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RESEARCH ARTICLE

Soil Microbial Community Composition During Natural Recovery in the Loess Plateau, China

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Abstract

This study aimed to determine the characteristics of soil microbial community composition and its relationship with soil chemical properties during natural recovery in the Loess Plateau. The soil microbial community composition was analyzed by comparing the soil microbial phospholipid fatty acids (PLFAs) of eight croplands abandoned for 1, 3, 5, 10, 13, 15, 20, and 30 yr in the Dunshan watershed, northern Loess Plateau, China. The results showed that soil organic carbon, total nitrogen, soil microbial biomass carbon, and soil microbial biomass nitrogen significantly increased with the abandonment duration, whereas the metabolic quotient significantly decreased. The Shannon richness and Shannon evenness of PLFAs significantly increased after 10 yr of abandonment. Gram-negative, Gram-positive, bacterial, fungal, and total PLFAs linearly increased with increased abandonment duration. Redundancy analysis showed that the abandonment duration was the most important environmental factor in determining the PLFA microbial community composition. The soil microbial PLFAs changed from anteiso- to iso-, unsaturated to saturated, and short- to long-chain during natural recovery. Therefore, in the Loess Plateau, cropland abandonment for natural recovery resulted in the increase of the soil microbial PLFA biomass and microbial PLFA species and changed the microbial from chemolithotrophic to a more heterotrophic community.

Key words: abandoned cropland, microbial diversity, phospholipid fatty acid (PLFA), redundancy analysis (RDA), Loess Plateau

INTRODUCTION

The degradation of ecological environments has accelerated in the last century because of population increase. Accordingly, strategies for controlling soil erosion and recovering fragile ecosystems have been proposed. Among them, revegetation is reported the most effective and useful (Hou *et al*. 2002). However, determining the effect of restoration is often limited to vegetation indicators such as plant diversity and coverage (Mummy *et al*. 2002), soil physicochemical properties, microbial biomass, as well as enzyme activities (Eaton *et al*. 2008; Fu *et al*. 2010; Wang *et al*. 2011). The soil microbial community is also an inherent factor in determining the biogeochemical cycles and organic matter turnover in soils (Harris 2009). Thus, investigating the soil microbial community composition is important in explaining the soil ecological processes during vegetation succession.

The structural composition of a soil microbial community can be evaluated by using phospholipid fatty acids (PLFAs). Given that phospholipids are rapidly degraded following cell death, PLFAs can reliably reflect the microbial community. Many researchers have used PLFAs to study the variations

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in soil microbial community composition during vegetation successions. For instance, McKinley (2005) reported that the prairie age is the most important environmental factor in determining the PLFA microbial community composition in native North American prairie grassland. Studies on the primary succession of a brown coal mine deposit found similar results (Baldrian *et al*. 2008). Other studies suggested that abiotic soil parameters such as organic matter content, C/N ratio, and pH have a more significant effect on the soil microbial PLFA biomass and microbial community composition than the soil age (Merilä *et al*. 2002, 2010; Welc *et al*. 2012). Moore *et al*. (2010) studied the long-term soil microbial community dynamics in subsurface soil horizons and discovered that older subsurface soils have a lower microbial community biomass, a higher fungal proportion, and a different community structure compared with younger subsurface soils. The soil texture, vegetation species, and meteorological factor also reportedly contribute substantially to studies on different ecosystems (Bach *et al*. 2010). However, few studies have evaluated the changes in microbial community composition during the natural restoration of a disturbed field, specifically in the Loess Plateau in China, one of the areas in the world that are most seriously affected by soil and water erosion.

The Loess Plateau belongs to arid and semi-arid regions that are subjected to the irrational use of land resources, which results in severe soil erosion, because of the increasing population (Wei *et al*. 2006). The Chinese government realized the severity of this problem and implemented the Conservation of Cropland to Forest and Grassland Project in 1999, in which numerous croplands were abandoned for natural recovery. Several studies have been conducted to investigate the changes in soil properties during natural recovery. Many researchers found that the soil bulk density significantly decreases, whereas the soil porosity, water-holding capacity, aggregate stability, and saturated hydraulic conductivity significantly increase during vegetation succession (Li and Shao 2006; Jiao *et al*. 2011). The following parameters also reportedly increase significantly during successional stages: soil fertility; soil organic carbon (SOC); total nitrogen (TN); microbial biomass C, N, and P; and enzyme activities (Liang *et al*. 2010; Wang *et al*. 2011; Jia *et al*. 2012). These changes in soil properties are mainly induced by the soil microbial community. Thus, the soil microbial community composition should be evaluated to understand the mechanism of the improvement in soil properties during vegetation succession in this region.

This study aimed to evaluate the changes in soil microbial community composition of abandoned croplands during natural recovery in the Loess Plateau. The soil chemical and microbial activities were also combined to identify the main factors that caused the changes in microbial composition during vegetation succession.

RESULTS

Soil chemical and microbial properties

The changes in soil chemical and microbial properties during natural recovery are presented in Fig. 1. The results showed that SOC, TN, soil microbial biomass carbon (SMBC), and soil microbial biomass nitrogen (SMBN) linearly increased with the restoration time. Compared with the 1-yr abandoned cropland, the values of SOC, TN, SMBC, and SMBN in the 30-yr abandoned cropland increased by 79.1, 65.0, 246.3, and 177.7%, respectively. The change in basal respiration (BR) with time was relatively small and no obvious trend was found. By contrast, qCO_2 significantly decreased with increased abandonment duration ($P<0.05$), and the value decreased by 72.1% in the 30-yr abandoned cropland compared with the 1-yr abandoned cropland.

Soil microbial community diversity

A total of 16 PLFAs were detected to characterize the microbial community composition of the eight abandoned croplands. The PLFA profiles were dominated by the group of normal saturated fatty acids: 14:0, 15:0, 16:0, 17:0, and 18:0. Terminally branched saturated fatty acids (i15:0, a15:0, i16:0, and

i17:0), cyclopropyl fatty acids (cy17:0 and cy19:0), monounsaturated fatty acids (16:1w9c, 18:1w9c, and 18:1w9t), a polyunsaturated fatty acid (18:2w6), and a hydroxyl fatty acid (3OH14:0) were also detected (Table 1).

H_{PLFA} and E_{PLFA} analyses by restoration ages showed a significant difference during vegetation succession ($P<0.05$) (Fig. 2). In particular, H_{PLFA} increased and showed a significant difference among the restoration ages before 10 yr. By contrast, no significant difference was found between restoration ages after 10 yr. For E_{PLFA}, no significant difference was found in the first 5 yr of abandonment, but a significant increase was found within 10 yr. No significant difference was found among the restoration ages after 10 yr.

