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Effects of vegetation type on soil microbial community structure and catabolic diversity assessed by polyphasic methods in North China

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Abstract

Soil microbes play a major role in ecological processes and are closely associated with the aboveground plant community. In order to understand the effects of vegetation type on the characteristics of soil microbial communities, the soil microbial communities were assessed by plate counts, phospholipid fatty acid (PLFA) and Biolog microplate techniques in five plant communities, i.e., soybean field (SF), artificial turf (AT), artificial shrub (AS), natural shrub (NS), and maize field (MF) in Jinan, Shandong Province, North China. The results showed that plant diversity had little discernible effect on microbial biomass but a positive impact on the evenness of utilized substrates in Biolog microplate. Legumes could significantly enhance the number of cultural microorganisms, microbial biomass, and community catabolic diversity. Except for SF dominated by legumes, the biomass of fungi and the catabolic diversity of microbial community were higher in less disturbed soil beneath NS than in frequently disturbed soils beneath the other vegetation types. These results confirmed that high number of plant species, legumes, and natural vegetation types tend to support soil microbial communities with higher function. The present study also found a significant correlation between the number of cultured bacteria and catabolic diversity of the bacterial community. Different research methods led to varied results in this study. The combination of several approaches is recommended for accurately describing the characteristics of microbial communities in many respects.

Key words: Biolog; legume; microbial biomass; plant diversity; phospholipid fatty acid (PLFA); soil microbes

Introduction

Soil microbes play an important role in many critical ecosystem processes (Wei *et al*., 2006), including nutrient cycling (Zak *et al*., 2003) and homeostasis (Carney and Matson, 2005), decomposition of organic matter (Wardle *et al*., 2004; Liao *et al*., 2005), as well as promoting plant health and growth as bio-fertilization (Shen, 1997). It is also clear that plant diversity can influence the characteristics of the soil microbial community through providing suitable habitats and food sources, e.g., litter (Kourtev *et al*., 2003). Legumes enhance soil quality through the significant effect of nitrogen fixation of nitrogen-fixing bacteria (Shen, 1997). Soil disturbance is also a major factor that affects soil microbial communities (Lupwayi *et al*., 1998). The changes in magnitude, structure, and catabolic capability of soil microbial community due to plant diversity, legumes, and soil disturbance may have implications for microbe-mediated processes (Griffiths *et al*., 2001). Measures of microbes responding to aboveground plants could be expected to give insights into ecosystem restoration.

Recently, the effects of aboveground plants on microbial parameters including microbial biomass, community structure and catabolic capacity have been studied (Lupwayi *et al*., 1998; Broughton and Gross, 2000; Malý *et al*., 2000; Kowalchuk *et al*., 2002; Zak *et al*., 2003). However, the results of the studies are controversial. Some studies (Zak *et al*., 2003; Carney and Matson, 2005) found significant effect of plant diversity on soil microbial biomass, community structure and catabolic capacity while other studies (Broughton and Gross, 2000; Malý *et al*., 2000) did not find discernable effect of plant diversity on these microbial parameters. Similarly, positive or zero impact of legumes on microbial biomass or catabolic capability has also been reported (Lupwayi *et al*., 1998; Spehn *et al*., 2000; Kirk *et al*., 2005). Furthermore, varied effects of disturbance due to anthropogenic activities on soil microbial properties have been documented (Lupwayi *et al*., 1998; Degens *et al*., 2001; Lupwayi *et al*., 2001). These discrepancies may result from different research methods or varied sampling sites. To elucidate the effects of aboveground plants on underground microbial communities, further studies are necessary.

