SOILS, SEC 5 • SOIL AND LANDSCAPE ECOLOGY • RESEARCH ARTICLE

Effects of land utilization patterns on soil microbial communities in an acid red soil based on DNA and PLFA analyses

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Received: 29 November 2012 / Accepted: 16 April 2013

Responsible editor: Yanfen Wang

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Abstract

Purpose Disturbances such as cultivation, logging and plantation occurred widely in acid red soil area of China, yet little is known about their effects on soil microbial community which is closely related to soil function. In this study, microbial community compositions were investigated in a red soil with different long-term land utilization patterns to understand the potential effects of cultivation and vegetation successions on relevant soil functions.

Materials and methods Land utilization patterns include restoration, degradation (logging), cropland and pine plantation. Both DNA and phospholipid fatty acid (PLFA) based methods were used to measure the abundance and community structure of microorganisms.

Results and discussion In general, DNA and PLFA based methods showed similar

results of microbial composition, but for some parameters, only one approach showed significant differences between different land utilization patterns. Land utilization patterns showed significant effects on abundance of total microbial community, bacteria, fungi and actinomycetes which were all lowest in the cropland plot either by PLFA or DNA analyses. 17:0 cyclo/16:1 ω7c and 19:0 cyclo/18:1 ω7c which are possibly associated with environmental stresses also varied among different land utilization patterns. Both PLFA and T-RFLP analysis showed that each land utilization pattern possessed a specific microbial community structure.

Conclusions These results revealed significant effects of different land utilization patterns especially cultivation and logging on soil microbial communities, and suggested that we should be cautious in utilizing red soils to sustain soil properties and functions. Combination of DNA and PLFA based methods is effective to provide precise results of microbial composition.

Keywords Acid soil • Cultivation • Land use • Microbial community • Vegetation succession

1 Introduction

Acid red soils are widespread in south China and are important for food supply and environmental protection. These soils have frequently been disturbed by human activities such as farming, logging and plantation which can have great effects on physicochemical and biological characteristics of the soils. For example, decline of organic carbon and increase of nitrification usually happened in cultivated soils (Compton and Boone 2000), while logging can lead to soil compaction and reduce

nitrification (Greacen and Sands 1980; Goodale and Aber 2001). However, it is difficult to fully understand and predict the changes of complicated soil properties and functions based on physical and chemical parameters.

Microorganisms inhabit soil with immense abundance and diversity and play key roles in decomposing of organic matter and cycling of nutrients. There are great variations between functions of different microbial groups. For instance, bacterial and fungal based energy channels are considered as relating to different ecosystem functions (Wardle et al. 2004). Besides the important functions of microbial community, microbial composition is sensitive to various environmental factors (Torsvik and Øvreås 2002; He et al. 2008, 2009, 2012; Khan et al. 2010). Therefore, microbial composition can be used as a good indicator to evaluate the influence of human activities including soil management practices on soil properties and functions (Joergensen and Emmerling 2006; Acosta-Martínez et al. 2008; Ge et al. 2010; Zhang et al. 2012).

Variations of soil microbial community between land utilization types including cultivation (Sall et al. 2006; Chaer et al. 2009; Zheng et al. 2010; Montecchiaa et al. 2011) and vegetation successions (Chan et al. 2008; Macdonald et al. 2009; Li et al. 2013) were observed in several studies. However, very few studies were based on a small-scale field sharing very similar natural conditions. This is important because high degree of spatial variability of many soil properties may happen which will influence the detection of variations caused by different treatments (Lauber et al. 2008; Ying et al. 2010).

Many culture-independent methods have been developed in recent decades and provided powerful tools to study soil microbial community. DNA based methods especially those PCR based methods greatly improved sensitivity and specificity in detecting different groups of microorganisms, but bias of PCR primers can reduce the

precision of the results. Microbial phospholipid fatty acid (PLFA) compositions have been widely used to measure the abundance of main microbial groups. Besides, PLFA compositions were related to physiological conditions of microorganisms. For example, PLFA ratio such as 17:0 cyclo/16:1ω7c and 19:0 cyclo/18:1ω7c are considered related to environmental stress (Guekert et al. 1986; Ratledge and Wilkinson 1988). But the specificity between PLFA components and certain microbial groups is weak. Considering the limitations of different methods, using multiple approaches is appealing to improve our understanding of the complex microbial communities.