Microbial PLFA biomass and composition

Significant differences were found between G^-PLFAs, G^+PLFAs, F-PLFAs, B-PLFAs, and total PLFAs among different restoration ages ($P<0.05$). Regression analysis showed a linear correlation between the soil PLFA characteristics and time since abandonment (Fig. 3-A-E). The soil G^-PLFAs, G^+PLFAs, B-PLFAs,

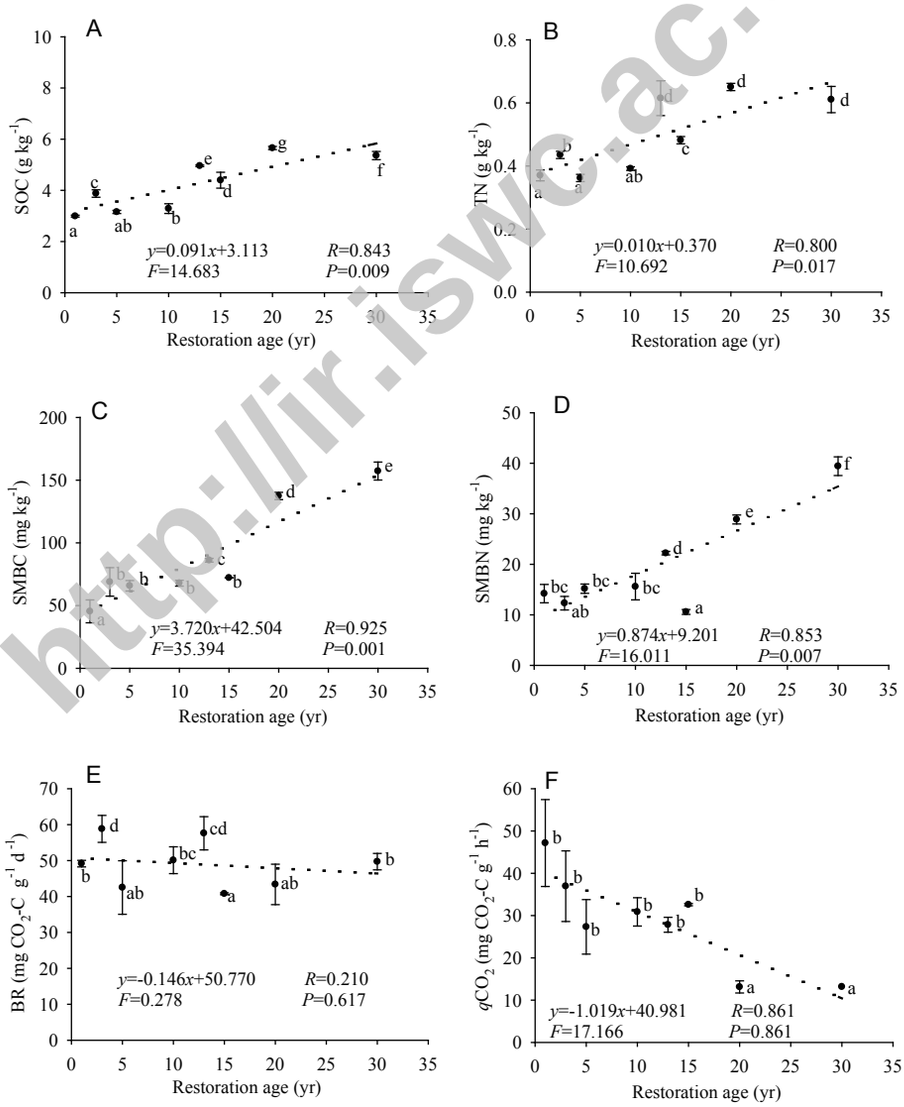

Fig. 1 Changes in SOC (A), TN (B), SMBC (C), SMBN (D), BR (E) and qCO_2 (F) with time. Different lowercase letters indicate significant difference at $P<0.05$ among restoration ages. The same as below.

Table 1 Concentrations of marker PLFAs (nmol g^{-1} dry soil) in the eight abandoned croplands

PLFA	Restoration age (yr)							
	1	3	5	10	13	15	20	30
14:0	0.256±0.044	0.248±0.034	0.267±0.081	0.181±0.014	0.252±0.127	0.349±0.024	0.405±0.155	0.412±0.050
i15:0	0.129±0.004	0.194±0.030	0.154±0.030	0.191±0.023	0.236±0.006	0.182±0.033	0.407±0.048	0.389±0.021
a15:0	0.204±0.042	0.249±0.021	0.221±0.041	0.260±0.022	0.243±0.022	0.257±0.048	0.320±0.043	0.326±0.016
15:0	0.205±0.062	0.207±0.064	0.188±0.062	0.209±0.111	0.167±0.115	0.202±0.011	0.244±0.099	0.244±0.044
3OH14:0	0.669±0.013	0.639±0.073	0.667±0.072	0.633±0.040	0.612±0.010	0.739±0.033	0.811±0.130	0.800±0.043
i16:0	0.098±0.001	0.117±0.012	0.110±0.018	0.131±0.012	0.151±0.024	0.147±0.011	0.284±0.036	0.252±0.020
16:1w9	0.593±0.076	0.595±0.104	0.601±0.089	0.546±0.074	0.680±0.132	0.725±0.034	1.084±0.310	1.336±0.200
16:0	1.130±0.154	1.172±0.146	1.290±0.129	1.171±0.125	1.431±0.467	1.755±0.144	2.049±0.515	2.099±0.139
i17:0	0.000±0.000	0.211±0.094	0.141±0.052	0.303±0.147	0.182±0.031	0.224±0.105	0.317±0.037	0.327±0.075
cy17:0	0.000±0.000	0.121±0.012	0.108±0.016	0.095±0.018	0.175±0.025	0.205±0.060	0.334±0.076	0.359±0.042
17:0	0.000±0.000	0.000±0.000	0.00±0.000	0.101±0.033	0.138±0.035	0.102±0.044	0.253±0.067	0.240±0.027
18:2w6	0.235±0.018	0.238±0.028	0.259±0.010	0.223±0.017	0.255±0.060	0.399±0.148	0.345±0.061	0.339±0.016
18:1w9c	0.558±0.039	0.585±0.064	0.613±0.032	0.558±0.049	0.748±0.158	0.835±0.049	1.124±0.229	1.054±0.110
18:1w9t	0.220±0.041	0.307±0.054	0.283±0.048	0.275±0.054	0.330±0.050	0.308±0.043	0.518±0.088	0.469±0.026
18:0	0.281±0.022	0.249±0.091	0.334±0.146	0.416±0.133	0.391±0.192	0.419±0.066	0.462±0.080	0.481±0.022
cy19:0	0.000±0.000	0.098±0.048	0.092±0.016	0.079±0.024	0.114±0.030	0.115±0.012	0.193±0.057	0.148±0.040

Data were expressed as mean±SD.

