African Journal of Biotechnology Vol. 10(73), pp. 16554-16565, 21 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1682
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Soil microbial community response to land use and various soil elements in a city landscape of north China

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Accepted 1 April, 2011

A field experiment in north China was conducted to study the effects of land use and soil elements at a moderate concentration on the amounts of various microbial populations, microbial community structure and catabolic activity which were analyzed by cultural method, phospholipid fatty acid (PLFA) and biolog techniques, respectively. The results showed that, land use type might be the primary control on soil microbial community structure. Legumes or soil N was hypothesized as the secondary factor influencing the soil microbial community structure. Legumes played an important role in stimulating the growth and reproduction of various soil microbial populations, accordingly promoting the microbial catabolic activity. Plant diversity had a significant positive effect on the amount of soil bacteria. Soil Cd at moderate concentration had a significant positive effect on microbial biomass. The majority of soil elements at moderate concentration except for Cr had no marked effects on microbial catabolic activity.

Key words: Biolog, land use, microbial biomass, phospholipid fatty acid, soil element.

INTRODUCTION

Soil microbes play a central role in many critical ecosystem processes (Wei et al., 2006), including geochemical cycling (Zak et al., 2003) especially nitrogen and carbon cycling (Wardle et al., 2004; Liao et al., 2005; Ushio et al., 2008) and homeostasis (Carney and Matson, 2005), as well as promoting plant health and growth as biofertilization (Shen, 1997). On the other hand, soil microbes are influenced by land use as well as the elemental composition in the soils they inhabit. It is clear that, land use can influence the characteristics of the soil microbial community by providing habitats and food sources, for example, litter (Kourtev et al., 2003). Soil elements provide nutrients for the growth of microbes.

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Abbreviations: AWCD, Average well color development; **PLFA,** phospholipid fatty acid; **MPN,** most probable number; **FAME,** fatty acid methyl esters.

Some heavy metals at high concentration have adverse effects on cellulose decomposition, carbon mineralization, nitrogen cycling, enzyme activity, size, biomass and the structure and diversity of the microbial community (Ghosh et al., 2004; Renella and Mench, 2004; Shentu et al., 2008; Li et al., 2009). Land use including secondary shrub forest, cultivated and urban plant communities, legumes and plant diversity as well as soil elements including composition and content, alone and in concert, can influence the characteristics of the soil microbial community and vice-versa. The driven changes in magnitude, activity, structure and function of soil microbial community may have implications for microbially-mediated ecosystem processes (Griffiths et al., 2001). An understanding of microbes responding to land use and soil elements could be expected to give insights into both ecosystem restoration and health evaluation.

Recently, the effects of land use on microbial parameters including microbial biomass, community structure and catabolic capacity have been studied. Chen and He (2003) investigated the microbial biomass in eroded fallow

land, woodland, tea garden, citrus grove, fallow grassland, vegetable field and paddy field. The lowest microbial biomass was found in eroded fallow land and the highest in vegetable and paddy fields. Xue et al. (2008) assessed soil microbial community characteristics in a chrono-sequence of tea garden systems (8-, 50-, and 90-year-old tea gardens), an adjacent wasteland and a 90-year-old forest and found that land-use change had a greater effect on soil microbial community structure than tea garden age. Steenwerth et al. (2002) evaluated soil microbial community composition for irrigated and non-irrigated agricultural sites, non-native annual grasslands and relict, never tilled or old field perennial grasslands and regarded that, a given land use type could be identified by soil microbial community composition. The study by Jangid et al. (2008) indicated that, the structure and composition of bacterial communities in forest soil were significantly different from that in agricultural soils. Li et al. (2007) analyzed the average well color development (AWCD) and thus, functional diversity indices of microbial community under different land uses in the subtropical region of China using the biolog technique. The result showed the following order: Paddy fields > fruit trees > forestry. However, few studies have focused on north China especially within city areas where there is a threat from ecosystem degradation. In contrast with aboveground communities, few studies were focused on soil microbial populations on restoration or rehabilitation projects despite the essential functions they perform (DeGrood et al., 2005). To elucidate the effects of different land uses on soil microbial communities in a city landscape of north China and therefore, give insights into restoring local ecosystem and evaluating soil health, further studies are necessary.

The effects of high concentrations of heavy metals on soil microbial community characteristics have been investigated in some recent studies (Knight et al., 1997; Shi et al., 2002; Yao et al., 2003; Dai et al., 2004; Schipper and Lee, 2004; Liao et al., 2005). However, there has been a lot of research looking at heavy metals in mine waste or ultra mafic soils. In general, heavy metals in soils exist under moderate concentration levels. The effects of heavy metals at moderate concentration were not well elucidated (Wei and Yang, 2010). Except for soil heavy metals, it is also clear that soil microbes were influenced by other soil elements. Thus, the studies of the effect of various soil elements under moderate conditions on the soil microbial community parameters are necessary. Our research area focuses on low level of heavy metal contamination and therefore, is appropriate to use to investigate the drivers of microbial biomass, community structure and catabolic activity under lightly polluted environments.

Traditional cultural method has been and frequently is still used for quantification of certain microbial populations (Tabacchioni et al., 2000; Luo et al., 2004; Triolo et al., 2008). Phospholipid fatty acid (PLFA) analysis provides a broad based description of the entire microbial community with information obtained about viable biomass concentrations and community composition. (Bligh and Dyer, 1959; Ringelberg et al., 1989; Werker and Hall, 2001). Biolog microplate technology is increasingly used to characterize microbial communities by determining the ability of the communities to utilize different sole carbon sources (Smalla et al., 1998) and has been applied to assess catabolic activity of microbial community from various environments, since Garland and Mills (1991) introduced it to characterize and classify mixed microbial communities. In this study, the earlier stated methods were employed to investigate changes in microbial biomass, community structure and function in a series of temperate soils in north China and accordingly, determine the effects of land use and soil elements on characteristics of soil microbial community. The following questions were addressed: First, what are the most important drivers in land use for a range of soil microbial parameters? Second, are there detectable effects of various soil elements at moderate concentration on soil microbial community?

