

# Effects of simulated acid rain on microbial characteristics in a lateritic red soil

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**Abstract** A laboratory experiment was performed to examine the impact of simulated acid rain (SAR) on nutrient leaching, microbial biomass, and microbial activities in a lateritic red soil in South China. The soil column leaching experiment was conducted over a 60-day period with the following six SAR pH treatments (levels): 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 and one control treatment (pH=7). Compared with the control treatment, the concentrations of soil organic matter, total nitrogen, total phosphorus, total potassium, soil microbial biomass carbon (MBC), soil microbial biomass nitrogen (MBN), and average well color density (AWCD) in the Ecoplates were all significantly decreased by leaching with SAR at different pH levels. The decrease in MBC and MBN indicated that acid rain reduced the soil microbial population, while the decrease in AWCD revealed that acid rain had a negative effect on soil bacterial metabolic function. Soil basal respiration increased gradually from pH 4.0 to 7.0 but decreased dramatically from pH 2.5 to 3.0. The decrease in soil nutrient was the major reason for the change of soil microbial functions. A principal

component analysis showed that the major carbon sources used by the bacteria were carbohydrates and carboxylic acids.

**Keywords** Acid rain · Lateritic red soil · Microbial characteristics · Soil nutrients

## Introduction

Acid rain, global warming, and ozone depletion are recognized as the world's three largest disasters to ecological environments. Acid rain can damage environments and ecosystems through acidification (Larssen et al. 2006; Fan et al. 2010; Liu et al. 2010; Wang et al. 2014). Acid rain primarily originates from anthropogenic activities which produce a large amount of sulfur dioxide (SO<sub>2</sub>) and nitrate and nitrite oxide (NO<sub>x</sub>) gases in the atmosphere. In recent year, China has become the third largest acid rain impaired region, only next to Europe and North America. The acid rain pH in China typically ranges from 3.0 to 4.5 (State Environmental Protection Administration of China 2005). At present, acid rain has been reported to cover at least one third of Chinese territory (Liu et al. 2011) and the cost of acid rain damage is estimated to be over 110 billion Chinese Yuan each year (Wu et al. 2006; Zhang et al. 2010).

South China is adjacent to the north of the Nanling Mountain and to the south of the South China Sea with high elevation in the north and low elevation in the south. Such geographical environment is favorable for the accumulation of polluted air, thus enhancing the formation of acid rain. During the past decades, the area, especially the Pearl River Delta in Guangdong Province, has experienced a rapid development of economy and society with intensive urbanization. The harmful impacts of acid rain resulted from anthropogenic activities in this area are increasingly severe (Qing et al. 2006;

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Wen et al. 2011). According to the acid rain control area delineated by the Chinese State Council, the Pearl River Delta accounts for 71.6 % of the total acid rain control area in Guangdong Province and for 16 % of the acid rain control area in China (Qing et al. 2006).

Lateritic red soil, a kind of the zonal acid soil distributed in south China, is thin and barren with acidic property, and the exchangeable aluminum is a dominant cation. Recent studies reveal that soil microorganism could control a number of chemical and biological processes in lateritic red soil. For example, as an important driving force for soil carbon and nitrogen recycles, microbes directly participate in fixation and mineralization of organic carbon in soil (Garcia-Pausas and Paterson 2011; Tang et al. 2011). To date, the soil microbial community structure and function have become the new indicators for soil quality and fertility. These indicators are affected by environmental conditions such as soil pH and soil carbon/nitrogen ratio. The slight changes to the composition of microbial community and microbial growth efficiency can have remarkable impacts on soil carbon and nitrogen dynamics (Acosta-Martínez et al. 2008). Estimating impact of different acid rain pH levels on soil nutrient and carbon status can accurately forecast carbon cycle.

A number of studies have been performed to investigate the impacts of acid rain on soil quality and microorganisms in recent years (Walna et al. 1998; Liao et al. 2007; Ling et al. 2007; Zhang et al. 2010; Liu et al. 2009). Zhang et al. (2007) demonstrated that there is a linear increase between the leachate  $K^+$  concentration and the simulated acid rain (SAR) with  $pH < 3.0$ , whereas there is an exponential decrease in leachate  $Na^+$  concentration at all levels of the SAR pH in a latosol soil. Ling et al. (2007) reported that after 21 days, a latosol treated with the SAR at  $pH = 2.5$  can leach about 34, 46, 20, and 77 % of the original exchangeable calcium ( $Ca^{2+}$ ), magnesium ( $Mg^{2+}$ ), potassium ( $K^+$ ), and sodium ( $Na^+$ ), respectively. Liao et al. (2007) reported that the release of heavy metals from the natural soils is zinc (Zn) > copper (Cu) > cadmium (Cd) and from the acid contaminated soils is Cd > Zn > Cu, indicating the leaching selectivity of each metal in acid impacted soils. Acid rain can restrain some microorganism activities through its effects on enzyme activity (Ling et al. 2010), which results in the decline of soil community function. Although the above studies have provided useful insights into the impacts of acid rain on soil cations, available phosphorus, heavy metals release, and enzyme activities, more efforts are needed to fully investigate the influence of SAR on soil nutrient leaching and microbial community structure and function. The objectives of this study were to (1) estimate impacts of SAR on soil nutrient leaching from the lateritic red soil, (2) examine the ecological functional changes of microorganisms on soil organic carbon (SOC) conversion after leaching with SAR, and (3) analyze impacts of the SAR on