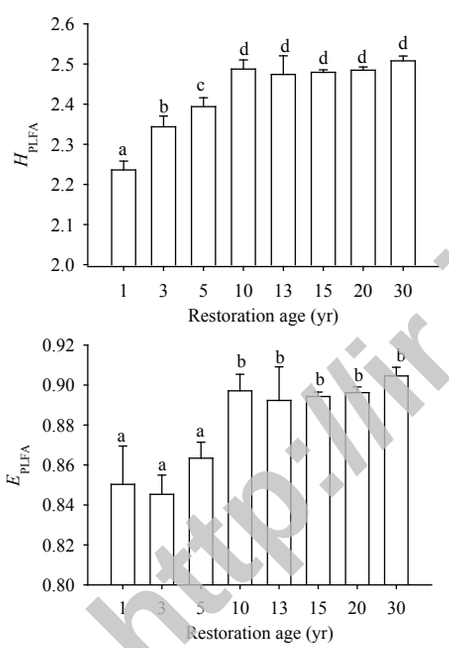

Fig. 2 Changes in Shannon richness (H_{PLFA}) and Shannon evenness (E_{PLFA}) of the abandoned cropland during natural recovery.

and total PLFAs linearly increased with time since abandonment commenced. Compared with the values in the 1-yr abandoned cropland, the soil G^-PLFAs, G^+PLFAs, B-PLFAs, and total PLFAs in the 30-yr abandoned cropland increased by 120.3, 200.5, 105.8, and 102.7%, respectively (Fig. 3-A-E). F-PLFAs also increased with the abandonment duration but not significantly (P=0.057). Compared with the value in the 1-yr abandoned cropland, the amount of F-PLFAs in the 30-yr abandoned cropland increased by 44.6% (Fig. 3-C). Change in F-/B-PLFAs ratio across time was relatively small and slightly decreased with increased abandonment duration (Fig. 3-F). The ratio in the 30-yr abandoned cropland was 29.5% less than that in the 1-yr abandoned cropland. Change in G^+/G^- ratio was comparatively small and showed a slightly increasing during the natural recovery (Fig. 3-G).

Thus, all PLFA groups exhibited an increasing trend during natural recovery. To compare the rate of increase among different groups, the relative abundances of different microbial groups were calculated from the PLFAs. Based on the total PLFA percentage, G^-PLFAs accounted for almost half of the detected PLFAs and were approximately constant during the successional stages. By contrast, the G^+PLFAs and B-PLFAs percentages slightly increased with the restoration time. F-PLFAs also decreased during natural recovery (Table 2).

Multivariate analyses

RDA was used to examine the patterns in the PLFA data from both the sampling sites and measured environmental gradients. Fig. 4-A shows the sampling points and environmental gradient arrows indicating the RDA ordination of the PLFA data. In Fig. 4-B, the PLFAs are shown with the sampling points. SOC, TN, SMBC, and SMBN increased in the general direction of restoration age, whereas qCO_2 increased toward the opposite direction (Fig. 4-A) as expected from the

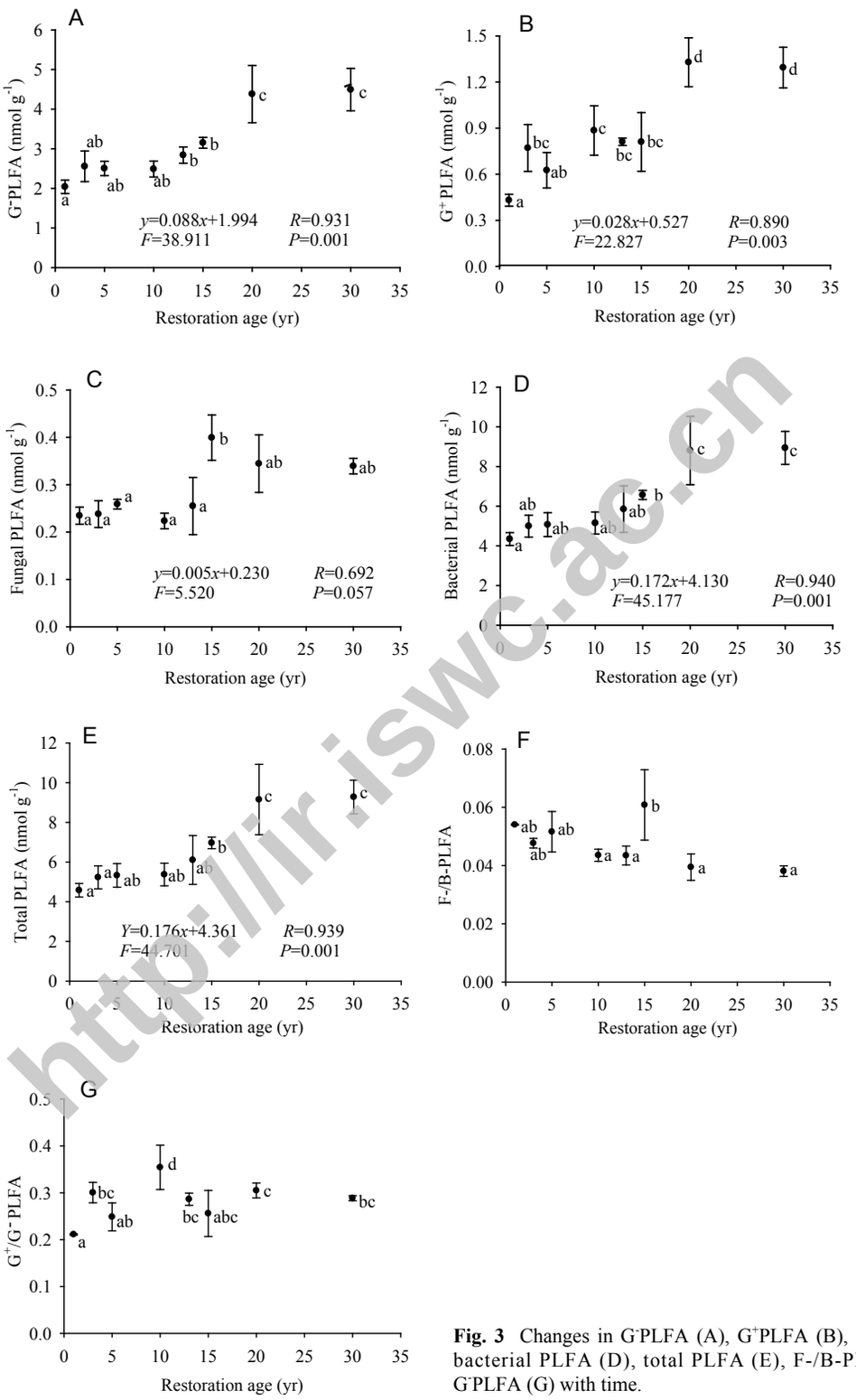

Fig. 3 Changes in G^-PLFA (A), G^+PLFA (B), fungal PLFA (C), bacterial PLFA (D), total PLFA (E), F-/B-PLFA (F) and G^+/G^-PLFA (G) with time.

sampling point comparisons (Fig. 1). Proportions of certain PLFAs (Fig. 4-B) were highly correlated with the age, SOC, TN, SMBC, and SMBN (i15:0, i16:0, 17:0, and cy17:0), C:N (i17:0, 18:1w9t, and cy19:0), BR (a15:0), or qCO_2 (3OH14:0, 15:0, and 18:2w6).