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In the present study, polyphasic methods including plate counts, phospholipid fatty acid (PLFA) and Biolog microplate techniques were employed to investigate the microbial biomass, community structure and catabolic diversity in soils beneath five typical plant communities in Jinan, North China, based on the following considerations. First, in the north of China where there is a threat from ecosystem degradation, the study on the relationship between vegetation type and soil microorganisms may give insight into the process of restoration and ecosystem management. Second, the selected five plant communities: soybean field (SF), artificial turf (AT), artificial shrub (AS), natural shrub (NS), and maize field (MF), are common native plant communities which are representative of natural and artificial communities with varied species diversity including legumes and non-legumes, so they can be used to investigate the effects of plant diversity, legumes, and human disturbance on soil microbial properties. In this study, the following main questions were addressed: (1) Are there any consistent effects of the aboveground plant diversity on a range of soil microbial parameters? (2) Is the microbial biomass and catabolic capability higher in the presence of legumes? (3) Is the catabolic capability of the soil microbial community beneath natural vegetation greater than that beneath artificial vegetation?

1 Materials and methods

1.1 Study site and experimental method

Five typical plant communities were selected: SF, AT, AS, NS, and MF. These plant communities were located in Jinan (36°40′N; 117°00′E), North China. The area is located in the North Temperate Zone, and has a continental monsoon climate with four distinctive seasons: dry and rainless in spring, hot and rainy in summer, crisp in autumn, and dry and cold in winter. The annual average temperature is 14°C, and average annual rainfall is 650–700 mm.

The field experiments and soil sample collections were carried out in August 2005. Plant species composition and the projective covers of all plant species in five plant communities were determined. Six soil cores were randomly taken from the top 10 cm of the profile in each plant community and immediately placed in sealed plastic bags on ice. After being sieved through 2-mm mesh, soil samples from the same plant community were mixed and homogenized thoroughly. All soil samples were analyzed within one week of field collection. The basic characteristics of the soil samples were measured using air-dried soils. Total N was estimated by the Kjeldahl method (Kjeldahl, 1883), and available phosphorus by the Olsen method (Homer and Pratt, 1961). Gravimetric soil moisture was determined through weight loss after drying 10–15 g fresh soil at 105°C for 24 h. Diversity indices (Shannon's *S*, *H* and *E*) were calculated from plant species and their projective covers. The basic characteristics of the soil samples and vegetation from five plant communities are shown in Table 1.

1.2 Characterization of the soil microbial community

1.2.1 Microbial plate counts

The numbers of cultural bacteria, fungi, actinomycetes and nitrogen-fixing bacteria were used as an estimate of biomass of certain microorganism populations. Bacteria, fungi, actinomycetes and nitrogen-fixing bacteria were enumerated in three replicates using the method described by Dong (1997) and Wei *et al*. (2006). Bacteria, fungi, actinomycetes and nitrogen-fixing bacteria were cultured on beef extract peptone medium, Martin's medium, Gause's No.1 synthetic medium and Waksman's medium for 2, 3, 7, and 5 d, respectively. The numbers of the four cultured populations were determined according to colony forming units (CFUs) on agar plates.

1.2.2 Phospholipid fatty acid (PLFA) analysis

PLFA analysis was used to gain insight into the biomass of certain microorganism populations and microbial community structure. PLFAs were measured in three replicates using the method of Bligh and Dyer (1959) with modifications by White *et al*. (1979). Lipids were extracted from 3 g of freeze-dried soil using a single-phase mix of chloroform, methanol, and citrate buffer (1:2:0.8 by volume). After suspension, the supernatant was recovered. Then the supernatant was split into two phases by adding chloroform and citrate buffer, and the lower chloroform phase containing the lipids was collected and concentrated to 1 ml through evaporation under a stream of N_2 gas. Neutral lipids, glycolipids and phospholipids were eluted in succession with chloroform, acetone and methanol on Supelclean LC-Si tubes (0.5 g) (Supelco, Bellefonte, PA, USA). The phospholipids were recovered, concentrated and methyl estered. The fatty acid methyl esters (FAME) obtained were concentrated again and stored at –20°C until analysis by gas chromatography (GC).

