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Effect of lime application on microbial community in acidic tea orchard soils in comparison with those in wasteland and forest soils

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Abstract

Lime application is a conventional technology to control acidification in tea orchard soils. We investigated the effect of lime application on soil microbial community diversity in the soils of three tea orchards, wasteland and forest. The BIOLOG data showed that both the average well color development of all carbon sources and the functional diversity index increased with the liming rate in the tea orchards and the forest, but decreased in the wasteland. The phospholipid fatty acid (PLFA) analysis showed that the structural diversity index of soil microbial community increased with the liming rate in all the tea orchards, the wasteland and the forest. Lime application also increased the soil-bacterial PLFA content in all the soils. Soil fungal and actinomycete PLFAs in the tea orchards showed an increasing trend from 0 to 3.2 g $CaCO_3$/kg application and then a decreasing trend from 3.2 to 6.4 g $CaCO_3$/kg application. The principal component analysis of BIOLOG and PLFA data suggested that lime application had a significant effect on soil microbial community structure, and land use had a greater effect on soil microbial community structure compared to lime application.

Key words: microbial community diversity; phospholipid fatty acid; lime; tea orchard soil
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Introduction

Tea (*Camellia sinensis*) is an important economic crop, which is widely planted in acidic red soils in the tropical and subtropical zones of China. The optimal pH range for tea growth is 4.5–6.0. However, the soil of tea orchards is often strongly acidified due to the high level of nitrogen fertilizer applied, and the acidity in tea orchard soil gradually increases with the tea-orchard age (Konishi, 1991; Tachibana et al., 1995; Liao, 1998; Shi et al., 1999; Yu et al., 2003, 2004). Xue et al. (2006) reported that the pH values of 8-, 50- and 90-year-old tea orchard soils are 4.22, 4.01 and 3.71, respectively, which indicates that the pH of tea orchard soils gradually decreases with the age after the wasteland is reclaimed for tea orchards. Thus, the soil pH should be adjusted for highly acidic tea orchards. Currently, the most economic and popular method is to apply lime to the soil.

Soil microbial community diversity is an important measure of sustainable land use and sensitive to changes in the soil chemical properties. Several studies indicated that lime application could directly or indirectly change soil microbial community structure (Nodar et al., 1992; Bååth and Arnebrandt, 1994; Lehle, 1994; Bardgett et al., 1996; Treonis et al., 2004; Spiegelberger et al., 2006; Kennedy et al., 2004, 2005). However, there are few studies about the effect of lime application on microbial community structure in tea orchard soil. In this study, we measured the microbial community structure and diversity of the soils in three tea orchards, a neighbouring wasteland and a forest that were amended with lime at the rates of 0, 1.6, 3.2 and 6.4 g $CaCO_3$/kg dry soil, respectively. The objective was to evaluate the effects of lime application on soil microbial community diversity in tea orchard soils and assess the relative importance of soil pH versus land use in structuring soil microbial community diversity.

1 Materials and methods

1.1 Soil sampling

The soil samples were collected from three sampling plots that were randomly chosen within an 8-year-old tea orchard, a 50-year-old tea orchard, a 90-year-old tea orchard, a neighbouring wasteland, and a 90-year-old forest in Meijiawu tea area, in Hangzhou, China. From each sampling plot, 20 cores (5 cm in diameter × 20 cm in length) were taken and mixed. All soils investigated were classified as red soils by the China Classification System (Ultisols in USA soil taxonomy) and were derived from the same parent material, namely quartzose sandstone interbedded with shale.

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The 15 bulked samples were transported on ice to the laboratory where they were sieved through a 2-mm mesh to remove plant debris and soil fauna. Each bulked sample was then separated into two parts. One part was air dried for chemical analysis (except that mineral-N was immediately analyzed); and another was stored at 4°C until the incubation experiment.

1.2 Soil chemical analysis

Soil pH was measured by a combination glass electrode (soil:water, 1:2.5, *W/W*). Total nitrogen was determined by Kjeldahl digestion (Keeney and Nelson, 1982) and quantified using a continuous flow analyzer (SA5000, Skalar Inc., the Netherlands), and the total organic carbon was determined by dichromate oxidation (Nelson and Sommers, 1982). Available phosphorus analysis was undertaken following the method by Olsen and Sommers (1982). Inorganic N (NH_4^+-N and NO_3^--N) was extracted with 2 mol/L KCl by shaking for 1 hr at 200 r/min and filtering through a 0.45-μm polysulfone membrane. The KCl-extracted N was determined colorimetrically in a continuous flow analyzer (SA5000, Skalar Inc., the Netherlands).