The first axis of RDA (Fig. 4) significantly corresponded to the variation in PLFAs ($P<0.05$). A total of 76.5% of the fitted PLFA data can be

Table 2 Relative abundances of microbial groups from PLFA

Microbial groups	Restoration age (yr)							
	1	3	5	10	13	15	20	30
G^+PLFA	9.41±0.15 a	14.67±1.53 cd	11.70±1.18 ab	16.41±1.93 d	13.60±2.26 bcd	11.60±2.41 ab	14.69±1.27 cd	13.94±0.16 bcd
G^-PLFA	44.55±0.42 a	48.75±2.00 a	47.15±1.84 a	46.41±1.26 a	47.32±5.93 a	45.25±0.96 a	48.12±2.52 a	48.35±1.43 a
Bacterial PLFA	94.88±0.01 ab	95.45±0.15 ab	95.10±0.62 ab	95.84±0.17 b	95.86±0.29 b	94.3±1.94 a	96.21±0.42 b	96.33±0.17 b
Fungal PLFA	5.12±0.01 ab	4.55±0.15 ab	4.90±0.63 ab	4.17±0.19 a	4.16±0.30 a	5.70±1.94 b	3.79±0.42 a	3.67±0.17 a

Different lowercase letters in the same row indicate significant difference at $P<0.05$.

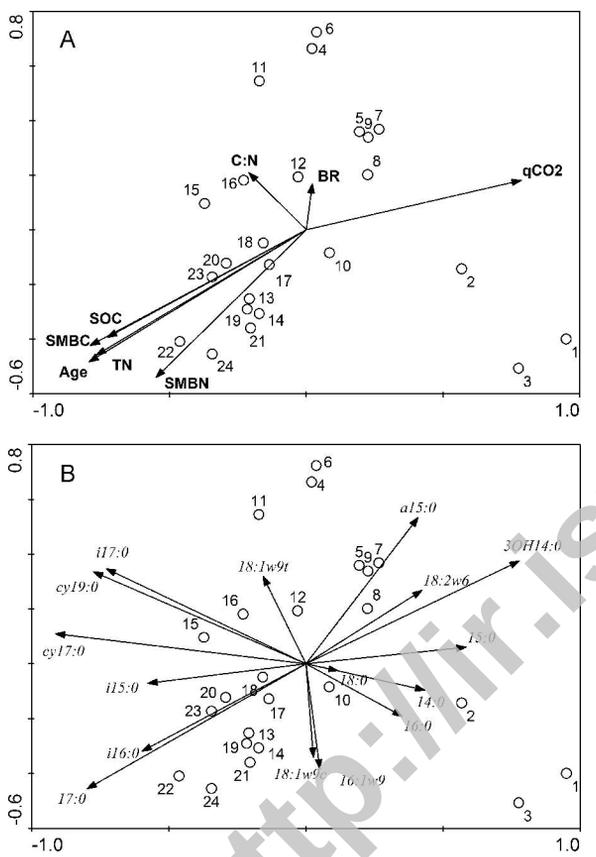

Fig. 4 Redundancy analysis (RDA) of the PLFA date for the 24 soil samples, using 16 PLFAs as species and 8 environmental variables. A, the relationship between environmental variables. B, the relationship between PLFA markers and the sampling points.

explained by the model (from the canonical sum of the eigenvalues), with the first two axes corresponding to 65.9% of this variation. For the four axes (cumulative species-environment variation), the percentages of this variation that can be explained using the obtained environmental variation were 72.1, 86.2, 93.2, and 96.5%, respectively. Axis 1 was negatively correlated with SOC, TN, age, SMBC, and SMBN. By contrast, axis 1 was positively correlated with qCO_2.

All 16 PLFAs were included in the RDA ordination to examine the relationships between samplings, species, and environmental gradients on the same diagram. Among the normal saturated PLFAs, the short-chain fatty acids (14:0, 15:0, and 16:0) were on the positive end of axis 1, whereas the long-chain fatty acid (17:0, not 18:0) was on the negative side. The cyclopropyl fatty acids (cy17:0 and cy19:0) were on the negative side of axis 1. The terminally branched saturated fatty acids (i15:0, i16:0, and i17:0) were on the negative side, whereas a15:0 was on the positive side of axis 1. For the monounsaturated fatty acids, 18:1w9t was on the upper left quadrant, whereas 16:1w9c and 18:1w9c were found on the lower right quadrant. The hydroxyl fatty acid (3OH14:0) and polyunsaturated fatty acid (18:2w6) were on the upper right quadrant.

DISCUSSION

Soil chemical and microbial properties

The soils sampled in this study had different chemical, microbial biomass, and microbial activities. SOC, TN, SMBC, and SMBN linearly increased with increased abandonment duration, consistent with the observation of Wang *et al*. (2011). The increasing trends of these parameters corresponded to the trends in the enhanced accumulation of organic C, as well as the improvement in N nutrition and microbial community in the soils as vegetation succession proceeded (Arunachalam and Pandey 2003; An *et al*. 2009; Zhu *et al*. 2012). BR was relatively stable from the beginning of cropland abandonment, similar to the finding of Merila *et al*. (2010). They found that BR remained unchanged along a boreal forest successional gradient located on the land-uplift coast of the Gulf of Bothnia, western Finland. The stable C/N ratio may account for the stability of BR during vegetation succession (Nohrstedt 1985).

Microbial qCO_2 is considered as an index for evaluating the soil microbial community efficiency for substrate utilization (Insam 1990). The decreased qCO_2 with the abandonment duration indicated that the ecosystem was stabilized by natural self-restoration (Anderson and Domsch 1993; Wardle and Ghani 1995).