FAME with a known amount of internal standard methyl nonadecanoate (Sigma-Aldrich Co. Ltd, Poole, UK) analyses was carried out using a VARIAN CP-3380 gas

Table 1 Basic characteristics of soil samples and vegetation from plant communities

Vegetation type	Soil textural class	Total N (%)	Available P (%)	Dominant plant species	*S*	*H*	*E*
SF	Sandy soil	0.244	0.225	*Glycine max*	16	0.425	0.353
AT	Sandy clay	0.033	0.050	*Festuca arundinacea*	11	0.241	0.232
AS	Sandy clay	0.068	0.072	*Rosa chinensis*	6	0.401	0.516
NS	Sandy clay	0.069	0.064	*Vitex negundon* var. *heterophylla* and *Zizyphus Jujuba* var. *spinosa*	22	0.467	0.338
MF	Sandy clay	0.201	0.088	*Zea Mays*	16	0.257	0.205

SF, AT, AS, NS and MF represent soybean field, artificial turf, artificial shrub, natural shrub, and maize field. *S*: Shannon richness; *H*: Shannon diversity; *E*: Shannon evenness indices.

chromatography with a 30 m × 0.32 mm × 0.25 μm capillary column (Varian, Inc. Amherst, MA, USA). A splitless injection was employed (injector at 300°C) and the oven was kept at 150°C for 4 min after injection. The oven temperature was then increased to 250°C at 4°C/min and kept for 6 min. Peaks were qualified and quantified by comparison with the bacterial acid methyl esters CP mix (Supelco, Bellefonte, Pennsylvania, USA) and methyl nonadecanoate. Among 26 PLFAs identified in this study, the following were chosen as bio-indicators. Gram negative bacteria: 12:0, 2-OH 12:0, 3-OH 12:0, 14:0, 15:0, 16:1ω7c, 18:1ω7c; Gram positive bacteria: i15:0, a15:0, i16:0, i17:0; and fungi: 18:2ω6, 9c (Kourtev *et al*., 2003).

1.2.3 Biolog procedure

The catabolic diversity of microbial communities was assessed using the Biolog procedure. Fresh soil sample 10 g (dry weight) in three replicates was dissolved in 100 ml of sterile saline (0.85% NaCl). After 10 min of suspension, the supernatant was recovered. Soil solution was obtained by diluting the supernatant 100-fold. Then, 150 ml aliquots of soil dilution were inoculated into ECO Microplates. The plates were incubated at 28°C for 168 h, and absorbance values of microplate wells were read at 590 nm every 12 h during incubation using the Biolog Microplate Reader (Biolog, Hayward, CA).

Absorbance values after 72 h of incubation were used to calculate indices. Raw absorbance values of test wells were corrected by subtracting the absorbance value of the control well, and the negative corrected values were set to zero. For principal components analysis, the corrected values were normalized as the quotient of values divided by the average well color development (AWCD) value. The Shannon diversity (H) index of catabolic diversity was calculated according to the equation:

$$H = -\sum p_i \ln p_i \quad (1)$$

where, p_i is calculated by dividing the corrected value by the total color change recorded for all 31 substrates. The Shannon evenness (E) index of catabolic diversity was calculated according to the equation:

$$E = H/\ln S \quad (2)$$

where, S (Shannon richness) was the number of substrates used by the microbial communities

1.3 Statistical analyses

Principal component analysis (PCA) based on correlation matrix and cluster analysis (single linkage algorithm based on Euclidean distances) was carried out to discriminate soil microbial communities beneath the five vegetation types. Classification of PLFAs and that of the substrates in microplates were also made with PCA. The PLFAs were more abundant and the substrates that were utilized more markedly beneath a vegetation type can be found by their positions that correspond to the position of the vegetation type in PCA scatter plots. Differences among microbial communities were inspected using Fisher's least significant difference multiple comparisons. Spearman nonparametric correlation was made among various parameters. The above statistical analyses were done using STATISTICA software. Plant diversity indices were calculated with BioDiversity Pro. Software.

