −0.91 | −0.65 | −0.32 | −0.75 | −0.40 | 0.09 | −0.75 | 0.61 | 0.69 |
| V | −0.84 | −0.80 | −0.91 | −0.62 | −0.31 | −0.82 | −0.31 | −0.06 | −0.74 | 0.63 | 0.69 |
| Zn | −0.67 | −0.63 | −0.83 | −0.43 | −0.12 | −0.65 | −0.12 | −0.04 | −0.56 | 0.49 | 0.55 |
| Se | 0.04 | 0.03 | −0.07 | 0.34 | 0.39 | 0.09 | 0.28 | 0.10 | 0.23 | −0.43 | −0.32 |
| N | 0.93 | 0.87 | 0.83 | 0.85 | 0.68 | 0.93 | 0.53 | 0.17 | 0.89 | −0.84 | −0.84 |
| S | −0.52 | −0.78 | −0.63 | −0.70 | 0.14 | −0.39 | −0.78 | −0.03 | −0.73 | 0.54 | 0.69 |
| $SiO_2$ | 0.72 | 0.74 | 0.78 | 0.49 | 0.12 | 0.68 | 0.32 | 0.03 | 0.61 | −0.41 | −0.52 |
| $Al_2O_3$ | −0.78 | −0.78 | −0.84 | −0.55 | −0.20 | −0.76 | −0.31 | −0.09 | −0.67 | 0.50 | 0.59 |
| $TFe_2O_3$ | −0.85 | −0.87 | −0.92 | −0.68 | −0.25 | −0.75 | −0.43 | −0.09 | −0.79 | 0.63 | 0.72 |
| MgO | −0.81 | −0.81 | −0.83 | −0.56 | −0.22 | −0.77 | −0.36 | −0.11 | −0.68 | 0.48 | 0.59 |
| CaO | −0.79 | −0.82 | −0.84 | −0.59 | −0.18 | −0.72 | −0.40 | −0.05 | −0.71 | 0.51 | 0.62 |
| $Na_2O$ | 0.74 | 0.72 | 0.80 | 0.45 | 0.17 | 0.72 | 0.23 | 0.05 | 0.59 | −0.40 | −0.50 |
| $K_2O$ | −0.71 | −0.70 | −0.78 | −0.44 | −0.13 | −0.70 | −0.22 | −0.05 | −0.57 | 0.40 | 0.50 |

that promote microbial growth, which in turn leads to a microorganisms number decrease. Nitrogen-fixing bacteria has a significant positive correlation with N and P, but with other elements, which showed that the existence of N and P can promote soil nitrogen fixation. Ammonifying bacteria had a significant negative correlation with As, Cd, Co, Cr, Cu, F, Hg, Mn, Mo, Ni, Pb, V, Zn, $Al_2O_3$, $TFe_2O_3$, MgO, CaO, and $K_2O$, but a significant positive correlation with N, P, $SiO_2$, and $Na_2O$ ($P < 0.05$). This demonstrated that, in this soil profile, As, Cd, Co, Cr, Cu, F, Hg, Mn, Mo, Ni, Pb, V, Zn, $Al_2O_3$, $TFe_2O_3$, MgO, CaO, and $K_2O$ inhibited the growth of ammonifying bacteria, and suppressed the soil-aminating function; However, N, P, $SiO_2$, and $Na_2O$ promoted the growth of ammonifying bacteria, and promoted the soil-aminating function. Nitrifying bacteria only had a significant negative correlation with S. Denitrifying bacteria did not have any correlation with the 25 elements ($P < 0.05$). These proved that the soil nitrify function depending bacteria had significant negative correlation to S, and the soil denitrifying function had not remarkable correlation to mineral element.

Our data showed that the number of actinomyces in the soil had remarkable correlations with As, Cr, F, Mn, Pb, V, and $TFe_2O_3$ ($P < 0.01$). Ammonifying bacteria had remarkable correlations with As, Co, Cr, Cu, F, Mn, Ni, Pb, V, Zn, $Al_2O_3$, $TFe_2O_3$, MgO, CaO, $K_2O$, $SiO_2$, and $Na_2O$ ($P < 0.01$). These indicated that in the profile actinomyces and ammonifying bacteria were sensitive to those chemical elements. We expected that the sensitive microbial indicator of soil health could be screened out from actinomyces or ammonifying bacteria.

## 4 Conclusions

In summary, we observed variations in microbial diversity of one agricultural soil depth profile in northern China, and analyzed the correlations between soil microbes and geochemistry. The numbers and proportions of various physiological groups of microorganisms changed with depth. The bacterial functional diversity for SCSU decreased with increasing depth, but evenness of the substrate utilization increased. Although the microbial metabolic diversity for SCSU patterns was different in every depth, it could be classified into three main groups by PCA and cluster analysis. The various physiological groups of microorganisms had remarkable correlation to relevant soil elements. We expected that the sensitive microbial indicator of soil health could be screened out from actinomyces or ammonifying bacteria.

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