Soil microbial community diversity

In this study, H_{PLFA} and E_{PLFA} significantly increased after cropland was abandoned for 10 yr. The greater PLFA diversity in the older abandoned cropland agreeded with the finding of Odum (1969), who posited that the species number increased during community development. This greater PLFA diversity also concurred with other studies on vegetation succession (Ahn and Peralta 2009; Card and Quideau 2010). Increases in microbial diversity corresponded to increased microbial biomass, in agreement with the result of DeGrood *et al*. (2005). They found that increases in microbial diversity corresponded to the increases in microbial biomass after the restoration of degraded serpentine soils. This increase in biomass should be accompanied by an increase in diversity to facilitate sustainable restoration (Bradshaw 1984). The results indicated that the microbial diversity was positively correlated with the increase in SOC during vegetation succession (Zhou *et al*. 2008).

The increase in microbial diversity during natural recovery can be attributed to microorganisms that are mainly *r* strategists, or fast colonizers with high growth rates, because they characterize altered environments during early stage of cropland abandonment. Under these conditions, all microbial groups can colonize the environment with a proportional increase in their abundance, which depends on the availability of substrates. However, with progressing succession, the food webs become more complex. Slower growing specialists, or *K* strategists, then occupy specific niches. This phenomenon may explain why H_{PLFA} and E_{PLFA} remained stable after 10 yr of abandonment (Zornoza *et al*. 2009).

Microbial PLFA biomass and composition

Distinct differences among the microbial PLFA biomass in the croplands abandoned for different durations were also found in this study. PLFA analysis results showed that the Gram-negative, Gram-positive, bacterial, and total PLFAs linearly increased with the restoration time, which corresponded to the significant increase in SMBC and SMBN. Mckinley *et al*. (2005) also found a positive correlation between the PLFA biomass and successional stages. Numerous studies have shown that the level of activity and size of the soil microbial community are C-limited (Grayston *et al*. 1997; Langer and Rinklebe 2011). SOC increased with increased chronosequence, which resulted in a higher net amount of resources that can support the microbial biomass. Soil moisture is also a determinant of soil microbial community composition because it regulates the rates of aerobic or anaerobic processes in the soil and the resulting community structure (Gutknecht *et al*. 2006). Zaady (2010) reported that the PLFA-based total biomass is twice higher in a semi-arid site than in arid and hyper-arid sites; G^+PLFAs is also significantly higher in a semi-arid site (with the highest rainfall) than in arid and hyper-arid sites. Other studies found that the soil moisture content increases during vegetation succession in semi-arid abandoned croplands in the Loess Plateau (Jiang *et al*. 2009). Thus, the improvement in soil moisture content during vegetation succession can also be attributed to the increase in PLFA biomass. F-PLFA biomass also increased with the restoration time, in accordance with a previous study (Vries *et al*. 2007). In addition to the changes in organic matter quantity and quality, reduced disturbance may also play an important role in stimulating fungal biomass, which has repeatedly been shown to be higher in no-tillage systems than in conventional tillage systems because tillage destroys the mycelial network (Wardle 1995; Helgason *et al*. 1998). The increasing trend of G^+/G^- ratio during the natural recovery suggest a shift of soil microbial composition from a G^- to a more G^+ dominated bacterial community. It might also be interpreted as a shift from a more chemolithotrophic (many of them are Gram-negatives) to a more heterotrophic community with increasing carbon input (Tscherko *et al*. 2004).

In the present study, the relative abundance of G^+PLFAs increased with the restoration time, and

F-PLFAs decreased during natural recovery. However, Potthoff *et al*. (2006) found opposite results after studying a grassland in California. The proportion of different PLFA groups differed from those in other studies (Moore *et al*. 2010). The history of the grassland, soil texture, pH, and vegetation species possibly contribute to the relative abundances of different PLFA groups and their changes during vegetation succession (Bach *et al*. 2010; Rousk *et al*. 2010).

RDA was performed to examine the patterns in PLFA data in terms of the sampling sites and obtained environmental gradients. The environmental variables obtained in this study corresponded to 96.5% of the PLFA variability. Some interesting PLFA patterns were correlated with the ordination axes in terms of chain length, iso- *vs*. anteiso-branching, and saturated *vs*. unsaturated groups among certain PLFAs. The results indicated that the soil microbial PLFA changed from anteiso- to iso-PLFAs, unsaturated to saturated PLFAs, and short- to long-chain PLFAs during natural recovery, in agreement with the results of McKinley *et al*. (2005). Changes in the PLFA ratios may be associated with nutrient stress (Peacock *et al*. 2001; Fierer *et al*. 2003) or oxygen limited stress. The high levels of organic matter and mature development of the extensive root systems of the naturally recovered grasses on the abandoned cropland may be unable to provide sufficient nutrients, root exudates, and growth factors to the bacterial communities in these ecosystems for balanced growth.

CONCLUSION

In arid and semi-arid areas, revegetation is very important in controlling soil erosion and recovering fragile ecosystems. This study indicated that vegetation succession after cropland abandonment in the Dunshan Watershed in the Loess Plateau resulted in a significant improvement in soil chemical properties, microbial diversity, microbial biomass, and microbial community composition changes. SOC, TN, SMBC, SMBN, G^-PLFAs, G^+PLFAs, B-PLFAs, F-PLFAs, total PLFAs and G^+/G^- ratio increased with increased abandonment duration. By contrast, qCO_2 and F/B ratio decreased after the cropland was abandoned. H_{PLFA} and E_{PLFA} significantly increased after 10 yr of abandonment. RDA showed that the abandonment duration was the most important environmental factor in determining the PLFA microbial community composition. The soil microbial PLFAs changed from anteiso- to iso-, unsaturated to saturated, and short- to long-chain during natural recovery. Therefore, in the Loess Plateau, cropland abandonment for natural recovery resulted in the increase of the soil microbial PLFA biomass and microbial PLFA species and changed the microbial from chemolithotrophic to a more heterotrophic community.

MATERIALS AND METHODS

Study area

The study was conducted at the Dunshan watershed (109°19′23′′E, 36°51′30′′N), Ansai Research Station of the Institute of Soil and Water Conservation, Chinese Academy of Sciences, Loess Plateau, China. This area is characterized by a semi-arid climate with a mean annual temperature of 8.8°C and average frost-free period of 203 d. The average annual precipitation is 510 mm, of which more than 60% falls between July and September. The annual evaporation ranges from 1 500 to 1 800 mm. The present soil type is primarily Huangmian soil (Calcaric Cambisols, FAO), which originates from wind deposits and is characterized by a yellow color, absence of bedding, silty texture, looseness, macroporosity, and wetness-induced collapsibility (Zhang *et al*. 2011). These characteristics make the soil particularly susceptible to wind erosion.