A long-term land utilization pattern experiment was established in a red soil since 1995, which is ideal to study the effect of cultivation and vegetation successions caused by human activities on the soil characteristics. In this paper, the abundance and community structure of main microbial groups such as bacteria and fungi were investigated under different land utilization patterns including the restoration, degradation, cultivation and pine plantation. Both PLFA analysis and DNA based methods such as real-time PCR and T-RFLP were used to give robust results.

2 Materials and methods

2.1 Site description and sampling

The long-term land utilization pattern experiment is located at a hillside of Taoyuan Experimental Station (E 111°26′, N 28°55′) in Hunan province of China which has been described in details by Ying et al. (2010). Before the experiment, the vegetation was natural forest which was cleared in 1995. Since then, the restoration plot (R) recovered to be a forest without human disturbance, and the degradation plot (D) was grown by grass and mowed twice in May and November every year. The cropland plot (C) was planted with different crops in different seasons, and conventional agricultural

management such as fertilization and tillage were employed. The pine plot (P) was scarcely disturbed after plantation. Soils were sampled in October of 2007 at the upper, middle and lower positions of the slope independently at each plot with soil depth of 0-10 cm. Three cores were mixed for each sample. Soil samples were transported on ice to laboratory, sieved (2mm), and stored at -80°C for further molecular analyses or air-dried for chemical analysis.

2.2 DNA extraction and real-time PCR

DNA was extracted with the MoBio UltraClean soil DNA isolation kit (San Diego, CA) from 0.5 g soil according to the manufacturer's instruction with three parallels for each sample, and the extracted DNA concentration was determined with Nanodrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies).

Real-time PCR was conducted with an iCycler iQ 5 thermocycler (Bio-Rad). The bacterial 16S rRNA genes were amplified (Premix Ex Taq™, TaKaRa) with primers BACT1369F and PROK1492R, and the 6-FAM labeled TAMRA probe TM1389F was used for fluorescence detection (Suzuki et al. 2000). SYBR® Premix Ex Taq™ (TaKaRa) was used for amplification of fungi. NS1 was used as forward primer for the 18S rRNA gene of fungi (White et al. 1990), and the reverse primer was the same as the GCFung used by May et al. (2001) but without the GC clamp. Recombinant plasmid (pGEM®-T Easy Vector, Promega) inserted with a target gene was used as standard template for construction of standard curve to calculate the copy number of each gene.

2.3 T-RFLP assay

The reverse primers for T-RFLP analysis of bacterial community were the same as used for the real-time PCR, while the forward primer was replaced by 27f (Edwards et al.

1989), and labeled with 6-FAM for fluorescence detection. PCR primers for fungi were the same as used for the real-time PCR. The amplified PCR fragments were digested by *Hha*I for 3hrs, then purified by ethanol precipitation and dissolved in distilled water before determination by the ABI 3700 capillary DNA sequencer. Each sample was measured twice, and some unstable peaks were deleted. Relative fluorescence of each peak was calculated from the peak area and averaged from the two replicates. Terminal restriction fragments (T-RFs) under 50bp or with relative fluorescence less than 1% in each sample were neglected.

2.4 PLFA analysis

PLFA was extracted from 8 g soil according to Bossio and Scow (1998). The resultant fatty acid methyl esters were separated, quantified, and identified using capillary gas chromatography (GC). Qualitative and quantitative fatty acid analyses were performed using an Agilent 6850 gas chromatograph and the MIDI Sherlock Microbial Identification System (6.0B). FAME 19:0 was added to the extracted PLFA solution as an internal standard to calculate the PLFA mass. According to Bardgett et al. (1996) and Zelles (1999), peaks of 14:0, 15:0, 16:0, 18:0, i15:0, a15:0, i16:0, i17:0, a17:0, 16:1ω7c, 18:1w5c, cy17:0a and cy19:0 were included to calculate bacterial fatty acids; i15:0, a15:0, i16:0, i17:0, a17:0 were regarded as G^+ bacteria; 16:1ω7c, 18:1w5c, cy17:0a and cy19:0 were regarded as G^- bacteria; 18:2ω6c was considered as fungal biomarkers; 16:0 10 methyl, 17:0 10 methyl and 18:0 10 methyl were considered as fatty acids of actinomycetes.