1.3 Incubation experiment

The soil samples were incubated at room temperature for one week. Then they were put in sterile polyethyleneglycol bottles (500 mL) and amended with lime at the rates of 0 (Ca0), 1.6 (Ca1), 3.2 (Ca2) and 6.4 (Ca3) g $CaCO_3$/kg dry soil, respectively, and they were further adjusted to the moisture content of 45% water holding capacity (Shi et al., 2006; Xue et al., 2006). All the bottles were sealed with a polyethylene film to delay moisture loss but allow gas exchange, and incubated under constant temperature of 25°C and relative humidity of 95%. The bottles were periodically weighed, and the initial moisture content was restored by adding distilled water. At the end of the two-month incubation experiment, the characteristics of the soil microbial community were assessed by the BIOLOG and phospholipid fatty acid (PLFA) analyses.

1.4 Community level substrate utilization analysis

BIOLOG Eco plates (MicroPlate™, BIOLOG Inc., Hayward, USA) were used to study the substrate utilization pattern of soil microbial communities as described by Girvan et al. (2003). Briefly, 10 g fresh soil was added to 100 mL distilled water in a 250 mL flask, and shaken at 200 r/min for 10 min, to achieve a 10^{-1} dilution. Ten-fold serial dilutions were prepared and the 10^{-3} dilution was used to inoculate the BIOLOG Eco plates. Plates were incubated at 25°C for seven days and color development value was read as absorbance daily with an automated plate reader (VMAX, Molecular Devices, Crawley, UK) at a wavelength of 590 nm, and the data were collected using Microlog 4.01 software.

1.5 PLFA analysis

Lipid extraction and PLFA analyses were performed using the modified Bligh and Dyer-method (Bligh and Dyer, 1959; Frostegård et al., 1993a). Briefly, 2.0 g soil (freeze-dried sample) was extracted with a chloroform-methanol-citrate buffer mixture (1:2:0.8, *V/V/V*), and the phospholipids were separated from other lipids on a silicic acid column. The phospholipids were subjected to a mild alkaline methanolysis and the resulting fatty acid methyl esters were prepared according to the MIDI protocol and analyzed using the MIDI Sherlock microbial identification system (MIS, MIDI Inc., USA). Fatty acids nomenclature follows that of Tunlid and White (1992). Individual fatty acids were designated in terms of total number of carbon atoms to the number of double bonds, followed by the position of the double bond from the methyl-end of the molecule. The prefixes i and a indicate iso and anteiso branching, respectively, and cy indicates cyclopropane fatty acid; Me refers to the position of methyl group from the carboxyl-end of the chain.

The sum of the PLFAs considered to be predominantly bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω7c, 16:1ω5c, i17:0, a17:0, cy17:0, 17:0, 18:1ω7c, cy19:0) was chosen to represent bacterial biomass (Frostegård et al., 1993b). The fatty acid 18:2ω6,9c was used to represent fungal biomass (Frostegård and Bååth, 1996; Olsson, 1999; Yao et al., 2006a). The fatty acid 18:0 (10Me) was used as an indicator of actinomycetes (Zogg et al., 1997).

1.6 Statistics

All values reported are the arithmetic means of the three determinations expressed on an oven-dried soil basis (105°C). Means and least significant differences (LSD) of 5% level were calculated by a one-way ANOVA.

The average well color development (AWCD) value of BIOLOG data was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates), as described by Garland (1996). Plates were read once daily and ANOVA of the AWCD over time was used to select comparable time points to avoid confounding effects of inoculum density differences between treatments (Garland, 1997; Yao et al., 2006b). The data were normalized by dividing the absorbance values by the AWCD values before multivariate analysis. The PLFA data were expressed as mole percent of individual fatty acids.