Experimental design and soil sampling

To study the changes in soil microbial community composition of abandoned cropland for natural recovery, eight croplands abandoned for 1, 3, 5, 10, 13, 15, 20, and 30 yr were selected as the experimental sites in September 2008. The ages of the abandoned croplands were determined by interviewing local farmers and village elders, as well as by reviewing rental contracts between farmers and the government. These sites slightly differ in terms of aspect, gradient, elevation, and previous farming practices. All investigated soils were developed from the same parent materials. Prior to abandonment, the main crops grown on the eight croplands were millet (*Setaria italica*) and soybean (*Glycine max*). One crop was grown each year and fertilizer was applied before planting. The croplands had been

cultivated using a hoe and a plow for more than 40 yr. After abandonment, the croplands were not subjected to human interference, and natural secondary vegetation commenced. Detailed information on the sample sites is shown in Table 3 (Zhang *et al*. 2012).

Three 20 m×20 m plots were established at each site and considered to be true replicates because the distance among them exceeded the spatial dependence (<13.5 m) of most soil chemical and microbial variables (Mariotte *et al*. 1997). Undisturbed soil samples were collected from each site at the top 20 cm layer using a stainless steel cylinder (5 cm in inner diameter). Litter horizons were removed before soil sampling. Ten soil cores were collected in an 'S'-type pattern from each site, mixed to form a composite sample, and taken to the laboratory for analysis. Roots, stones, and debris were removed from the soil samples, and each soil sample was divided into two parts. One part was air dried, crushed, and passed through a 0.25 mm mesh for SOC and TN analysis. The other part was immediately sieved through a 2 mm mesh and stored at 4°C for the analysis of biological properties. Subsamples for PLFA evaluation were frozen at -20°C until analysis.

Table 3 Description of the sampling plots

Age (yr)	Slope gradient	Slope aspect	Altitude (m)	Dominant species
1	25°	E	1 274	*Artemisia capillaries*
3	22°	E10°N	1 273	*Artemisia capillaries*
5	25°	W10°N	1 298	*Artemisia capillaries*, *Heteropappus altaicus*
10	24°	E30°N	1 312	*Heteropappus altaicus*, *Artemisia capillaries*
13	22°	N30°E	1 282	*Artemisia capillaries*, *Artemisia sacrorum* Ledeb
15	28°	N25°W	1 256	*Artemisia sacrorum* Ledeb, *Stipa bungeana* Trin
20	22°	N35°E	1 287	*Stipa bungeana* Trin, *Artemisia sacrorum* Ledeb
30	30°	E30°N	1 258	*Artemisia sacrorum* Ledeb

Laboratory analysis

Soil chemical and microbial properties

The SOC, TN, soil microbial biomass C and N (SMBC and SMBN, respectively), basal respiration (BR), and metabolic quotient (qCO_2) were analyzed as described by Zhang *et al*. (2011).

Microbial community structure

The PLFA content is used as an index of the viable microbial biomass (Calderón *et al*. 2000) because cellular enzymes hydrolyze and release the phosphate group of phospholipids within minutes or hours following cell death (Vestal and White 1989; Marie *et al*. 1999). In the present work, phospholipid extraction and PLFA analysis were performed based on the method of Bligh and Dyer (1959) as modified by White *et al*. (1979) and described by Frostegard *et al*. (1993).

Freeze-dried soil (3 g) was weighed and placed in glass tubes. Lipids in the soil samples were extracted twice by adding 3.6 mL of citrate buffer (pH 4.0), 4 mL of chloroform, and 8 mL of methanol. Suspensions were shaken for 1 h and centrifuged for 10 min (1 258×g). The supernatants of both extraction cycles were collected in new tubes, in which 4.8 mL of citrate buffer and 6 mL of chloroform were added to enhance phase separation. On the next day, the lipid layers were transferred to new tubes, dried under N_2, and redissolved in chloroform. The lipid extracts were separated into neutral, glycolipids, and phospholipids by chloroform, acetone, and methanol, respectively, in a silica-filled solid-phase extraction column. The methanol fractions (PLFAs) were dried under N_2. The dried lipids were then redissolved in 1 mL of methanol:toluene (1:1, v/v) and 1 mL of 0.2 mol L^{-1} methanolic KOH. Samples were incubated at 35°C for 15 min to allow *trans*-esterification to methyl esters. After cooling the samples to room temperature, 2 mL of hexane:chloroform (4:1, v/v), 1 mL of 1 mol L^{-1} acetic acid, and 2 mL of water were added. After vortexing, the samples were centrifuged for 5 min (805×g). The hexane layers containing the methylated fatty acids were transferred to pointed tubes. The aqueous phases were washed twice with hexane:chloroform, and the combined hexane phases were dried under N_2. The fatty acid methyl esters were finally redissolved in 0.3 mL of hexane containing methyl nonadecanoate fatty acid (19:0) as an internal standard.

PLFAs were detected using gas chromatography (GC) coupled with a flame ionization detector (FID). An Agilent 7890A GC system was used and fitted with a split/splitless injector and an HP-5 (Agilent Technologies, Santa Clara, USA) capillary column (30 m length, 0.25 mm ID, 0.25 μm film; containing 5% phenylmethyl siloxane). Helium was used as the carrier gas (20 cm s^{-1}). Fatty acid methyl esters (FAMEs) were separated under the following temperature conditions: 150°C for 4 min, increased at a rate of 4 to 250°C per min, and finally maintained for 5 min. Samples (1 μL) were injected using an autosampler at an injector temperature of 250°C. FAMEs were detected using FID operating at 280°C, and compared with known retention times to identify individual PLFAs using a Supelco 26 PLFA peak standard (Sigma Aldrich, Dorset, UK). The fatty acids

were quantified by comparing the individual PLFA peak areas with those of the internal standard (19:0). PLFAs were expressed in nanomole PLFA per gram dry soil. The results represent the arithmetic means of three replicates.

The fatty acid nomenclature used was in accordance with Ratledge and Wilkinson (1988). Certain lipids can serve as indicators of specific microbial groups. In particular, branched saturated (iso- and anteiso-) lipids, including i15:0, a15:0, i16:0, and i17:0, have been found to be indicators of Gram-positive bacteria (G^+PLFA). The lipids used as indicators of Gram-negative bacteria (G^-PLFA) in this study were 16:1w9, cy17:0, 18:1w9c, 18:1w9t, and cy19:0, and saturated fatty acids containing an -OH group. The bacterial PLFA was assumed to be represented by the sum of the marker PLFAs for G^+PLFA, G^-PLFA and normal saturated fatty acids: 14:0, 15:0, 16:0, 17:0, and 18:0. The lipid 18:2w6 indicated fungal PLFAs (F-PLFAs) (Zelles 1997). The F-/B-PLFA ratio was used as an index of the fungal/bacterial biomass ratio in the soil (Strickland and Rousk 2010).