MATERIALS AND METHODS

Study region

Soil samples were collected in Jinan city (117°00′E, 36°40´N, with an area of approximately 3257 km^2), Shandong province, north of China (Figure 1). The area is situated 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 to 700 mm.

Plant investigation

In this study, sixteen sampling sites were selected every kilometer from northwest to southeast along the beeline (spanning from 117°1′18″E to 117°8′20″E and from 36°43′55″N to 36°37′11″N) through Jinan city of China in August, 2005. These sampling sites from northwest to southeast are given numbers as J01, J02, …, J16 in sequence (Figure 1). All sixteen sampling sites were on luvisols derived from limestone material. There were eight land use types where urban wasteland (J01 and J04), parkland (J03, J06 and J10), residential grass garden (J05 and J09) and flowerbed (J07) were classified as urban plant community; soybean field (J02), maize field (J12, J14, J15 and J16) and maize and soybean mixed field (J13) were considered as cultivated plant community; site J08 and J11 were secondary shrub forests. The plant species composition and projective covers of all plant species in each sampling site were recorded. The Shannon richness (S_p), diversity (H_p) and evenness (E_p) indices of plant diversity were calculated from plant species and their projective covers (Table 1).

Soil sampling and processing

Three replicates were taken for each sampling site. One replicate consisted of six soil cores taken randomly from the surroundings of each spot. All soil cores were taken from the top 10 cm of the profile

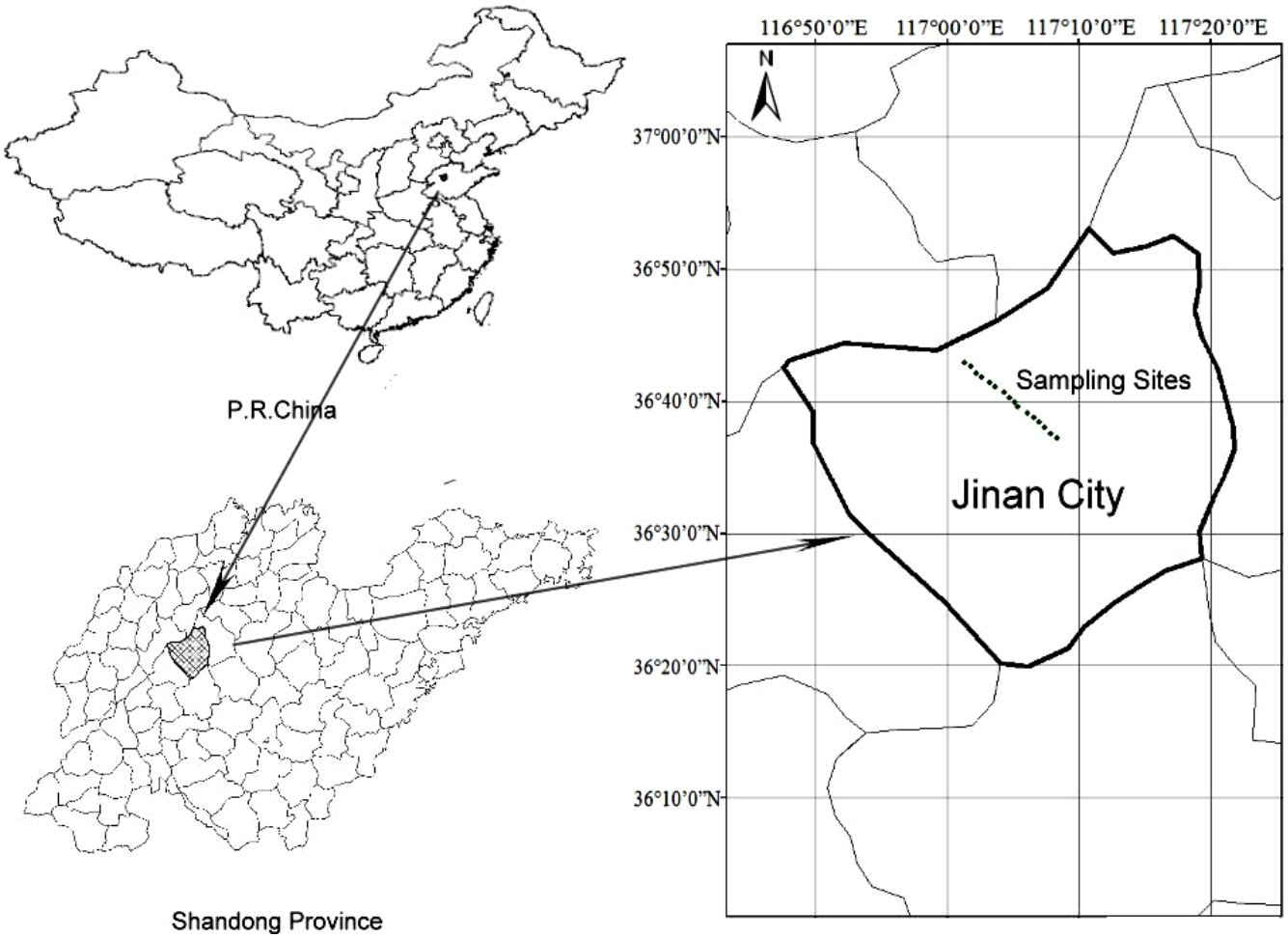

Figure 1. Locations of the study region and sampling sites from different land use types. The sixteen sampling sites from northwest to southeast are given numbers as J01, J02, …, J16 in sequence. The basic characteristics of sampling sites see Table 1.