The diversity of microbial community was assessed by the Shannon index (Shannon and Weaver, 1949), calculated for each soil sample using the following equation:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where, H' is the value of the Shannon index, p_i is the number of individuals of species i, and s is the number of species found in the community profile. For BIOLOG data, p_i is the proportional color development of the ith well relative to the total color development of all wells. In the case of PLFA data, p_i is the concentration of ith individual fatty acid relative to the concentration of all fatty acids.

2 Results

The basic physico-chemical properties of soils are shown in Table 1. The pH of the tea orchard soils decreased gradually with increasing age after wasteland was reclaimed as tea orchard. The pH in the 90-year-old tea orchard soil was even lower than that in the 90-year-old forest.

Lime application caused a significant increase in soil pH (Table 2). Figure 1 shows that the average well color development (AWCD) of carbon sources in the tea orchard soils with different lime treatments were all close to zero

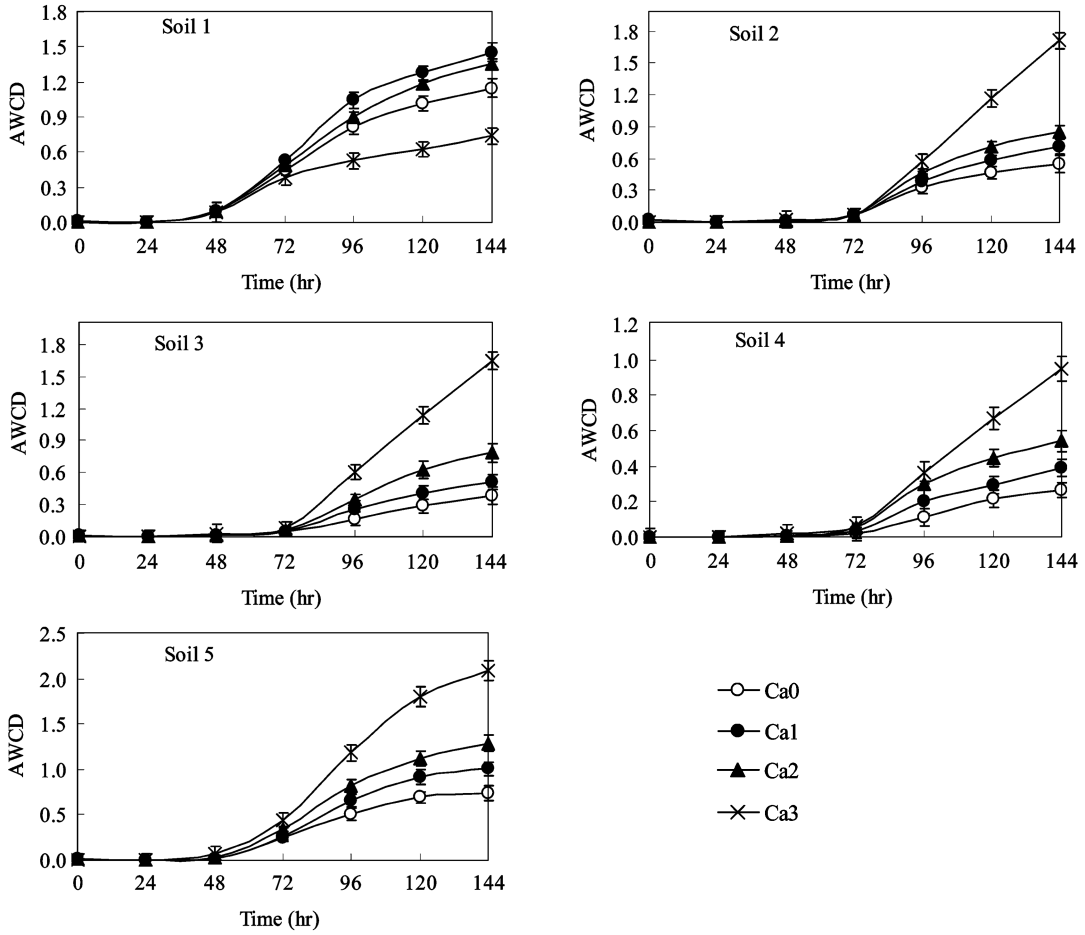

Fig. 1 Effects of lime application on the average well color development (AWCD) of the BIOLOG Eco plates at 590 nm for the tested soils collected from wasteland (soil 1), 8-year-old tea orchard (soil 2), 50-year-old tea orchard (soil 3), 90-year-old tea orchard (soil 4), and 90-year-old forest (soil 5). Ca0–3 stand for 0, 1.6, 3.2, 6.4 g $CaCO_3$/kg dry soil, respectively.