PLFA diversity indices

The diversity of the fatty acids was calculated using the Shannon richness index H (i.e., H_{PLFA}): $H=-\sum_{i=1}^{n}[p_i(\ln p_i)]$, where p_i is the relative abundance of each fatty acid in the total PLFAs and n is the number of detected fatty acids. The equitability of the fatty acids was calculated with the Shannon evenness index E (i.e., E_{PLFA}): $E=H/\ln(R)$, where R is the total number of fatty acids tested in the community (Shannon 1948).

Statistical analysis

The results are reported as the mean±standard deviation (SD). All data were analyzed by one-way ANOVA with restoration age as the factor. The Duncan test at a probability level of $P<0.05$ was used to perform multiple comparisons. Curve estimation was used to choose fitting curve types. Changes in SOC, TN, SMBC, SMBN, qCO_2, and soil microbial community groups of PLFAs were evaluated by linear regression. One-way ANOVA was carried out to test the goodness of fit of the regressions. All statistical analyses were performed using SPSS 15.0 software. Differences at $P<0.05$ were considered statistically significant.

A redundancy analysis (RDA) was conducted using Canoco 4.5 to analyze the response of microbial community composition to soil characteristics during natural recovery. Microbial community compositions (PLFAs) were used as species data, and soil factors were used as environmental variables. RDA, a linear canonical community ordination method, was used to visualize the relationships among the PLFAs, environmental variable gradients, and samples. RDA is similar to the canonical correspondence analysis (CCA), except that RDA is a linear model whereas CCA is unimodal (the PLFA data in this study did not conform to a unimodal distribution). A log ratio transformation, $Y'=\log(100\times Y+1.00)$, was used because the PLFA data were compositional in nature (mole percentages of the total sample). The arrows in the resulting ordination diagrams pointed to the direction of maximum variation in the PLFAs, and the arrow length was proportional to the rate of change. PLFAs near the edge of the plot were the most important PLFAs that indicated site differences, whereas those near the center were less important. The PLFA arrows that pointed to the same general direction as the environmental arrows indicated good correlation with that variable. Longer arrows indicated that higher confidence can be obtained in that correlation. The longest environmental gradient arrows indicated higher confidence in the inferred correlations, approximately corresponding to a greater effect of that variable on the total species variation, and pointed to the direction where the site scores would move if the value of that environmental variable increased (McKinley *et al*. 2005).

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References

Ahn C, Peralta R M. 2009. Soil bacterial community structure and physiochemical properties in mitigation wetlands created in the Piedmont region of Virginia (USA). *Ecological Engineering*, **35**, 1036-1042.

An S S, Huang Y M, Zheng F L. 2009. Evaluation of soil microbial indices along a revegetation chronosequence in grassland soils on the Loess Plateau, Northwest China. *Applied Soil Ecology*, **41**, 286-292.

Anderson T, Domsch K. 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to access the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biology and Biochemistry*, **25**, 393-395.

Arunachalam A, Pandey H N. 2003. Ecosystem restoration of jhum fallows in northeast India: Microbial C and N along altitudinal and successional gradients. *Restoration Ecology*, **11**, 168-173.

Bach E M, Baer S G, Meyer C K, Six J. 2010. Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology and Biochemistry*, **42**, 2182-2191.

Baldrian P, Trögl J, Frouz J, Šnajdr J, Valášková V, Merhautová V, Cajthaml T, Herinková J. 2008. Enzyme activities and microbial biomass in topsoil layer during spontaneous succession in spoil heaps after brown coal

mining. *Soil Biology and Biochemistry*, **40**, 2107-2115.
Bligh E G, Dyer W J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, **37**, 911-917.
Bradshaw A D. 1984. Ecological principles and land reclamation practice. *Landscape Planning*, **11**, 35-48.
Calderón, F J, Jackson L E, Scow K M, Rolston D E. 2000. Microbial responses to simulated tillage in cultivated and uncultivated soils. *Soil Biology and Biochemistry*, **32**, 1547-1559.
Card S M, Quideau S A. 2010. Microbial community structure in restored riparian soils of the Canadian prairie pothole region. *Soil Biology and Biochemistry*, **42**, 1463-1471.
DeGrood S H, Claassen V P, Scow K M. 2005. Microbial community composition on native and drastically disturbed serpentine soils. *Soil Biology and Biochemistry*, **37**, 1427-1435.
Eaton J M, McGoff N M, Byme K A, Leahy P, Kiely G. 2008. Land cover change and soil organic carbon stocks in the Republic of Ireland 1851-2000. *Climate Change*, **91**, 317-334.
Fierer N, Schimel J P, Holden P A. 2003. Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, **35**, 167-176.
Frostegård A, Tunlid A, Bååth E. 1993. Phospholipid fatty acid composition biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology*, **59**, 3605-3617.
Fu X L, Shao M A, Wei X R, Horton R. 2010. Soil organic carbon and total nitrogen as affected by vegetation types in Northern Loess Plateau of China. *Geoderma*, **155**, 31-35.
Grayston S J, Vaughan D, Jones D. 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**, 29-56.
Gutknecht J L M, Goodman R M, Balsar T C. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. *Plant and Soil*, **289**, 17-34.
Harris J A. 2003. Measurements of the soil microbial community for estimating the success of restoration. *European Journal of Soil Science*, **54**, 801-808.
Harris J. 2009. Soil microbial communities and restoration ecology: facilitators or followers? *Science*, **325**, 573-574.
Helgason T, Daniell T J, Husband R, Fitter A H, Young J P W. 1998. Ploughing up the wood-wide web? *Nature*, **394**, 431-431.
Hou F J, Xiao J Y, Nan Z B. 2002. Eco-restoration of abandoned farmland in the loess plateau. *Chinese Journal of Applied Ecology*, **13**, 923-929. (in Chinese)
Insam H. 1990. Are the soil microbial biomass and basal respiration governed by the climatic regime? *Soil Biology and Biochemistry*, **22**, 525-532.
Jia X X, Wei X R, Shao M A, Li X Z. 2012. Distribution of soil carbon and nitrogen along a revegetational succession on the Loess Plateau of China. *Catena*, **95**, 160-168.
Jiang J P, Xiong Y C, Jiang H M, Ye D Y, Song Y J, Li F M. 2009. Soil microbial activity during secondary vegetation succession in semiarid abandoned lands of Loess Plateau. *Pedosphere*, **19**, 735-747.
Jiao F, Wen Z M, Shao M A. 2011. Changes in soil properties across a chronosequence of vegetation restoration on the Loess Plateau of China. *Catena*, **86**, 110-116.
Langer U, Rinklebe J. 2011. Priming effect after glucose amendment in two different soils evaluated by SIR- and PLFA-technique. *Ecological Engineering*, **37**, 465-473.
Li Y Y, Shao M A. 2006. Change of soil physical properties under long-term natural vegetation restoration in the Loess Plateau of China. *Journal of Arid Environments*, **64**, 77-96.
Liang J, Wang X A, Yu Z D, Dong Z M, Wang J C. 2010. Effects of vegetation succession on soil fertility within farming-plantation ecotone in Ziwuling mountains of the Loess Plateau in China. *Agricultural Sciences in China*, **9**, 1481-1491.
Mariotte C A, Hudson G, Hamilton D, Neilson R, Boag B, Handley L L, Wishart J, Scrimgeour C M, Robinson D. 1997. Spatial variability of soil total C and N and their stable isotopes in an upland Scottish grassland. *Plant and Soil*, **196**, 151-162.
Maire N, Borcard D, Laczkó E, Matthey W. 1999. Organic matter cycling in grassland soils of the Swiss Jura mountains: biodiversity and strategies of the living communities. *Soil Biology and Biochemistry*, **31**, 1281-1293.
McKinley V L, Peacock A D, White D C. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. *Soil Biology and Biochemistry*, **37**, 1946-1958.
Merilä P, Strömmer R, Fritze H. 2002. Soil microbial activity and community structure along a primary succession transect on the land-uplift coast in western Finland. *Soil Biology and Biochemistry*, **34**, 1647-1654.
Merilä P, Malmivaara-Lämsä M, Spetz P, Stark S, Vierikko K, Derome J, Fritze H. 2010. Soil organic matter quality as a link between microbial community structure and vegetation composition along a successional gradient in a boreal forest. *Applied Soil Ecology*, **46**, 259-267.
Moore J, Macalady J L, Schulz M S, White A F, Brantley S L. 2010. Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California. *Soil Biology and Biochemistry*, **42**, 21-31.
Mummey D L, Stahl P D, Buyer J S. 2002. Microbial biomarkers as an indicator of ecosystem recovery following surface mine reclamation. *Applied Soil*