Table 1. The basic characteristics of land use from sampling sites.

Site no.	Land use type	Soil group	S_p	H_p	E_p
J01	Urban wasteland	Luvisols	14	1.827	0.692
J02	Soybean field	Luvisols	16	0.978	0.353
J03	Parkland	Luvisols	8	1.113	0.535
J04	Urban wasteland	Luvisols	8	1.748	0.841
J05	Residential grass garden	Luvisols	6	1.609	0.898
J06	Parkland	Luvisols	6	1.457	0.813
J07	Flowerbed	Luvisols	6	1.071	0.598
J08	Secondary shrub forest	Luvisols	11	2.112	0.881
J09	Residential grass garden	Luvisols	31	2.835	0.825
J10	Parkland	Luvisols	18	1.100	0.381
J11	Secondary shrub forest	Luvisols	24	1.353	0.426
J12	Maize field	Luvisols	17	0.526	0.186
J13	Maize and soybean mixed field	Luvisols	9	1.087	0.495
J14	Maize field	Luvisols	14	0.581	0.220
J15	Maize field	Luvisols	18	0.592	0.205
J16	Maize field	Luvisols	20	0.518	0.173

Sp, Hp and Ep represent the Shannon richness, diversity and evenness indices of plant diversity, respectively.

and immediately placed in sealed plastic bags on ice. After being sieved through 2 mm mesh, the soil cores from the same spot were mixed and homogenized thoroughly to form one composite spot sample. All soil samples were conducted for analyses within one week after field collection.

Soil elements analysis

Concentrations of 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 in soils were determined using standard analytical techniques as described in the technical specification for soil environmental monitoring (HJ/T 166-2004) by Wuhan synthetical analytical centre of rock and minerals. Briefly, concentrations of Co, Mn, Ni, V, Fe_2O_3, MgO and CaO were quantified by inductively coupled plasmaatomic emission spectroscopy (ICPS-7510, Shimadzu, Japan). Concentrations of As, Hg and Se were quantified by hydride generation-atomic absorption spectroscopy (AFS-230E, Haiguang, China). Concentrations of Cr, Cu, P, Zn, SiO_2, Al_2O_3, Na_2O and K_2O were quantified by x-ray fluorescence (Spectroscan makc-GV, Spectron, USA). Concentrations of B and Pb were quantified by emission spectrometry (WP-1, Modern Rayleigh, China). Concentrations of Cd were quantified by flame atomic absorption spectrophotometry (AA-6800, Shimadzu, Japan). Concentrations of F were quantified by ion-selective electrode (ISE, Thermo Orin, USA). Concentrations of Mo was quantified with polarography (797 VA Computrace, Metrohm, Switzerland). N and S were analyzed with the distillation method and the combustion method, respectively (Li, 1983). The concentrations of soil elements from the sampling sites are shown in Table 2.

Enumeration of soil microbes

Bacteria, fungi, actinomycetes, nitrogen-fixing bacteria, nitrifying bacteria, denitrifying bacteria and cellulose decomposing bacteria were cultured and enumerated using the method described by Dong (1996). Briefly, bacteria, fungi, actinomycetes and nitrogen-fixing bacteria were counted as colony forming units (CFUs) on particular agar plates, the others were counted with most probable number (MPN) method. The amounts of the stated microbial groups were used as an estimate of the biomass of certain microbial population.

PLFA analysis

PLFAs were measured 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/citrate buffer (1:2:0.8 by volume). The supernatant was recovered and split into two phases by adding chloroform and citrate buffer. The lower chloroform phase containing the lipids was collected and evaporated under a stream of N_2 gas. After a series of elution with chloroform, acetone and methanol on Supelclean LC-Si tubes (0.5 g) (Supelco, Bellefonte, PA, USA), the phospholipids were recovered, evaporated and methyl estered. The resulting fatty acid methyl esters (FAME) was dissolved in hexane and transferred to a Varian CP-3380 gas 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 as an inner standard. PLFA analysis was used to gain insight into microbial community structure.

Biolog procedure

10 g (dry weight) fresh soil sample was serially diluted in 100 ml sterile saline (0.85% NaCl) until a final dilution of 1:1000 was obtained. Then, 150 µl aliquots of this dilution were added to each well of the 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 after incubation using biolog microplate reader (Biolog, Hayward, CA). Raw absorbance values of test wells were corrected by subtracting absorbance value of the control well and the negative corrected values were set to zero. The AWCD at 72 h of incubation was calculated and used as the indicator of catabolic activity of soil microbial community.

Statistical analyses

Principal component analysis (PCA) based on correlation matrix was carried out to discriminate between the soil microbial community structures from sixteen sampling sites.
Various microbial parameters between the sites were compared at the 5% level of significance using Fisher's least significant difference multiple comparisons. Pearson correlation was made among various microbial parameters and plant diversity indices. Stepwise multiple linear regression analysis was made to find the key soil elements affecting microbial parameters. The stated statistical analyses were done using STATISTICA software. Plant diversity indices were calculated with Biodiversity Pro. software.

RESULTS

Characteristics of soil elements in sampling sites

Table 2 shows that the concentrations of soil elements were mostly under the threshold limit value of secondary environmental quality standard for soil in China (National Environmental Protection Agency of China, 1995, which applies to soils in farmland, orchards and rangeland). Total Cd concentrations in soil from J02 and J09 sites exceeded the standard limit by 91.3 and 4.6%, respectively. The concentrations of soil total Cu at J03 and soil total Ni at J15 exceed the standard limit by 1.6 and 9.25%, respectively. Among the sixteen sampling sites, the concentrations of soil total Cr at three sites (J01, J02 and J03) in the northwest area were high and the concentrations of soil total Cr decreased gradually from northwest to southeast as a result of a chemical plant located northwest.