2.5 Statistic analysis

One way ANOVA analysis was used to determine the effect of a single factor by SPSS

11.5, and tests with $P < 0.05$ were considered significant. Two-factor analysis of variance showed that variations along the slope gradient were minor compared with land utilization patterns. Considering that there was no replicate for each treatment, and the area of these plots was not large (*ca*. 1 ha totally) and soil properties were similar in different plots before the experiment, samples from the upper, middle and lower positions in the same plot were regarded as replicates of each land utilization pattern. This is important to give strong and meaningful results about the effect of land utilization patterns especially in this hilly area to cover the effect of landform. Bivariate Pearson correlation analysis was carried out to associate different parameters with SPSS 11.5. Bray-Curtis cluster analysis of T-RFLP and PLFA profile was operated by software BioDiversity Pro.

3 Results

3.1 Abundance of total microbial community, bacteria and fungi based on DNA analysis

Land utilization patterns showed significant effects on all soil chemical properties tested including soil pH, organic matter, total N, NH_4^+, NO_3^-, available P and K which were particularly evident for the cropland plot which possessed the lowest organic matter, pH and highest concentrations of available P and NO_3^- (Table 1). DNA concentrations and bacterial gene copies were both lowest in the cropland and highest in the pine plot. Bacterial gene copies were near 100 folds of the fungal gene copies. There is no significant difference in the fungal gene copy number between different land utilization patterns.

3.2 T-RFLP analysis of microbial community structure

A total of 16 T-RFs were used for community structure analysis of bacterial 16S rRNA gene, and the relative fluorescence was calculated to present the proportion of each T-RFs. Most differences were found between the cropland plot and others. For example, the cropland possessed several unique T-RFs of 160, 207 and 217bp which were not found in other plots (Fig. 1). In addition, the proportion of 190bp fragment was lowest while the 77bp fragment was highest in the cropland plot.

Cluster analysis of T-RFLP results based on the proportion of each T-RFs showed significant difference of bacterial community structure among different land utilization patterns (Fig. 2). The restoration and pine plots were closest, while the cropland was most distant from others which coinciding with results showed in Fig. 1.

3.3 PLFA analysis

Table 2 shows that concentration of many fatty acid components including 14:0, 15:0 iso, 16:1 w5c, 16:0, 16:0 10 methyl, 17:0 cyclo, 17:0 10 methyl, 18:1ω9t alcohol, 18:2ω6c, 18:1ω9c, 18:1ω5c and 18:0 10 methyl differed significantly among different land utilization patterns. Concentrations of most fatty acids were lowest in the cropland plot, while density of 18:1ω9t alcohol was particularly high in the cropland plot and rarely found in other plots. The fungal marker 18:2ω6c was highest in the degradation plot and lowest in the cropland and pine plots. Concentration of another possible fungal marker 18:1ω9c (Bååth 2003) was also highest in the degradation plot.

The calculated fatty acid concentrations of main microbial groups and some PLFA ratios are listed in Table 3. Fatty acid concentrations of fungi and actinomycetes differed significantly among different land utilizations and were both highest in the degradation plot and lowest in the cropland plot. Similar to the results of DNA analysis, the total and bacterial PLFA were both minimum in the cropland plot although the

effect was not significant at $P < 0.05$. Fungi/bacteria PLFA values were highest in the degradation plot and lowest in the cropland and pine plots. 17:0 cyclo/16:1ω7c values were highest in the cropland plot and lowest in the restoration and degradation plots. 19:0 cyclo/18:1ω7c values in the pine plot were much higher than other plots.

Cluster analysis of PLFA based on relative mass separated different land utilization patterns clearly (Fig. 3). Two main lineages were formed with the restoration plot clustered closely with the degradation plot and the cropland plot closest to the pine plot. Similarities between different land utilization patterns were above 84% which is much higher than T-RFLP analysis.

3.4 Correlations of microbial groups and abiotic properties

Table 4 shows significant correlations of fungal PLFA with soil pH negatively and with NO_3^- positively, which was opposite for 17:0 cyclo/16:1ω7c ratio. DNA concentration and bacterial gene copy correlated negatively with available P ($p < 0.01$). Besides, some other significant correlations were found such as the negative correlation between G^+ bacteria and NH_4^+ concentration, positive and negative correlations of fungal PLFA with organic matter and available P respectively, and negative correlations of actinomycetes with available P, NH_4^+ and NO_3^- concentration.