Table 1 Basic properties of the soil samples

Soil No.	pH	Organic C (g/kg)	Total N (g/kg)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	Available P (mg/kg)
1	5.16 ± 0.04 a	7.4 ± 0.2 d	0.85 ± 0.03 e	5.9 ± 0.6 c	6.6 ± 0.9 e	1.7 ± 0.4 e
2	4.22 ± 0.03 b	13.9 ± 0.2 c	1.35 ± 0.04 d	8.0 ± 0.6 b	46.6 ± 2.1 b	17.8 ± 1.3 d
3	4.01 ± 0.04 c	22.2 ± 0.1 b	2.05 ± 0.10 b	7.1 ± 0.5 b	56.1 ± 2.5 a	19.5 ± 1.5 c
4	3.71 ± 0.04 d	26.3 ± 0.5 a	2.29 ± 0.09 a	4.4 ± 0.2 d	40.3 ± 2.0 c	54.5 ± 2.3 a
5	3.94 ± 0.05 c	27.5 ± 0.5 a	1.75 ± 0.11 c	9.2 ± 0.9 a	13.5 ± 1.1 d	23.9 ± 1.8 b

Different letters within each column indicate significant difference of mean value.
Soil No. 1: wasteland; No. 2: 8-year-old tea orchard; No. 3: 50-year-old tea orchard; No. 4: 90-year-old tea orchard; No. 5: 90-year-old forest.

Table 2 Soil pH value after lime application

Soil No.	Ca0	Ca1	Ca2	Ca3
1	5.20 ± 0.11 d	6.23 ± 0.10 c	7.32 ± 0.13 b	7.77 ± 0.12 a
2	4.21 ± 0.06 d	4.87 ± 0.09 c	5.55 ± 0.10 b	7.34 ± 0.10 a
3	3.99 ± 0.05 d	4.50 ± 0.08 c	5.06 ± 0.08 b	6.67 ± 0.09 a
4	3.68 ± 0.05 d	4.26 ± 0.05 c	4.78 ± 0.09 b	5.92 ± 0.05 a
5	3.96 ± 0.07 d	4.21 ± 0.06 c	4.93 ± 0.06 b	6.20 ± 0.06 a

Different letters within each line indicate significant difference of mean value. Soil No.1–5 refer to table 1. Ca0–3 stand for 0, 1.6, 3.2, 6.4 g $CaCO_3$/kg dry soil, respectively.

before 72 hr. At 144 hr of the incubation, the AWCD of the three tea orchards and the forest gradually increased with the lime level, and reached the peak under the Ca3 treatment, whose maximal increased rates were 211%, 328%, 257% and 182%, respectively (Fig. 1).

The bacterial PLFA of the soils with different treatments all gradually increased with the lime level (Fig. 2). The fungal and actinomycete PLFAs from the soils in the tea orchards (Soil No. 2–4) showed a clear increasing trend from Ca0 to Ca2 and then decreased. On the other side, the fungal and actinomycete PLFAs of the wasteland and forest soils decreased or increased gradually with the lime level, respectively.

Based on the Shannon diversity index calculated from BIOLOG data, the soil microbial community diversity in the three tea orchards and the forest all significantly increased with the lime level (Fig. 3). Being distinct from the corresponding controls, the increased rate of the Shannon diversity index under the Ca3 treatment was significantly different not only between the tea orchards and the forest, but also among the tea orchards. The index order of different soils was as follows: 90-year-old tea orchard > 8-year-old tea orchard or 50-year-old tea orchard > forest. For the wasteland, the soil microbial community diversity was the highest under the Ca1 treatment and was lower under Ca3 than that of the control. For the PLFA data, the change of the Shannon diversity index in all the soils with the lime level followed the same pattern of Ca3 > Ca2 > Ca1 > Ca0.