Soil microbial community structures in sampling sites

The PLFA profiles in soil were used to discriminate between the total microbial community structures in sixteen soils by principal component analysis (Figure 2a, b). PC1, PC2 and PC3 accounted for 24.20, 20.58 and 12.29% of total variation, respectively, and their Eigen values were 4.11, 3.50 and 2.09, respectively. The PLFA profiles in the two secondary shrub forests (J08 and J11) discriminated from the other fourteen sampling sites

Table 2. The concentrations of soil elements from sampling sites (As-Se: mg kg^{-1}, N-K_2O: %).

Soil element	J01	J02	J03	J04	J05	J06	J07	J08	J09	J10	J11	J12	J13	J14	J15	J16
As	13.3	15.2	12.5	10.8	11.8	13.1	10.4	9.3	10.5	8.9	10.1	11.2	10	10.6	10.2	10.3
B	44.3	48	65.1	57.1	55.8	53.7	59.4	161	57	55.5	76.8	59.3	50.8	69.1	168.7	59.9
Cd	0.2	0.574*	0.28	0.093	0.168	0.134	0.177	0.164	0.314*	0.136	0.089	0.169	0.211	0.191	0.144	0.116
Co	10.8	11.6	13.3	13	15.1	16.2	16.5	11.6	14.2	12.8	13.1	11.2	14.2	13.4	15.7	13.8
Cr	300.5	288.6	151.9	71.3	85.2	83.1	86.2	71.4	76	65.8	62.7	69.3	72.2	71.8	142.2	65.4
Cu	24.1	38.4	50.8*	28.7	41.4	23.7	28.5	23.2	27.7	21.6	24.3	23.1	25.4	24.9	23.4	21.8
F	597	577	639	471	481	516	536	596	805	485	606	529	523	545	712	571
Hg	0.072	0.26	0.28	0.104	0.39	0.034	0.026	0.043	0.065	0.039	0.026	0.419	0.057	0.131	0.013	0.029
Mn	560	566	582	545	545	723	711	483	730	609	563	587	608	587	570	614
Mo	0.8	0.99	1.2	0.69	0.97	0.42	0.52	0.47	0.7	0.56	0.54	0.51	0.59	0.71	0.54	0.56
Ni	26.7	28.3	29.7	29.1	35.5	33.8	33.6	24.7	25.2	26.6	27.5	23.3	33.8	28.6	43.7*	28.9
P	797	2252	2575	934	1528	496	717	680	820	611	644	830	946	1010	878	581
Pb	35.2	49.8	69.3	25.2	45	21.1	28.7	22.3	32.5	23.1	18.6	24.8	27.7	29.2	19.1	24.1
V	75.4	80.6	81.8	79.3	85.1	93.2	99.5	73.7	81.4	74.9	94.9	78.2	80.1	81.6	90.9	81.4
Zn	70.2	111.2	158.3	65.3	107.6	60.5	89.6	47.8	103.8	62.9	56	79.7	98.4	151.6	55.6	62.3
Se	0.66	0.68	1.11	0.19	0.64	0.08	0.23	0.46	0.46	0.16	0.18	0.29	0.31	0.32	0.31	0.16
N	0.059	0.244	0.164	0.048	0.098	0.033	0.068	0.102	0.121	0.053	0.069	0.091	0.076	0.118	0.201	0.07
S	0.225	0.07	0.088	0.02	0.044	0.006	0.024	0.005	0.049	0.025	0.007	0.015	0.013	0.014	0.012	0.011
SiO_2	57.41	54.84	48.25	60.24	57.57	57.24	61.44	43.84	56.29	60.81	56.39	58.22	56.65	51.66	45.57	59.92
Al_2O_3	11.92	11.49	13	13.03	13.58	13.75	14.87	10.69	13.27	13.2	14.7	13.23	12.99	12.77	11.51	13.71
Fe_2O_3	4.16	4.19	4.43	4.16	4.44	4.82	5.43	4.07	5.07	4.18	4.74	4.38	4.41	4.4	4.47	4.62
MgO	2.32	2.25	2.14	2.01	1.91	2.14	1.96	2.93	2.01	1.91	1.81	2.02	2.17	2.39	3.17	2.06
CaO	6.66	7.11	10.65	5.11	5.5	5.35	2.27	17.07	5.75	4.5	6.35	5.21	6.37	8.98	13.46	4.22
Na_2O	1.87	1.82	1.38	1.45	1.59	1.26	1.27	1.03	1.28	1.48	1.17	1.43	1.46	1.21	1.13	1.32
K_2O	2.2	2.15	1.96	2.25	2.12	2.29	2.5	1.47	2.21	2.34	2.24	2.29	2.33	2.25	2.04	2.4

*Element contents exceed the threshold limit value of secondary environmental quality standard for soil in China (National Environmental Protection Agency of China, 1995, which applies to soils in farmland, orchards and rangeland).

which were classified as cultivated or urban plant communities and they differed from each other in ordination plot of PC1 versus PC2 (Figure 2a). Of the fourteen cultivated or urban plant communities, the PLFA profiles also revealed the differences in soil microbial community structure among soybean field (site J02) one maize field (J15) and the other twelve sampling sites in ordination plot of PC1 versus PC3 (Figure 2b).