4 Discussion

4.1 Effects of land utilization patterns on the abundance of main groups of microbial community

Coinciding with the predomination of bacteria in microbial community as indicated by real-time PCR, strong positive correlation between DNA concentration and bacterial gene copy ($r = 0.905$, $p < 0.01$) were found which implied reliability of these two

parameters. While PLFA mass is possibly less precise to measure total microbial or bacterial concentration because many PLFA components were not recognized and not included when calculating the abundance of total microbial community and bacteria. In contrast, PLFA analysis is likely to be a better method than PCR to measure fungal abundance since bias of many fungi primers was found (Gao et al. 2008), while 18:2ω6c and 18:1ω9c were common in fungi although rare in bacteria (Bååth 2003), and 18:2ω6c was most widely accepted and used as fungal marker (Frostegård and Bååth, 1996). In this study, 18:2ω6c correlated well ($r=0.948$, $p<0.001$) with 18:1ω9c which further supported the reasonableness of these fungal markers. Therefore, it seems appropriate to use DNA concentration, bacterial gene copy and fungal PLFA data to measure the variations of abundance of total microbial community, bacteria and fungi, respectively.

In this study, abundance of total microbial community, bacteria and fungi obtained by DNA method correlated positively with those obtained by PLFA analysis although it was not always significant (data not shown), but only one method showed significant differences between treatments for each parameter. These results suggested the feasibility of both methods as well as the importance of using multiple methods to improve precision to detect minor changes among different treatments. The small discrepancies between results obtained from DNA and PLFA analysis may due to the different characteristics between the two methods described above, but some other factors can also lead to different results between DNA and PLFA analysis. For example, different microbial groups possessed different PLFA mass, so the same microbial number as measured by gene copy doesn't means the same PLFA mass. According to the advantage of DNA and PLFA analysis in measuring microbial abundance as described above, the significant differences of abundance of total microbial community

and bacteria based on DNA analysis as well as fungal abundance based on PLFA analysis among different land utilization patterns seems reliable. Nevertheless, in order to give robust results, we tended to describe the similar results obtained by both PLFA and DNA methods here.

Both DNA and PLFA analysis showed that the abundance of total microbial community and main groups such as bacteria, fungi and actinomycetes were all lowest in the cropland plot although differences were significant only for one approach, which provided strong evidence of negative effect of cultivation on most microbial groups. This is likely due to the decrease of nutrients according to the lowest organic matter in the cropland plot which can be related to decreased plant litter and increased mineralization as happened in many cultivated soils. In addition, available P and NO_3^- concentration which was particularly high in the cropland plot correlated negatively with abundance of those microbial groups, and suggested possible inhibition of microbial growth by available P and N in the cropland plot. The effect of P seems true since influence of P fertilization on microbial composition was reported by other study (He et al. 2008; Ye and Wright, 2010; Zheng et al. 2013). Decrease in abundance of soil microbial communities was also found in some other soils (Bucldey and Schmidt 2001; Bossio et al. 2005; Ge et al 2008a,b; Montecchiaa et al. 2011; Bissett et al. 2011), and reflected widely happened decrease of soil quality in cultivated soils.

Fungi/bacteria PLFA, fungal PLFA and fungal gene copy were all highest in the degradation plot although difference of gene copy was not significant. Fungal based energy channel is considered associated with low mineralization rate and lead to carbon sequestration (Wardle et al. 2004) which was supported by the positive correlation between fungal PLFA and organic matter. Negative correlation between fungal abundance and N mineralization was also reported by Fraterrigo et al. (2006). Thus, the

highest fungi abundance and fungi/bacteria ratio in the degradation plot is likely associated with reduction of mineralization by logging. Coinciding with this, some other studies reported that logging can lead to compaction and increase soil bulk density (Greacen and Sands et al. 1980; Johnson et al. 1991), while bulk density correlated negatively with N mineralization (Fraterrigo et al. 2006). These information suggested associations of increased fungal abundance, increased bulk density, and decline of mineralization under logging, but further study is needed to give strong evidence.