2 Results

2.1 Numbers of cultured microbial populations

In general, the amount of cultured fungi was about three orders of magnitude lower than those of other microorganism populations in each soil (Table 2). The numbers of the four microorganism populations beneath SF were all higher compared to those beneath the other vegetation types. Except for SF, there were no significant differences in the numbers of bacteria and actinomycetes among the four vegetation types. However, among the four vegetation types expect for SF, the number of fungi was higher beneath AT and the number of nitrogen-fixing bacteria was higher beneath MF ($P < 0.05$). No correlations were found between the numbers of each cultured microbial population and Shannon diversity indices of vegetation.

2.2 Microbial community structure

Biomasses of Gram negative bacteria, Gram positive bacteria, and fungi in five soils are shown in Fig.1. Biomasses of Gram negative and Gram positive bacteria were both higher beneath the two agricultural fields than beneath the other three vegetation types, although the differences were not statistically significant for all vegetation types. However, the biomass of fungi in the soil beneath NS was the highest among these five soils. No correlations were found between Shannon diversity indices of vegetation and the biomass of microbes represented by PLFAs. Similarly, no correlations were found in the

Table 2 Quantities of four microbial populations beneath different vegetation types

Vegetation type	Bacteria (10^6/g dry soil) plate counts	Fungi (10^3/g dry soil) plate counts	Actinomycetes (10^6/g dry soil) plate counts	Nitrogen-fixing bacteria (10^6/g dry soil) plate counts
SF	13.76± 2.81^a	9.53 ± 1.20^a	4.05 ± 0.35^a	3.75 ± 0.18^a
AT	2.41 ± 0.85^b	7.87 ± 0.71^a	1.14 ± 0.22^b	1.20 ± 0.10^c
AS	2.31 ± 0.44^b	2.77 ± 0.59^b	2.11 ± 0.57^b	0.94 ± 0.07^c
NS	5.35 ± 3.85^b	3.74 ± 0.14^b	1.33 ± 0.04^b	1.26 ± 0.19^c
MF	4.39 ± 0.80^b	3.73 ± 0.14^b	2.53 ± 0.73^b	1.85 ± 0.02^b

Values are means ± SE. Means followed by the same superscript within treatment categories are not significantly different (Fisher PLSD test, $P < 0.05$). Refer to Table 1 for definitions of vegetation types.

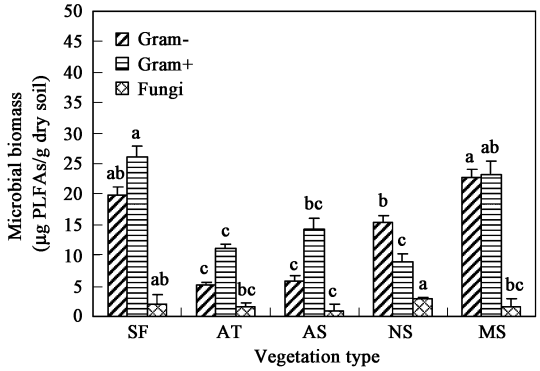

Fig. 1 Microbial biomass represented by total concentration of PLFAs contributed to various microbial communities (Gram negative bacteria, Gram positive bacteria and fungi). Bars with different letters are significantly different in Gram negative bacteria, Gram positive bacteria and fungi among different vegetation types (Fisher PLSD test, $P<0.05$). Values are means +SE. Refer to Table 1 for definitions of vegetation types.

biomass of bacteria or fungi between plate counts and PLFA analysis.