Based on BIOLOG data, the principal component analysis (PCA) revealed a significant effect of lime application on the patterns of potential carbon utilization and microbial community structure (Fig. 4a). The change trends of soil microbial community with the lime level were different among the tea orchards, the wasteland and the forest. For the three tea-orchard soils, the first principal component (PC1, accounting for 36.5% of the variance) showed a decreasing trend; whereas the second principal component (PC2, accounting for 9.6% of the variance) showed an increasing trend. For the forest soil, the change trend was not in agreement with the tea-orchard soils: PC2 gradually decreased with the lime level. Both PC1 and PC2 in the wasteland were lower under the Ca1 and Ca2 treatments than those under Ca0, and PC1 was higher under Ca3 than that under Ca0. The analysis of the loadings of the most influential carbon sources from the tea orchard

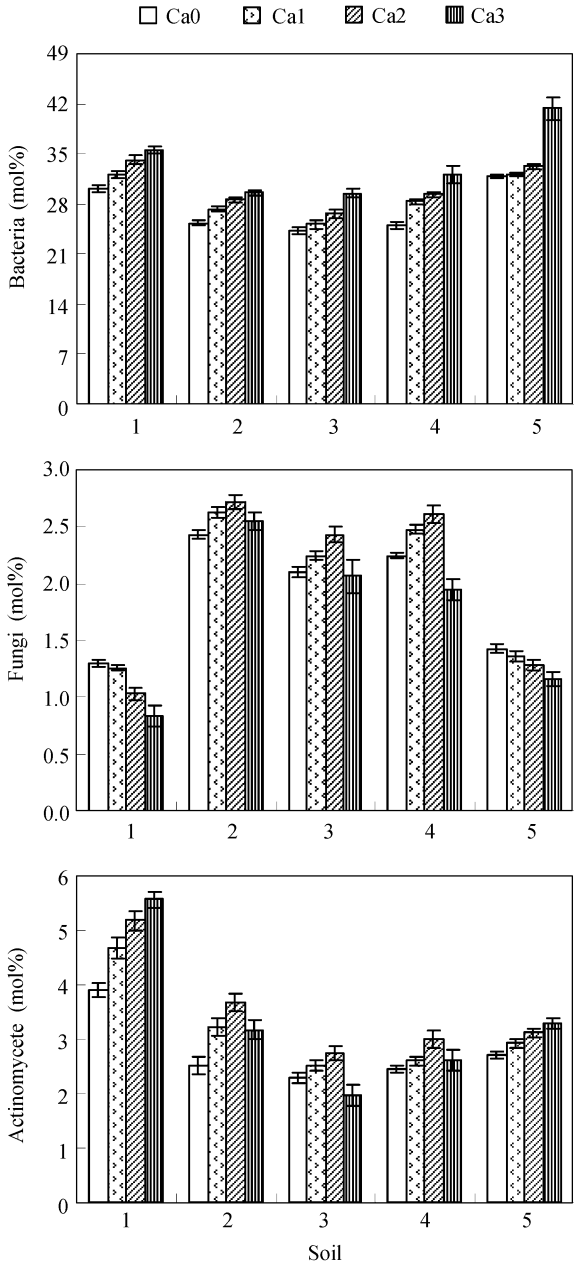

Fig. 2 Effects of lime application on mol% of the characterizing microbial PLFAs.