The pine plot possessed lowest fungi/bacteria PLFA and highest bacterial gene copy and bacterial PLFA although difference of bacterial PLFA between different treatments was not significant. These results implied increased function of bacterial based energy channel which lead to increased mineralization and carbon loss. It seems reasonable because soil of the pine plot received plenty of plant litter as pine leaves but the organic matter in this plot was relatively low. Besides, soil pH in the pine plot was also relatively low which is possibly related to the increased degradation of organic matter.

4.2 Variations in PLFA values related to environmental stress between different land utilization patterns

17:0 cyclo/16:1 ω7c and (or) 19:0 cyclo/18:1 ω7c value which is considered related to environmental stress such as soil pH, carbon content and oxygen level etc (Guekert et al. 1986; Ratledge and Wilkinson 1988) indicated increase of environmental stress to microbial community in the cropland and pine plots. According to the low pH in red soils and the high correlation between soil pH and 17:0 cyclo/16:1 ω7c value, it is possible that decrease of soil pH enhanced environmental stress and increased 17:0 cyclo/16:1 ω7c value. However, decrease of pH could not explain well the particularly

high value of 19:0 cyclo/18:1 ω7c in the pine plot. These results reflected sensitive and complicated responses of 17:0 cyclo/16:1 ω7c and 19:0 cyclo/18:1 ω7c to environmental factors and suggested a possible role of 17:0 cyclo/16:1 ω7c and 19:0 cyclo/18:1 ω7c as good indicators of environmental change.

4.3 Distance between different land utilization patterns based on microbial community structure analysis

T-RFLP analysis showed significant differences of bacterial community structure between different land utilization patterns. The cropland plot was quite different from others in bacterial community structure which suggested great effects of cultivation on soil properties compared with vegetation successions. In congruence with this, some other studies also showed significant changes of microbial communities caused by cultivation rather than plant composition (Bucldey and Schmidt 2001). P fertilization may play an important role in modifying the microbial community structure in the cropland as reported by Ye and Wright (2010) which coincide with the high P concentration in the cropland plots (data not shown). Some specific T-RFs were found in the cropland plot which can be used as good biomarkers to identify and predict the particular effects of cultivation on soil properties and functions. T-RFLP analysis of fungi community showed great variation between replicates, and no significant effect of land utilization patterns on fungal community structure was found (data not shown). High spatial heterogeneity of fungal community was reported in some other studies (Klamer et al. 2002; Girvan et al. 2004; He et al. 2005; Schwarzenbach et al. 2007) which may cover the effects of land utilization patterns on fungal community structure.

Cluster analysis of PLFA data separated different land utilization patterns clearly which confirmed the specific effect of each land utilization patterns on soil microbial

community. Relationships between different land utilization patterns based on PLFA dendrogram differed from T-RFLP analysis with the cropland plot closest to the pine plot which is possibly related to the effect of soil pH. The discrepancy between PLFA and T-RFLP results is not surprising because specificity between PLFA components and microbial taxa was low. For example, PLFA composition such as 17:0 cyclo/16:1 ω7c and 19:0 cyclo/18:1 ω7c can be affected by physiological change of microorganisms under environmental stress. Therefore, both T-RFLP and PLFA are effective to distinguish different treatments, but they provided different information of microbial community structure. These results reflected the important effect of method used when analyze distances between microbial community structures.

5 Conclusions

Results of this study revealed significant effects of land utilization patterns particularly cultivation and logging on microbial composition in a Chinese red soil, which indicated evident influence of these land utilization patterns on soil properties, and implied the necessary of cautious in utilization of soil to sustain soil quality and ecosystem function. Studies in more systems and analysis of other soil properties will be helpful to extend these results and associate changes of microbial community with soil functions. Both DNA and PLFA analysis provided important information on microbial community composition, but advantages or disadvantages varied between different methods. Actually, it is difficult to well understand the microbial composition using a unique method because of the complexity of microbial community and the shortcomings of many methods. So we should learn the advantages and disadvantages of different methods and be cautious to select the appropriate methods and also be cautious to describe the results. Using multivariate approaches is useful to improve the accuracy

and precision of the results. Besides, the fast development of high-throughput sequencing technology in recent years has reduced the cost of sequencing dramatically, thus it is possible to use the high-throughput sequencing in the near future to improve our understanding of soil microbial community in more detail.