The PLFA data from five sites were subjected to PCA (Fig.2a). PC1 and PC2 accounted for 37.25% and 27.17% of total variation respectively, and their eigenvalues were 5.59 and 4.08 respectively. The agricultural fields (SF and MF) differed from the other three vegetation types (AS, AT and NS) on PC1 dimension ($P < 0.05$). Among AS, AT and NS plots, NS differentiated from the others on PC2 dimension ($P < 0.05$). Cluster analysis (Fig.2b) shows that the microbial community structure beneath NS was different from those beneath the other vegetation types. The factor loadings plot (Fig.2c) shows that the PLFAs which were more abundant beneath NS than beneath the other vegetations included 10:0 2-OH, 18:2ω6, 9c, i15:0 and 14:0, while PLFAs like 14:0 3-OH were more abundant beneath the other vegetation types than beneath NS. Those PLFAs (e.g. a15:0, 18:1ω9c, 16:0 and i16:0) were more abundant beneath the two agriculture fields than beneath the other vegetation types.

2.3 Microbial community catabolic diversity

Biolog data from the five sites were subjected to PCA (Fig.3a). PC1 and PC2 accounted for 34.60% and 25.35% of total variation respectively, and their eigenvalues were 10.73 and 7.89 respectively. Biolog PC1 differed AT from the other four vegetation types ($P < 0.05$). Biolog PC2 differentiated MF from the other four vegetation types ($P < 0.05$). These vegetation types were classified into three categories by cluster analysis of Biolog data (Fig.3b): AT, MF and the rest of the vegetation types. The factor loadings plot (Fig.3c) shows that substrates such as phenylethylamine (G4), putrescine (H4) and 4-hydroxy benzoic acid (D3) were utilized more beneath AT than beneath the other vegetation types. The substrates which were utilized more by microorganisms beneath MF than beneath the other vegetation types included *i*-erythritol (C2), *d*-cellobiose (G1) and *d*,*l*-α-glycerol phosphate (H2). However, those substrates including *d*-xylose (B2), β-methyl-*d*-Glucoside (A2), *d*-mannitol (D2) and N-acetyl-*d*-glucosamine (E2)

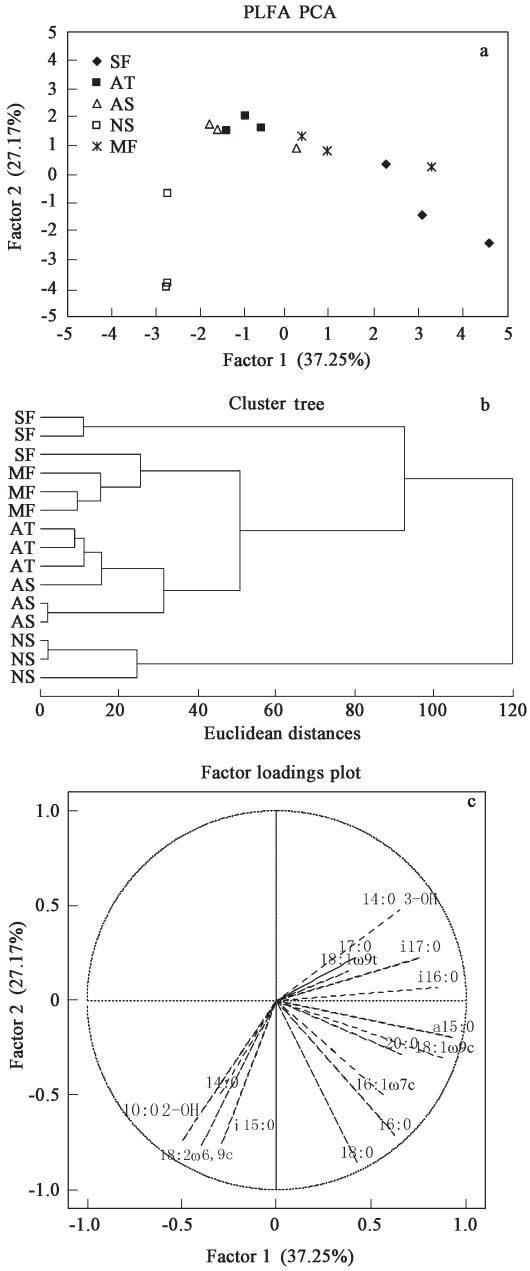

Fig. 2 Classification of treatments by principal component analysis (PCA) (a), cluster analysis (b) with PLFA data, and the factor loadings plot in PCA (c). Refer to Table 1 for definitions of vegetation types.

were utilized less beneath AT and MF than beneath the other three vegetation types.