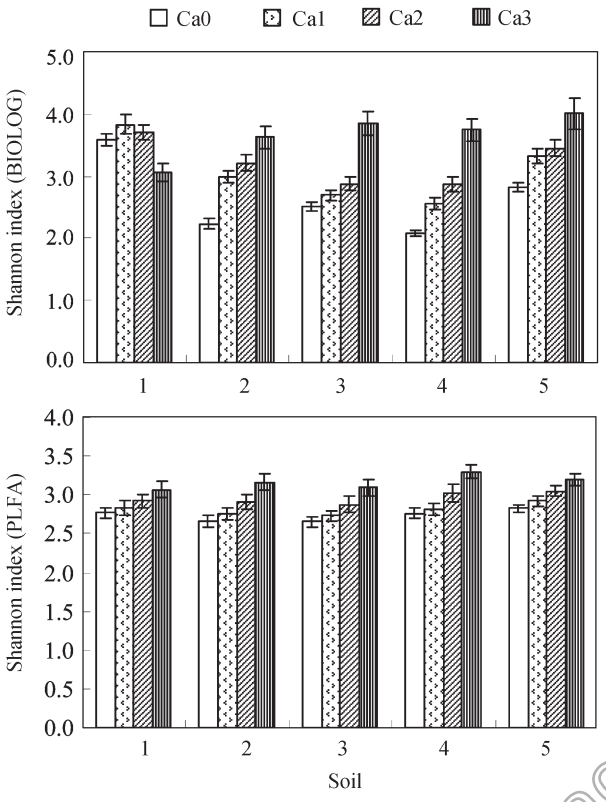

Fig. 3 Effects of lime application on Shannon index of soil microbial communities based on BIOLOG and PLFA data.

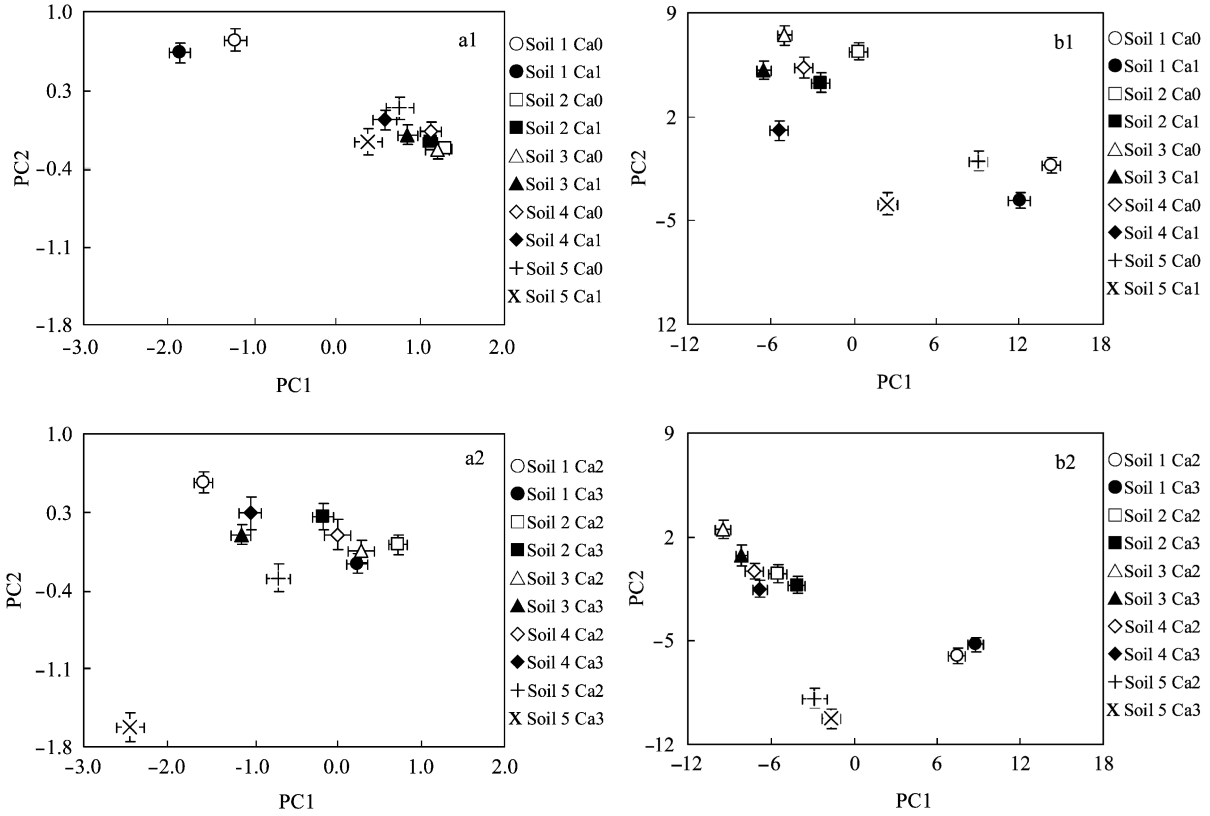

Fig. 4 Principal component analysis (PCA) of the tested soils with lime treatment based on BIOLOG (a) and PLFA (b) data.

soils indicated that lime application stimulated the utilization rate of D-mannitol, N-acetyl-D-glucosamine, pyruvic acid methyl ester and L-asparagine, but it repressed the utilization rate of D-cellobiose, 2-hydroxy benzoic acid, D-xylose and L-serine.