Amounts of soil microbial populations and catabolic activity of whole community in sampling sites

Table 3 shows that bacteria, actinomycetes, nitrogen- fixing bacteria and denitrifying bacteria were dominant microbial populations in soils in our research area. The amounts of the four microbial populations were all higher than those of the other three ones (fungi, nitrifying bacteria and cellulose decomposing bacteria) significantly ($P < 0.05$). Among the sixteen sampling sites, the amounts of cultured bacteria, actinomycetes, nitrogen-fixing

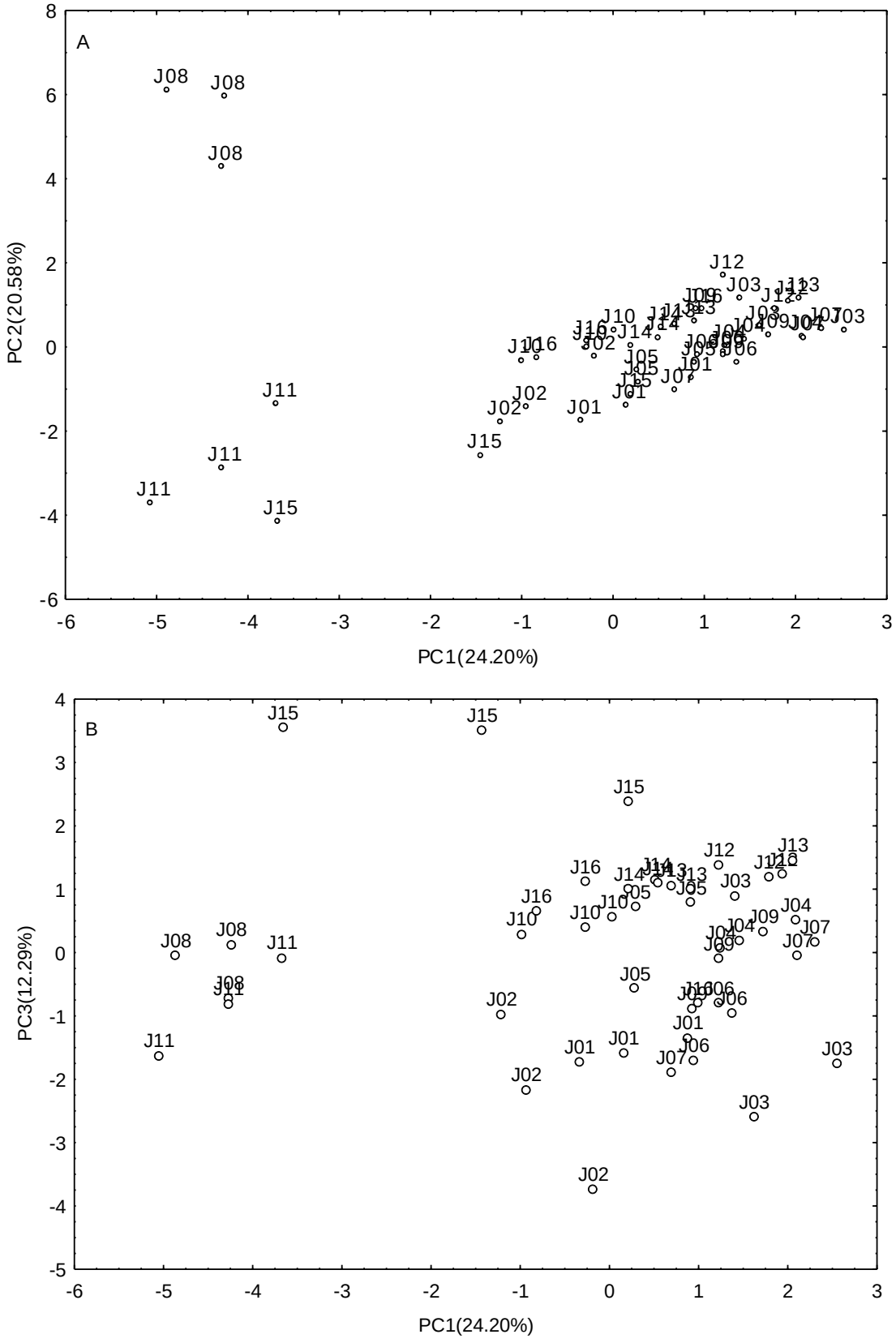

Figure 2. Ordination plots of PC1 versusPC2 (A) and PC 1 versus PC3 (B) of soil PLFA profiles in 16 sites. Values in parentheses indicate the percentage of total variation accounted for by each principal component axis.

Table 3. The amounts of seven cultured microbial populations (10^5 g^{-1} dry soil) and AWCD values from sampling sites. Values are expressed as mean (SE).