Acknowledgements This work was supported by the Natural Science Foundation of China (41025004, 40901121) and the CAS/SAFEA International Partnership Program for Creative Research Teams of "Ecosystem Processes and Services".

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Table 1 Soil chemical properties and quantification of microbial communities based on DNA analysis under different land utilization patterns

Parameters	Restoration	Degradation	Cropland	Pine
pH	4.22±0.05(ab)[a]	4.33±0.00(a)	4.08±0.11(c)	4.11±0.04(bc)
Organic matter (g kg^{-1} soil)	16.0±1.0(a)	16.2±3.2(a)	11.1±1.6(b)	13.6±2.6(ab)
Total N (mg kg^{-1} soil)	2.84±0.34 (a)	2.45±0.20(ab)	1.94±0.16(c)	2.20±0.30 (bc)
NH_4^+-N (mg N kg^{-1} soil)	4.37±2.21(b)	2.38±0.76(ab)	2.85±0.44(ab)	1.09±1.16(a)
NO_3^--N (mg N kg^{-1} soil)	13.05±0.84(b)	0.78±0.48(a)	47.37±3.47(d)	17.85±1.28(c)
Available P (mg kg^{-1} soil)	1.36±0.15(b)	0.99±0.08(b)	28.89±6.35(a)	1.10±0.48(b)
Available K	102.3±17.0 (a)	54.1±7.7(c)	70.3±8.8(bc)	82.8±14.7(ab)
DNA concentration (μg g^{-1} soil)	4.11±0.94(b)[a]	3.79±1.03(b)	1.90±0.35(c)	5.67±0.46(a)
Log bacterial gene copy (g^{-1} soil)	9.96±0.17(ab)	9.89±0.20(b)	9.58±0.11(c)	10.18±0.06(a)
Log fungal gene copy (g^{-1} soil)	8.06±0.20	8.37±0.11	8.12±0.12	8.26±0.24

[a]Mean± SD (n = 3). Letters in parentheses indicate significant differences of land utilization patterns with $P < 0.05$

Table 2 Concentrations of individual PLFA under different land utilization patterns

PLFA component	PLFA mass (mg kg^{-1} soil)			
	Restoration	Degradation	Cropland	Pine
14:0	29(ab)[a]	30(ab)	20(b)	34(a)
15:0 iso	350(ab)	322(bc)	224(c)	438(a)
15:0 anteiso	137	141	119	150
15:0	19	26	21	25
16:0 iso	175	192	180	221
16:1ω7c	136	103	90	105
16:1ω5c	86(a)	87(a)	47(b)	55(ab)
16:0	542(ab)	659(a)	482(c)	521(c)
16:0 10 methyl	233(ab)	226(ab)	182(b)	270(a)
17:0 iso	126	159	159	165
17:0 anteiso	82	86	83	82
17:0 cyclo	62(ab)	46(b)	83(a)	77(a)
17:0 10 methyl	33(ab)	32(ab)	30(b)	41(a)
18:1ω9t Alcohol	- [b] (b)	-(b)	38(a)	6(b)[c]
18:2ω6c	78(b)	105(a)	53(c)	54(c)
18:1ω9c	263(b)	336(a)	168(c)	209(ab)
18:1ω7c	163	128	135	103
18:1ω5c	42(ab)	40(ab)	57(a)	-(b)
18:0	128	149	115	129
18:0 10 methyl	118(ab)	193(a)	97(c)	145(b)
19:0 cyclo	281	264	228	342

[a] The number is the mean value, while letters in parentheses indicate significant differences of different land utilization patterns with $P < 0.05$

[b] "-" means below the detection limit

[c] PLFA detected only in one of the three samples

Table 3 Calculated PLFA concentrations of main microbial groups and PLFA ratios under different utilization patterns

PLFA componets	Restoration	Degradation	Cropland	Pine
Total (mg/kg soil)	4561±933[a]	4589±159	3913±525	4487±477
Bacteria (mg/kg soil)	2141±532	2250±94	1890±290	2313±254
G^+ (mg/kg soil)	870±216	900±26	765±119	1046±120
G^- (mg/kg soil)	554±181	485±84	487±73	561±68
Fungi (mg/kg soi)	78±17(b)	105±3(a)	53±16(c)	54±7(c)
Actinomycete (mg/kg soil)	382±28(ab)	451±12(a)	361±9(b)	467±10(a)
Fungi/Bacteria	0.037±0.004(b)	0.047±0.002(a)	0.027±0.004(c)	0.023±0.003(c)
17:0 cyclo/16:1ω7c	0.467±0.052(c)	0.449±0.031(c)	0.946±0.166(a)	0.729±0.088(b)
19:0 cyclo/18:1ω7c	1.732±0.078(b)	2.091±0.213(b)	1.685±0.120(b)	3.406±0.673(a)