The Shannon indices of catabolic diversity beneath

Table 3 Shannon richness *S*, diversity *H*, and evenness indices *E* calculated from the Biolog data

Soil sample	*S*	*H*	*E*
SF	27.33 ± 1.33^{a}	3.21 ± 0.03^{a}	0.97 ± 0.01^{a}
AT	19.33 ± 1.86^{c}	2.80 ± 0.05^{c}	0.95 ± 0.01^{ab}
AS	22.00 ± 0.00^{bc}	2.90 ± 0.04^{bc}	0.94 ± 0.01^{b}
NS	24.33 ± 1.20^{ab}	3.11 ± 0.03^{a}	0.97 ± 0.01^{a}
MF	22.33 ± 0.67^{bc}	2.98 ± 0.01^{b}	0.96 ± 0.01^{ab}

Values are means ± SE. Means followed by the same superscript within treatment categories are not significantly different (Fisher PLSD test, $P < 0.05$). Refer to Table 1 for definitions of vegetation types.

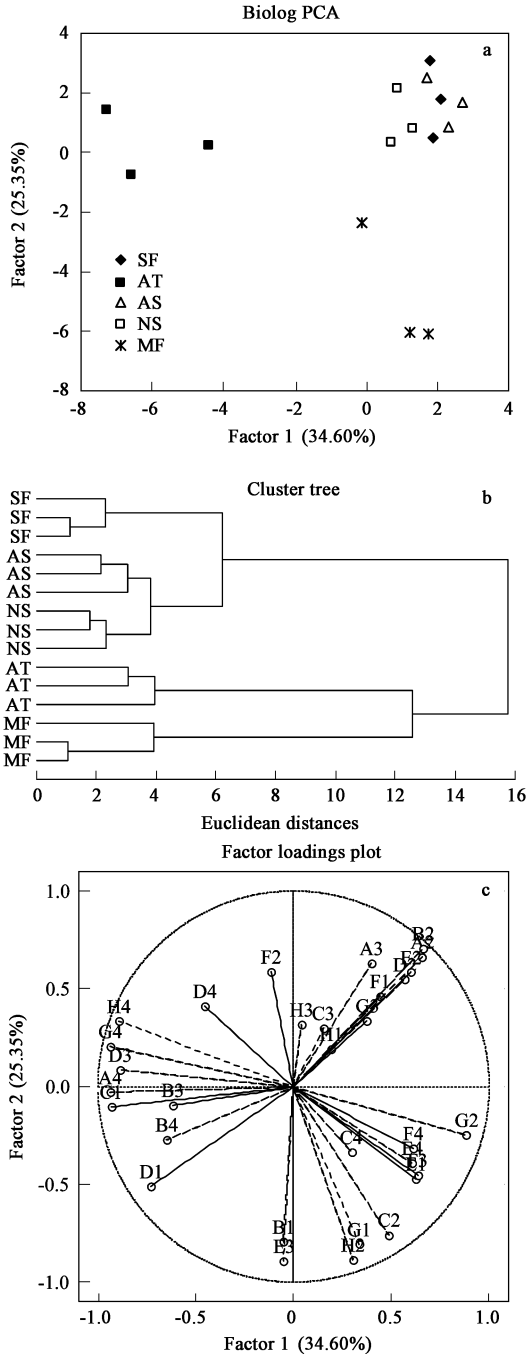

Fig. 3 Classification of treatments by principal component analysis (PCA) (a), cluster analysis (b) with Biolog data, and the factor loadings plot in PCA (c). Refer to Table 1 for definitions of vegetation types.