Site no.	Bacteria	Fungi	Actinomycetes	Nitrogen-fixing bacteria	Nitrifying bacteria	Denitrifying bacteria	Cellulose decomposing bacteria	AWCD
J01	14.314 (1.988)	0.044 (0.004)	7.027 (0.526)	5.465 (0.542)	0.012 (0.000)	3.253 (0.025)	0.124 (0.005)	0.913 (0.065)
J02	137.600 (28.106)	0.095 (0.012)	40.543 (3.459)	37.496 (1.774)	0.663 (0.006)	36.857 (0.226)	1.622 (0.049)	1.067 (0.021)
J03	11.066 (5.503)	0.003 (0.001)	10.074 (2.115)	2.862 (0.132)	0.011 (0.001)	1.088 (0.027)	0.003 (0.000)	0.180 (0.035)
J04	3.174 (1.431)	0.001 (0.000)	8.055 (0.572)	0.119 (0.000)	0.030 (0.000)	0.536 (0.008)	0.030 (0.002)	0.304 (0.031)
J05	33.519 (5.361)	0.052 (0.004)	9.851 (2.710)	8.339 (1.596)	0.552 (0.004)	55.184 (0.512)	0.307 (0.012)	0.716 (0.037)
J06	24.052 (8.541)	0.079 (0.007)	11.374 (2.189)	11.985 (0.971)	0.306 (0.005)	11.618 (0.154)	0.055 (0.002)	0.445 (0.097)
J07	23.052 (4.436)	0.028 (0.006)	21.124 (5.739)	9.388 (0.659)	1.195 (0.018)	5.658 (0.086)	0.566 (0.033)	0.681 (0.044)
J08	10.698 (0.650)	0.031 (0.004)	12.999 (1.778)	12.636 (2.056)	0.005 (0.000)	30.277 (0.173)	0.030 (0.003)	0.636 (0.041)
J09	87.718 (13.027)	0.105 (0.013)	12.102 (0.708)	20.508 (0.722)	0.030 (0.000)	1.827 (0.009)	0.030 (0.001)	0.658 (0.155)
J10	66.989 (7.988)	0.067 (0.003)	19.427 (4.264)	18.296 (2.892)	0.094 (0.002)	11.932 (0.075)	0.031 (0.002)	0.788 (0.045)
J11	53.502 (38.456)	0.037 (0.001)	13.275 (0.418)	12.551 (1.940)	0.115 (0.001)	3.017 (0.022)	0.005 (0.000)	0.827 (0.030)
J12	17.450 (1.430)	0.012 (0.004)	9.102 (0.927)	18.759 (1.926)	0.113 (0.017)	2.974 (0.284)	0.024 (0.008)	0.075 (0.019)
J13	28.135 (3.438)	0.032 (0.006)	20.595 (4.149)	12.886 (3.736)	0.051 (0.002)	16.881 (0.208)	0.028 (0.004)	0.238 (0.035)
J14	43.707 (5.073)	0.021 (0.003)	14.620 (0.483)	0.986 (0.100)	0.005 (0.000)	1.102 (0.008)	0.029 (0.003)	0.264 (0.042)
J15	43.921 (8.032)	0.037 (0.001)	25.303 (7.338)	18.482 (0.168)	0.087 (0.000)	5.247 (0.051)	0.175 (0.005)	0.615 (0.070)
J16	36.211 (4.080)	0.028 (0.009)	9.656 (2.846)	5.092 (1.176)	0.011 (0.000)	5.092 (0.048)	0.002 (0.000)	0.406 (0.065)

bacteria and cellulose decomposing bacteria in soil from J02 were significantly more than other sampling sites ($P < 0.05$). In addition, the amounts of cultured soil fungi, nitrifying bacteria and denitrifying bacteria from J02 were second (Table 4). Similarly, the catabolic activity of microbial community expressed as AWCD at J02 was the highest among all the sampling sites. The AWCD value was higher significantly than other sampling sites except for J01 ($P < 0.05$).

Correlation between various microbial parameters

In Table 5, it is shown that the amount of bacteria was correlated significantly with the amounts of fungi ($R = 0.76$, $P < 0.01$), actinomycetes ($R = 0.73$, $P < 0.01$), nitrogen-fixing bacteria ($R = 0.80$, $P < 0.01$) and cellulose decomposing bacteria ($R = 0.68$, $P < 0.01$). There were significant positive correlations between the amounts of nitrogen-fixing bacteria and fungi ($R = 0.69$, $P < 0.01$) and actinomycetes ($R = 0.76$, $P < 0.01$). Significant positive correlations were also found between the amounts of cellulose decomposing bacteria and actinomycetes ($R = 0.81$, $P < 0.01$), nitrogen-fixing bacteria ($R = 0.67$, $P < 0.01$) and nitrifying bacteria ($R = 0.66$, $P < 0.01$). Furthermore, significant positive correlations between AWCD and the amounts of bacteria ($R = 0.59$, $P < 0.05$), fungi ($R = 0.64$, $P < 0.01$), nitrogen-fixing bacteria ($R = 0.50$, $P < 0.05$) and cellulose decomposing bacteria ($R = 0.56$, $P < 0.05$) were found.

Relationship between plant diversity and various microbial parameters

The amount of bacteria was significantly correlated with S_p ($R = 0.55$, $P < 0.05$). Besides, no significant correlations were found between Shannon indices of plant diversity and amounts of different cultured microbial populations. No significant correlations were found between plant diversity indices and catabolic activity of microbial community expressed as AWCD (Table 6).

Relationship between soil elements and various microbial parameters

Stepwise multiple regressions showed a greater

Table 4. The sampling sites listed in ascending order of various microbial parameters [a)].

Sort order	Bacteria	Fungi	Actinomycetes	Nitrogen-fixing bacteria	Nitrifying bacteria	Denitrifying bacteria	Cellulose decomposing bacteria	AWCD
1	J04^{a}	J04^{a}	J01^{a}	J04^{a}	J14^{a}	J04^{a}	J16^{a}	J12^{a}
2	J08^{ab}	J03^{a}	J04^{a}	J14^{ab}	J08^{a}	J03^{b}	J03^{a}	J03^{ab}
3	J03^{ab}	J12^{ab}	J12^{a}	J03^{ab}	J16^{ab}	J14^{b}	J11^{a}	J13^{abc}
4	J01^{ab}	J14^{bc}	J16^{a}	J16^{bc}	J03^{ab}	J09^{c}	J12^{ab}	J14^{bc}
5	J12^{abc}	J07^{bcd}	J05^{a}	J01^{bc}	J01^{abc}	J12^{d}	J13^{ab}	J04^{bcd}
6	J07^{abc}	J16^{bcd}	J03^{a}	J05^{cd}	J04^{bc}	J11^{d}	J14^{ab}	J16^{cd}
7	J06^{abc}	J08^{cd}	J06^{ab}	J07^{cd}	J09^{c}	J01^{d}	J04^{ab}	J06^{de}
8	J13^{abc}	J13^{cd}	J09^{abc}	J06^{d}	J13^{d}	J16^{e}	J08^{ab}	J15^{ef}
9	J05^{abcd}	J15^{cde}	J08^{abc}	J11^{d}	J15^{e}	J15^{ef}	J09^{ab}	J08^{f}
10	J16^{abcd}	J11^{cde}	J11^{abc}	J08^{d}	J10^{ef}	J07^{f}	J10^{ab}	J09^{fg}
11	J14^{bcd}	J01^{de}	J14^{abc}	J13^{d}	J12^{fg}	J06^{g}	J06^{b}	J07^{fg}
12	J15^{bcd}	J05^{ef}	J10^{bcd}	J10^{e}	J11^{g}	J10^{g}	J01^{c}	J05^{fg}
13	J11^{cde}	J10^{fg}	J13^{bcd}	J15^{e}	J06^{h}	J13^{h}	J15^{d}	J10^{fgh}
14	J10^{de}	J06^{gh}	J07^{cd}	J12^{e}	J05^{i}	J08^{i}	J05^{e}	J11^{gh}
15	J09^{e}	J02^{hi}	J15^{d}	J09^{e}	J02^{j}	J02^{j}	J07^{f}	J01^{hi}
16	J02^{f}	J09^{i}	J02^{e}	J02^{f}	J07^{k}	J05^{k}	J02^{g}	J02^{i}