[a]Mean±SD, and letters in parentheses indicate significant differences between land utilization patterns with $P < 0.05$

Table 4 Correlations of microbial groups and abiotic properties

	pH	Organic matter	Total N	Available P	Available K	NH_4^+	NO_3^-
Total PLFA	.393	.459	.319	-.461	.158	-.369	-.516
Bacterial PLFA	.281	.369	.187	-.446	.086	-.526	-.466
G^+ PLFA	.107	.214	.026	-.471	.165	-.656(*)	-.402
G^- PLFA	-.034	.274	.210	-.185	.326	-.323	-.109
Fungal PLFA	.816(**)	.607(*)	.517	-.606(*)	-.182	-.061	-.817(**)
Actinomycetal PLFA	.284	.228	-.030	-.679(*)	-.187	-.657(*)	-.632(*)
17:0 cyclo/16:1 w7c	-.867(**)	-.582(*)	-.660(*)	.702(*)	-.165	-.146	.884(**)
19:0 cyclo/18:1ω7c	-.199	.052	-.055	-.417	.068	-.464	-.210
DNA concentration	.167	.425	.296	-.754(**)	.392	-.343	-.574
Bacterial gene copy	.162	.445	.392	-.725(**)	.308	-.312	-.577(*)
Fungal gene copy	.300	.422	.099	-.267	-.328	-.404	-.357

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

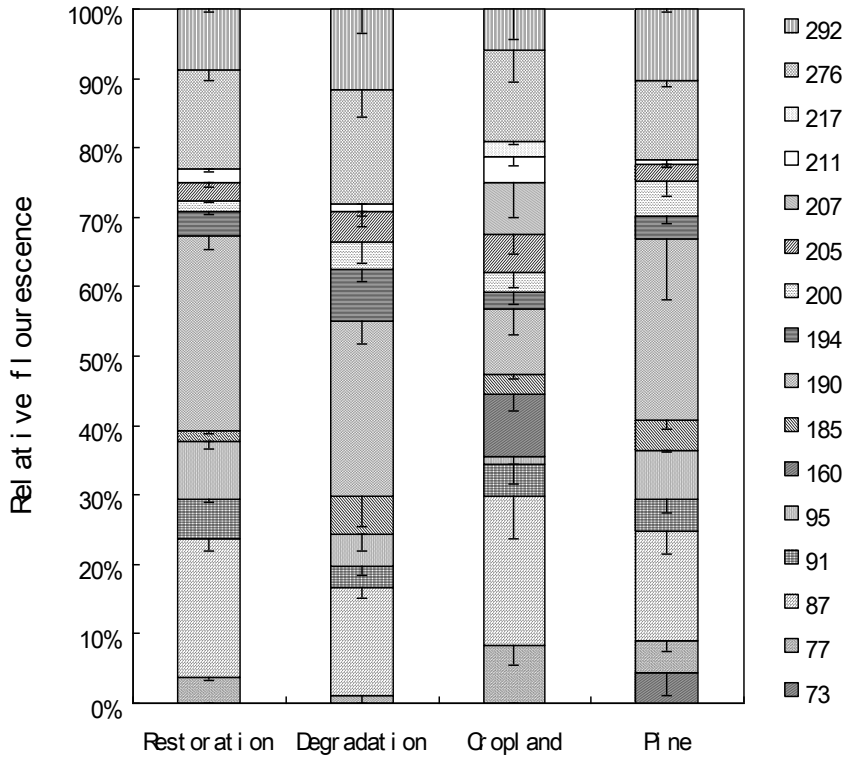
Figure legends

Fig. 1 Relative abundance of T-RFs under different land utilization patterns. Error bars means standard deviations

Fig. 2 Clustering of bacterial community structure under different land utilization patterns based on T-RFLP analysis. Letters of sample name mean land utilization patterns of restoration (R), degradation (D), cropland (C) and pine plantation (P), while the letter followed means samples from upper (U), middle (M) and lower (L) position of the hill side