The PLFA-based PCA also showed that lime application had an evident effect on soil microbial community structure (Fig. 4b). The change patterns of soil microbial community structure from the wasteland, the tea orchards and the forest with the lime level were perfectly consistent with the treatment order from Ca0 to Ca2; and both PC1 (accounting for 58.4% of the variance) and PC2 (accounting for 27.5% of the variance) showed a decreasing trend. PC1 showed an increasing trend and PC2 showed a decreasing trend for the tea orchards and the forest from the Ca2 treatment to the Ca3 treatment. In contrast, PC2 from the wasteland showed an increasing trend. The analysis of the loadings of the most influential fatty acid from the tea orchard soils indicated that upon lime application, fatty acids 16:0 (10Me), i15:0 and i16:0 increased, while 18:0 and 18:1ω9c decreased.

In order to compare the relative importance of land use versus lime application in structuring soil microbial community structure, we performed the PCA analysis based on different land uses (tea orchard, wasteland and forest) or different pH groups. The results showed the soil microbial communities in the wasteland, tea orchards and forest were significantly separated along PC1 and PC2 (Fig. 5). However, The trends of different groups with soil pH were no obvious different (Fig. 6), suggesting that upon the lime application with the range from Ca0 to Ca3, the soil pH change had a smaller effect on soil microbial community structure compared with the land use.

3 Discussion

The average well color development is often used to assess the whole activity of the microbial community based on the number and species of microbes. In this study, we designed three gradients of lime, and showed that the AWCD values in the tea orchards and the forest both increased with the lime level, while the lime effect on AWCD in the wasteland followed a specific pattern: the effect of Ca1 was the highest, and that of Ca3 was much lower than the control. This observation suggests that, as for tea orchards and forest, the treatments of Ca0, Ca1, Ca2 and Ca3 increase the activity of microbes; for wasteland, however, the Ca3 treatment has an inhibitory effect on microbes. Therefore, the background soil pH should be determined before lime can be applied for the pH adjustment.

Many studies have shown that soil microbial community structure not only responds to soil management practice but also to several environmental factors. A key factor in determining soil microbial community structure is soil pH. Bååth and Anderson (2003) found that the fungi/bacteria ratio measured with the selective inhibition technique significantly decreases with the increase of pH. Tang and Xiong (2003) reported that the tobacco-covered acidic soil with lime application results in the increases of soil pH, bacteria, actinomycetes and aerobic fungi of decomposing fibrin. This study assessed the changes of

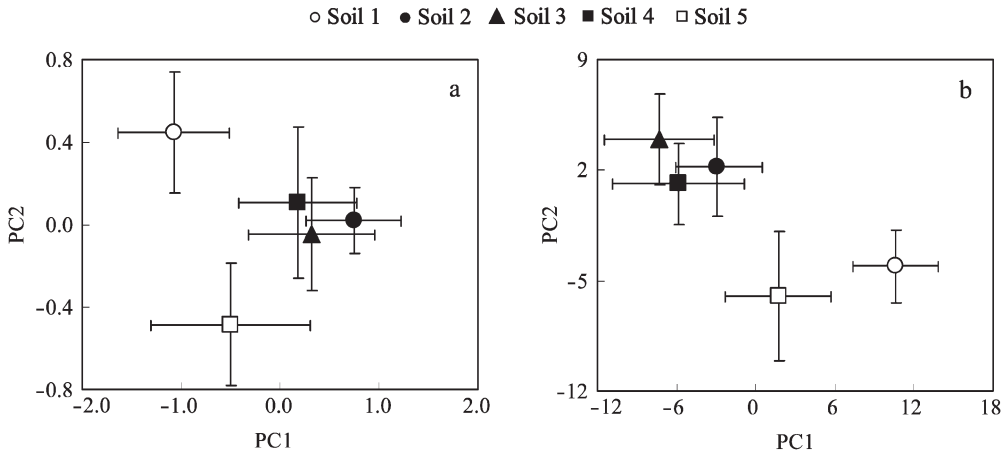

Fig. 5 Principal component analysis (PCA) of the tested soils with different land uses based on BIOLOG (a) and PLFA (b) data.