Ecology, **21**, 251-259.

Nohrstedt H. 1985. Biological activity in soil from forest stands in central Sweden, as related to site properties. *Microbial Ecology*, **11**, 259-266.

Odum E P. 1969. The strategy of ecosystem development. *Science*, 164, 262-270.

Peacock A D, Macnaughton S J, Cantu J M, Dale V H, White D C. 2001. Soil microbial biomass and community composition along an anthropogenic disturbance gradient within a long-leaf pine habitat. *Ecological Indicators*, **1**, 113-121.

Potthoff M, Steenwerth K L, Jackson L E, Drenovsky R E, Scow K M, Joergensen R G. 2006. Soil microbial community composition as affected by restoration practices in California grassland. *Soil Biology and Biochemistry*, **38**, 1851-1860.

Ratledge C, Wilkinson S G. 1988. Fatty acids related and derived lipids. In: Ratledge C, Wilkinson S G, eds., *Microbial Lipids*. Academic Press, San Diego. pp. 23-53.

Rousk J, Brookes P C, Baath E. 2010. The microbial PLFA composition as affected by pH in an arable soil. *Soil Biology and Biochemistry*, **42**, 516-520.

Shannon C E. 1948. A mathematical theory of communication. *The Bell System Technical Journal*, **27**, 379-423.

Strickland M S, Rousk J. 2010. Considering fungal:bacterial dominance in soils – Methods controls, and ecosystem implications. *Soil Biology and Biochemistry*, **42**, 1385-1395.

Tscherko D, hammesfahr U, Marx M C, Kandeler E. 2004. Shifts in rhizophere microbial communities and enzyme activity of *Poa alpine* across alpine chronosequence. *Soil Biology and Biochemistry*, **36**, 1685-1698.

Vestal J R, White D C. 1989. Lipid analysis in microbial ecology: quantitative approaches to the study of microbial communities. *BioScience*, **39**, 535-541.

Vries F T, Bloem J, Eekeren N V, Brusaard L, Hoffland E. 2007. Fungal biomass in pastures increases with age and reduced N input. *Soil Biology and Biochemistry*, **39**, 1620-1630.

Wang B, Liu G B, Xue S, Zhu B B. 2011. Changes in soil physico-chemical and microbiological properties during natural succession on abandoned farmland in the Loess Plateau. *Environmental Earth Sciences*, **62**, 915-925.

Wardle D A. 1995. Impacts of disturbance on detritus food webs in agroecosystems of contrasting tillage and weed management practices. *Advances in Ecological Research*, **26**, 105-185.

Wardle D A, Ghani A. 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. *Soil Biology and Biochemistry*, **27**, 1601-1610.

Wei J, Zhou J, Tian J L, He X B, Tang K L. 2006. Decoupling soil erosion and human activities on the Chinese Loess Plateau in the 20th century. *Catena*, **68**, 10-15.

Welc M, Bünemann E K, Fließbach A, Frossard E, Jansa J. 2012. Soil bacterial and fungal communities along a soil chronosequence assessed by fatty acid profiling. *Soil Biology and Biochemistry*, **49**, 184-192.

White D C, Davis W M, Nickels J S, King J D, Bobbie R J. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia*, **40**, 51-62.

Zaady E, Ben-David E A, Sher Y, Tzirkin R, Nejidat A. 2010. Inferring biological soil crust successional stage using combined PLFA, DGGE, physical and biophysiological analyses. *Soil Biology and Biochemistry*, **42**, 842-849.

Zelles L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere*, **35**, 275-294.

Zhang C, Liu G B, Xue S, Song Z L. 2011. A comparison of soil qualities of different revegetation types in the Loess Plateau, China. *Plant and Soil*, **347**, 163-178.

Zhang C, Liu G B, Xue S, Zhang C S. 2012. Rhizosphere soil microbial properties on abandoned croplands in the Loess Plateau, China during vegetation succession. *European Journal of Soil Biology*, **50**, 127-136.

Zhou J, Guo W H, Wang R Q, Han X M, Wang Q. 2008. Microbial community diversity in the profile of an agricultural soil in the northern China. *Journal of Environmental Sciences*, **20**, 981-988.

Zornoza R, Guerrero C, Mataix-Solera J, Scow K M, Arcenegui V, Mataix-Beneyto J. 2009. Changes in soil microbial community structure following the abandonment of agriculture terraces in mountainous areas of Eastern Spain. *Applied Soil Ecology*, **42**, 315-323.

Zhu H H, He X Y, Wang K L, Su Y R, Wu J S. 2012. Interactions of vegetation succession, soil bio-chemical properties and microbial communities in a Karst ecosystem. *European Journal of Soil Biology*, **51**, 1-7.

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