Fig. 3 Clustering of microbial community structure under different land utilization patterns based on PLFA analysis. Letters of sample name mean land utilization patterns of restoration (R), degradation (D), cropland (C) and pine plantation (P), while the letter followed means samples from upper (U), middle (M) and lower (L) position of the hill side

Fig. 1


Relative flourescence
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90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Restoration
Degradation
Cropland
Pine
292
276
217
211
207
205
200
194
190
185
160
95
91
87
77
73

Fig. 2

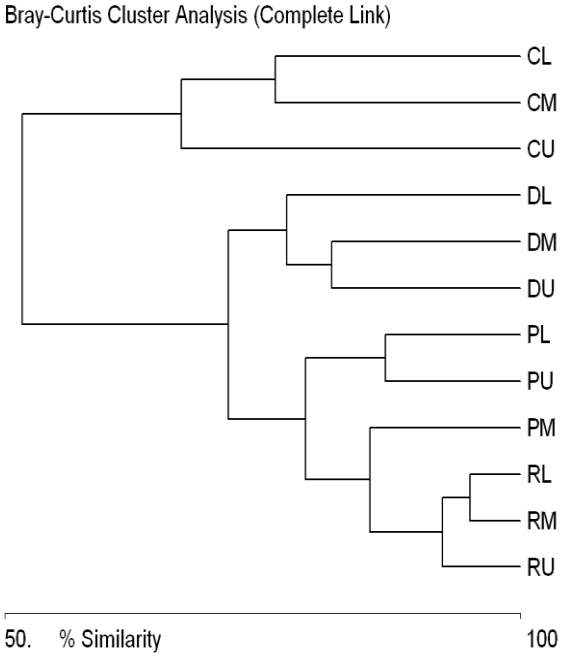
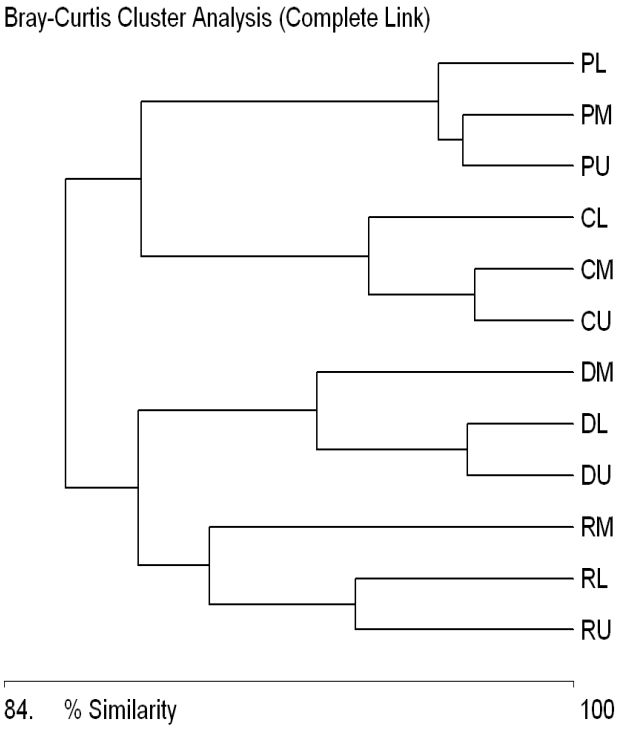

Bray-Curtis Cluster Analysis (Complete Link)
CL
CM
CU
DL
DM
DU
PL
PU
PM
RL
RM
RU
50.
% Similarity
100

Fig. 3


Bray-Curtis Cluster Analysis (Complete Link)
PL
PM
PU
CL
CM
CU
DM
DL
DU
RM
RL
RU
84.
% Similarity
100

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Title:
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Date:
2013-08

Citation:
Ying, J. -Y., Zhang, L. -M., Wei, W. -X. & He, J. -Z. (2013). Effects of land utilization patterns on soil microbial communities in an acid red soil based on DNA and PLFA analyses. JOURNAL OF SOILS AND SEDIMENTS, 13 (7), pp.1223-1231. https://doi.org/10.1007/s11368-013-0704-4.

Persistent Link:
http://hdl.handle.net/11343/283106