SF and NS were higher than those beneath the other vegetation types, although the differences were not statistically significant for all vegetation types. There was no significant difference between SF and NS. The lowest Shannon indices appeared in AT or AS (Table 3). A positive correlation was found when linking evenness index of catabolic diversity to richness index of plant diversity, i.e. the number of plant species (R^2 = 0.86, P < 0.05). Meanwhile, a positive but decelerating correlation was also found between catabolic diversity index (H) and the number of cultured bacteria (R^2 = 0.56, P < 0.01) (Fig.4).

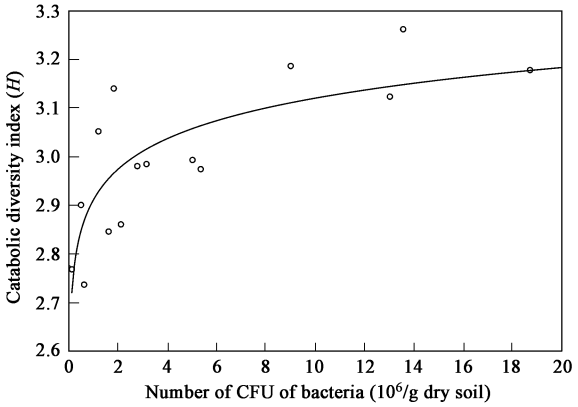

Fig. 4 Relationship between the catabolic diversity index (H) and number of CFU of bacteria. The equation describing the best-fit line is: H = 1.6559 + 0.2092 × lg (CFUs of bacteria) (n = 15, R^2 = 0.56, P < 0.01).

3 Discussion

The effects of plant diversity on microbial biomass and catabolic capability are controversial (Broughton and Gross, 2000; Malý *et al*., 2000; Carney and Matson, 2005). The present result shows that there was no discernible effect between plant diversity and the numbers of the cultured microbial populations beneath five vegetation types in Jinan. However, a positive correlation was found between the evenness index of catabolic diversity in soil and the richness index of plant diversity. Plant litter provides the habitat, food and energy for microorganisms in the soil. The microbial biomass depends on the quantity of the available supplies that are not determined by plant diversity. Nevertheless, the microbial physiological group that utilizes special nutritious resource is restricted by the plant species that control the quality of available resources (Wardle *et al*., 2004). High number of plant species can produce a high diversity of litter and consequently causes the formation of a species-rich microbial community (Zak *et al*., 2003; Bartelt-Ryser *et al*., 2005) that leads to the even utilization of the substrates.

Besides plant diversity, the specific plant functional group such as legumes was also considered as an important factor influencing the soil microbial community (Spehn *et al*., 2000; Scherer-Lorenzen *et al*., 2003). In this study, it is noteworthy that the numbers of four types of cultured microorganisms, microbial biomass represented by PLFAs, and microbial community catabolic diversity in SF were very high. The result indicated that it was the presence of a particular functional group i.e. legumes that was responsible for stimulating microbial community size and function. These results are in agreement with earlier studies that found microbial biomass, respiration, and catabolic activity could be stimulated when legumes were present (Lupwayi *et al*., 1998; Spehn *et al*., 2000). Aboveground plants can provide nutritious resources for soil microbial communities, so it is likely that soil microbial communities respond differently to various plant diversity and identity, especially specific plant functional groups such as legumes which can enhance litter quality by increasing organic matter and decreasing the C/N ratio

(Drinkwater *et al*., 1998; Schutter and Dick, 2000).