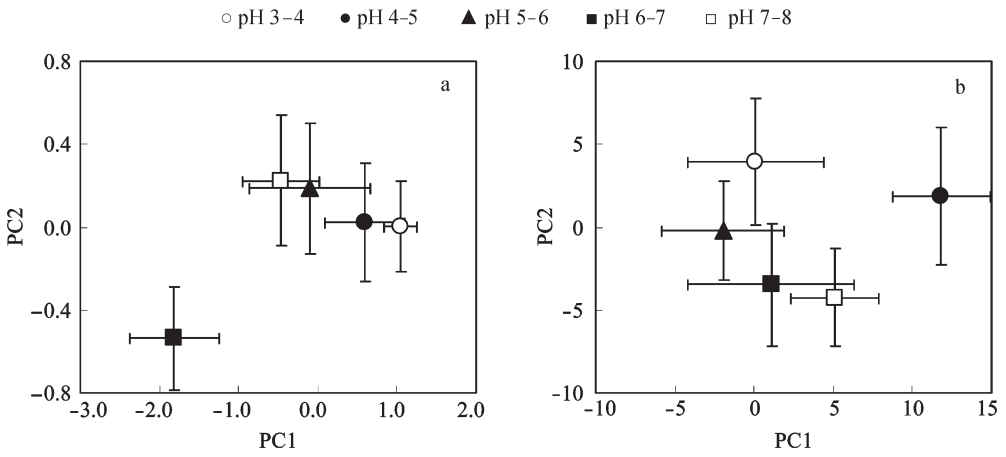

Fig. 6 Principal component analysis (PCA) of the tested soils with different pH groups based on BIOLOG (a) and PLFA (b) data.

three main microbes-bacteria, fungi and actinomycetes using the PLFA method and found that all these microbes had significant changes. Bacteria showed an increasing trend with the amount of lime input in the tea orchards, the wasteland and the forestland. Interestingly, the change of fungi in the tea orchards was quite different from that in wasteland or forest. The amount of actinomycetes increased with the lime application in the wasteland and the forest, which is consistent with the general concept that the environment with neutral or slightly alkali pH is suitable for actinomycetes. Howerer, in all the tea-orchard soils, the amount of actinomycetes increased from Ca0, Ca1 to Ca2 and significantly declined from Ca2 to Ca3. The results may suggest that some acid-tolerant actinomycetes have higher activity in low pH condition than in neutral condition in the tea orchard soils (Nioh et al., 1993, 1995).

The Shannon index is a widely-used community diversity measurement, which reflects the richness and homogeneousness of a community. According to the analysis on BIOLOG data, the effect of lime application on the microbial diversity index is similar to what is observed in AWCD: the soil microbial diversity index of the tea orchards and the forest increased with the amount of lime; while the diversity index in the wasteland was the highest under the Ca1 treatment and was lower than the control under Ca3. The contrasting effects may be due to the background pH in the wasteland, which is much higher than that in the tea orchards or in the forestland. Thus, with the application of high-amount lime, the wasteland may not fit some microbes. According to our PLFA analysis, all the soil microbial diversity indexes increased with the lime amount. The pattern is a little different from that based on BIOLOG data, and this is probably because these two methods reflect different aspects of the microbes (Xue et al., 2008).

Land use involving different plant species and soil properties, can affect the soil microbial community structure (Nüsslein and Tiedje, 1999). Plant species have a large effect on microbial community structure (Grayston et al., 1998, 2001; O'Donnell et al., 2001; Nüsslein and Tiedje, 1999; Marschner et al., 2001). Because a large amount of organic compounds are secreted by the root and continuously supplied by the litter, the number and species of soil organic matters may be changed and therefore have a significant influence on microbial community structure. This study showed that the influence of lime on soil microbial community was less than that of land use at least

in the lime range between Ca0 and Ca3. However, several studies argue that the influence of artificial cultivation management on soil microbial community structure is more important than plant species. Buckley and Schmidt (2001) found that cultivation and chemical pesticides are the main factors that influence on soil microbial community structure in terms of plant species. Thus, it seems different results are obtained in these studies, which may be due to different plant types, management styles and management intensity.

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