[a)]Sampling sites within each column with different superscript are significantly different (Fisher PLSD test, $P < 0.05$)

Table 5. The correlation coefficients between various microbial parameters [a)].

Parameter	Bacteria	Fungi	Actinomycetes	Nitrogen-fixing bacteria	Nitrifying bacteria	Denitrifying bacteria	Cellulose decomposing bacteria	AWCD
Bacteria	1.00	0.76**	0.73**	0.80**	0.22	0.27	0.68**	0.59*
Fungi	0.76**	1.00	0.42	0.69**	0.21	0.35	0.44	0.64**
Actinomycetes	0.73**	0.42	1.00	0.76**	0.46	0.32	0.81**	0.46
Nitrogen-fixing bacteria	0.80**	0.69**	0.76**	1.00	0.27	0.35	0.67**	0.50*
Nitrifying bacteria	0.22	0.21	0.46	0.27	1.00	0.38	0.66**	0.38
Denitrifying bacteria	0.27	0.35	0.32	0.35	0.38	1.00	0.49	0.40
Cellulose decomposing bacteria	0.68**	0.44	0.81**	0.67**	0.66**	0.49	1.00	0.56*
AWCD	0.59*	0.64**	0.46	0.50*	0.38	0.40	0.56*	1.00

[a)] Significant correlations between various microbial parameters are noted by * $P < 0.05$; ** $P < 0.01$

positive effect of Cd in soils on amounts of cultured bacteria, fungi, actinomycetes, nitrifying bacteria, denitrifying bacteria and cellulose decomposing bacteria ($P < 0.01$) than any other soil elements. A significant positive effect of Cr in soils on AWCD was also found ($P < 0.05$). No marked effects of

Table 7. The dependency of various cultured microbial populations and AWCD on the soil elements according to stepwise multiple regression analysis.

Dependent	Factors in model	Standardized coefficients	Multiple R	t	Significance
Bacteria	Cd	1.034	0.845	5.670	0.000
	Se	-0.531		-2.911	0.012
Fungi	Cd	1.021	0.733	3.880	0.002
	P	-0.727		-2.763	0.016
Actinomycetes	Cd	1.083	0.901	7.137	0.000
	Zn	-0.634		-4.349	0.001
	S	-0.305		-2.313	0.039
Nitrogen-fixing bacteria	Zn	1.411	0.879	6.347	0.000
	Pb	-1.118		-5.022	0.000
	Mn	-0.337		-2.334	0.038
Nitrifying bacteria	none				
Denitrifying bacteria	Cd	0.801	0.801	5.001	0.000
Cellulose decomposing bacteria	Cd	0.801	0.801	4.998	0.000
AWCD	Cr	0.517	0.517	2.258	0.040

soil elements except for Cr on AWCD were found (Table 7).

DISCUSSION

A number of land use factors may concomitantly cause the differentiation of soil microbial communities. In this study, the primary differences in microbial community structure were found between the secondary shrub forests and cultivated and urban plant communities (Figure 2a). It was consistent with some earlier studies in which changes in land use resulted in dramatic changes in microbial community structure (Steenwerth et al., 2002; Jangid et al., 2008; Xue et al., 2008). Conversion of secondary plant communities to cultivated and urban plant communities can cause dramatic effects on soil physical and chemical properties (Davidson and Ackerman, 1993; Swift et al., 1998). Therefore, land use type (secondary shrub forest or cultivated and urban plant communities) might have a more important influence on soil microbial community structure than other land use factors. Furthermore, the two secondary shrub forests differed from each other, whereas cultivated and urban plant communities congregated and could not be clearly discriminated from each other in Figure 2a. It indicates that, secondary plant communities could promote the formation of a high diversity of microbial community composition (Bossio et al., 2005). Soil microbes drive decomposition (Ushio et al., 2008), nitrogen cycling (Wardle et al., 2004; Liao et al., 2005; Ushio et al., 2008) and homeostasis (Carney and Matson, 2005), thus, secondary plant communities with a high diversity of microbial composition play an important role in terrestrial ecosystem health (Wei et al., 2006; Ushio et al., 2008) and resistance of stress and disturbance (Degens et al., 2001).