Soil disturbance was another factor that may affect soil microbial communities (Lupwayi *et al*., 1998). In this study, the biomasses of bacteria beneath two agricultural fields were higher while the biomass of fungi was higher in the soil beneath NS than other soils, which is consistent with earlier studies (Moore, 1994; Hedlund, 2002) that found fungi would account for a larger proportion in less disturbed soils than in frequently disturbed soils. Fungi and bacteria differ in their responses to changes in agricultural management practices. Fungi apply plant residues as mulch, thus, fungi beneath NS prosper through their hyphal growth into the litter layer. However, tillage destroys the fungal hyphae and incorporates plant residues into the soil, thereby bacteria in agricultural fields thrive because the contact surface between the substrate and bacteria is increased. Except for SF which was significantly influenced by legumes, the catabolic diversity, substrate richness, and substrate evenness beneath less disturbed NS were higher than beneath the other vegetation types, although the differences were not statistically significant for all vegetation types. The lowest catabolic diversity indices appeared in AT or AS. In less disturbed soils, different microbial populations interact with each other and over time they can combined into a compatible community which can utilize more substrates. However, in frequently disturbed soils, the previous equilibrium is broken which will lead to less utilization of substrates. The low catabolic diversity due to anthropogenic activities could lead to the reduction in resistance of microbial communities to stress or disturbance (Degens *et al*., 2001). So, the stability, adaptability, and resilience of microbial communities beneath natural vegetation are greater than those beneath artificial vegetation.

The relationship between plant diversity and ecosystem function has received much attention in ecology (Loreau *et al*., 2001; Bell *et al*., 2005), but the relationship between the number of microorganism and microbial function is often ignored. In the present study, there was a significant correlation ($R^2 = 0.56$, $P < 0.01$) between the number of cultured bacteria and their function expressed by catabolic diversity of the bacterial community. The number of cultured bacteria posed a positive but decelerating impact on the catabolic diversity of the bacterial community. It demonstrates the existence of bacterial functional redundancy with increasing number of bacteria in soils. There was a decelerating relationship between microbial community function and increasing species richness (Bell *et al*., 2005) and the microbial community with a high number of organisms has a great chance of including more species. Thus, it is reasonable that catabolic diversity increases at a decelerating rate with an increase in the number of bacteria.

Different research methods usually lead to varied results, which were also found in this study when characteristics of soil microbial community were assessed with different analytical techniques. First, there were no correlations in the biomass of bacteria or fungi between plate counts, a culture-based method, and PLFA analysis, a direct extraction technique. This reflects the discrepancy between the culture-dependent method and the culture-independent method. Second, PLFA profiles and Biolog profiles of the five vegetation types could be distinguished but in different patterns. PLFA profiles distinguished the microbial community structure beneath NS from those beneath the other vegetation types; however, Biolog profiles classified the microbial community function into three categories i.e. AT, MF and the rest of the vegetation types. The result suggests that different vegetation types associated with specific litter foster corresponding microbial community. But the shifts are inconsistent between microbial community structure and microbial community function. Many previous studies also showed discrepancies in microbial parameters especially between microbial community structure and function, both of which were obtained by different approaches (Embley and Finlay, 1994; Buyer and Drinkwater, 1997; Widmer *et al*., 2001). So, the combination of several microbial approaches is required to obtain accurate information on the characteristics of the soil microbial community in many respects (Widmer *et al*., 2001). Plants, the soil, and soil microorganisms interact and accordingly construct a holistic system (Wardle *et al*., 2004). The soil microbial community may in turn affect the growth and composition of the aboveground plant community through controlling decomposition of organic matter (Wardle *et al*., 2004; Liao *et al*., 2005) and element cycling (Zak *et al*., 2003; Wardle *et al*., 2004). The interplay of plants and soil microorganisms determines the process of ecosystem restoration (Zak *et al*., 2003; Carney and Matson, 2005). This study of the relationship between vegetation type and soil microbial community is expected to give some insights into ecosystem restoration in the north of China.

4 Conclusions

Vegetation types influence the soil microbial community magnitude, structure, and catabolic diversity through difference of plant diversity, legumes, and human disturbance. The present study confirmed that plant diversity and natural vegetation types had a positive effect on soil microbial function in terms of catabolic capability. Legumes could significantly enhance soil microorganisms in many respects including the number of cultural microorganisms, microbial biomass, and community catabolic capability. It implied that high plant species richness, legumes, and natural vegetation types support soil microbial community with higher catabolic diversity. The study of the effects of vegetation type on soil microorganisms has implications for ecosystem restoration in the north of China.

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