Of the fourteen cultivated and urban plant communities with the exception of two secondary shrub forests, the differences in soil microbial community structure described by PLFA profiles were found among soybean field (site J02), one maize field (J15) and other twelve sampling sites in Figure 2b. The concentrations of soil total Cd at J02 and Ni at J15 exceeded the threshold limit of secondary environmental quality standard for soil in China. The contamination indices of Cd at J02 and Ni at J15 were 1.91 and 1.09, respectively. It might be one of the reasons for the differences in soil microbial community structure among J02, J15 and the other twelve sampling sites with the exception of two secondary shrub forests. Although, many reports on the effects of high concentrations of heavy metals on soil microbial community structure and composition (Dai et al., 2004; Liao et al., 2005), the study of Yang et al. (2004) showed that light heavy metal pollution might not result in any change in soil microbial community structure. Further-more, the total concentrations of Cd at J09 and Cu at J03 were also slightly high (the contamination indices were 1.05 and 1.02, respectively), while no discriminations were found between these two sampling sites and other twelve sampling sites with the exception of two secondary shrub forests. So Cd and Ni in soil were not considered as the

main one responsible for the differences in microbial community structure in our research area. The concentrations of soil total N at J02 and J15 were both high, legumes for example, the dominant plant species of J02 or soil N were speculated as the responsible factors for the differences in microbial community structure. It has been reported in some studies that, soil N influenced microbial community structure and composition (Peacock et al., 2001; Bradley et al., 2006). Some studies found that, soil microbial communities were influenced significantly when legumes were present (Lupwayi et al., 1998; Spehn et al., 2000). Aboveground plants can provide nutritious resources for soil microbes, so soil microbial communities respond differently to various plant compositions, especially legumes because of the significant effect of nitrogen-fixing rhizobia bacteria on soil condition (Drinkwater et al., 1998). Besides land use type (secondary shrub forests or cultivated and urban plant communities), the presence of legumes or soil N was hypothesized as the secondary factor influencing the soil microbial community structure in our study. Nevertheless, further research is required for a better understanding of the response of microbial communities to legumes and some soil elements such as N, Cd, Ni, etc.

The obvious effects of legumes on microbial biomass were found in recent studies (Spehn et al., 2000; Scherer-Lorenzen et al., 2003). In the present study, it was evident that the amounts of all cultured microbial populations in soybean field were very high. It revealed that, the presence of legumes was responsible for stimulating the growth and reproduction of soil microbes. Our results were consistent with some earlier studies that found that microbial biomass, respiration and catabolic activity could be stimulated when legumes were present (Lupwayi et al., 1998; Spehn et al., 2000). Besides the contribution of legume nitrogen fixation to soil microbial biomass, a positive correlation was found between the richness index of plant diversity and the amount of soil bacteria. The number of plant species determines available inhabitation, food and energy for microbes in the soil (Wardle et al., 2004; Ushio et al., 2008), thus, the rich plant species can produce a high diversity of litter and consequently, lead to the high amount of soil bacteria (Zak et al., 2003; Bartelt-Ryser et al., 2005).

Similarly, the microbial catabolic activity expressed as AWCD of soybean field was highest in all soils. Moreover, positive correlations were found between AWCD and the amounts of some microbial populations, such as bacteria, fungi, nitrogen-fixing bacteria and cellulose decomposing bacteria. These results are in agreement with the earlier stated result that a high number of microbial populations thrive in soybean field. It indicated that, legumes might promote catabolic activity of microbial community through stimulating the soil microbial population. The fact that a positive correlation between size of fungi and AWCD was interesting as biolog ECO plates contain a fungicide to prevent fungal growth. The significant positive correlations were found between sizes of bacteria and fungi, nitrogen-fixing bacteria and cellulose decomposing bacteria. Thus, it was possible that AWCD was correlated with sizes of such microbial populations.

In this study, the significant positive effects of Cd in soils on amounts of cultured microbial populations were found, including bacteria, fungi, actinomycetes, nitrifying bacteria, denitrifying bacteria and cellulose decomposing bacteria. It was consistent with the study of Shentu et al. (2008) who found that, soil microbial biomass was enhanced at low Cd levels (0.5 to 1 mg/kg). It has been reported that, some certain microbial species may even prosper under heavy metal polluted conditions especially the slight heavy metal pollution (Sandaa et al., 2001; Dai et al., 2004; Lazzaro et al., 2008). It indicated that the increase of soil Cd at moderate concentration level (from 0.089 to 0.574 mg/kg in this study) could enhance the sizes of some microbial populations in our research area. The significant positive effect of Cr in soils on AWCD was also found in our study. It was in agreement with the recent study that found the positive correlation between the Cr content and AWCD in a sewage irrigation area (Zhang et al., 2008). It indicated that, microbial activity could be stimulated by soil Cr in appropriate concentrations (Zhang et al., 2008). However, the majority of soil elements had no marked effects on microbial catabolic activity, thus, soil elements at moderate concentration might place less constraint on microbial catabolic activity. The increased substrate utilization efficiency by the microbes can explain the stimulating effects of Cd or Cr under appropriate concentrations on soil microbial biomass or activity because the increased substrate utilization efficiency advances microbial physical activities including both anabolic and catabolic processes (Shentu et al., 2008; Zhang et al., 2008).

Conclusion

Land use type (secondary shrub forest or cultivated and urban plant communities) was considered as the primary control on soil microbial community structure in our research area. Secondary shrub forest could promote the soil microbial diversity, thereby played an important role in terrestrial ecosystem health and resistance of stress and disturbance. It was considered that legumes or soil N was the secondary factor influencing soil microbial community structure. Legumes could stimulate significantly the sizes of soil microbial populations and accordingly, promote the catabolic activity of the whole community. Plant diversity also had a positive effect on the amount of soil bacteria. Soil Cd or Cr at moderate concentrations had significant positive effects on microbial biomass or activity, respectively. Nevertheless, the majority of soil elements at moderate concentrations placed less constraint on microbial biomass and catabolic activity of microbial community.

ACKNOWLEDGEMENTS

This work was supported by the program for new century excellent talents in the University of China (No. NCET-07-0511), key project of natural science foundation of Shandong Province (No. 2009ZRB01875) and natural science foundation of Shandong Province (No. ZR2011DL007).

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