metabolism diversity of microorganisms in lateritic red soil and their utilization of carbon sources.

## Materials and methods

The top 20 cm of a lateritic red soil collected from a garden located in a district of Guangzhou City, Guangdong Province, China was used for the experiment. This soil has  $pH$  4.78 with an organic matter content of  $16.50 \text{ g kg}^{-1}$ , total N (TN) of  $0.93 \text{ g kg}^{-1}$ , total P (TP) of  $1.13 \text{ g kg}^{-1}$ , and total K (TK) of  $22.08 \text{ g kg}^{-1}$ . Analytical grade sulfuric acid ( $H_2SO_4$ ), hydrochloric acid (HCl), nitric acid ( $HNO_3$ ), and other chemical reagents were purchased from the Guangzhou Chemical Reagent Inc., Guangdong Province, China. In South China, acid rain primarily consists of  $H_2SO_4$ ,  $HNO_3$ , and HCl. A mass ratio of 3.5:1.5:1.6 for  $H_2SO_4$ ,  $HNO_3$ , and HCl, respectively, was used to prepare the stock acid solution. The working solutions of the SAR with  $pH$  2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 were prepared in the volumetric flasks by diluting the stock solution with deionized water.

A plastic cylinder with a 15-cm inner diameter was used to make 30-cm-long soil columns. For each column, 3000 g of air-dried soil was passed through a 2-mm sieve mixed thoroughly and stirred to prevent layering. Prior to and after filling the column, a piece of plastic filter and two pieces of paper filters were placed at both ends of the column to prevent the leakage of the soil. A total of six SAR treatments (i.e.,  $pH$  levels at 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0) with a control treatment at  $pH$  7 were investigated with each treatment replicated four times for a total  $n = 28$ . To reflect the natural rainfall conditions, an intermittent influent application method was employed. More specifically, a 250-ml influent of the SAR was slowly sprayed at a rate of  $6 \text{ cm}^3 \text{ min}^{-1}$  to the top of the column every 72 h. The total volume of the SAR solution represents the average annual rainfall at the location where the soil samples were collected. After the column spraying experiment was terminated, the soil samples were air-dried at ambient temperature and sieved through a 1 mm mesh for the determination of soil properties. The remaining soil was kept moist in the dark at  $4^\circ\text{C}$  to assess soil microbial parameters. The experiment lasted for 60 days.

The air-dried soil was analyzed for soil nutrients using the methods by NSICS (1978); soil organic matter (SOM) was determined using the potassium dichromate-external heating method; alkaline hydrolysis nitrogen (AHN) was determined using the alkaline hydrolysis diffusion method; and available P was determined with sodium bicarbonate ( $NaHCO_3$ ) extract method (NSICS 1978).

Soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN) were determined by the fumigation-extraction method (Vance et al. 1987). Three subsamples of fresh soil (equivalent to 50 g dry soil) were

extracted with 100 ml 0.5 M potassium sulfate ( $K_2SO_4$ ). The samples were shaken for 30 min and then filtered, and three subsamples (also equivalent to 50.0 g dry soil) were simultaneously fumigated in a 25 °C dark room with ethanol-free chloroform for 24 h and then extracted. The filtered liquid samples were stored at 4 °C for the analysis of SOM and TN. SMBC were calculated as  $SMBC = EC/0.45$  (Wu et al. 1990), where EC (extractable carbon) is the difference between carbon extracted from fumigated and non-fumigated samples. Similarly, the TN extracted from the fumigated and the non-fumigated soils were multiplied by MBN ( $MBN = EN/0.54$ ), where MBN is microbial biomass N and EN is extractable N.

Soil basal respiration (SBR) was determined by carbon dioxide ( $CO_2$ ) evolution rate from the soil incubated for 24 h at 22 °C, using a gas chromatograph equipped with a thermal conductivity detector. Briefly, soil samples (10 g) were placed in a vial (15 ml) and water ( $0.1 \text{ ml g}^{-1}$  soil) was added dropwise with 0.1 ml. The vials were incubated for 24 h at 25 °C and, subsequently, the  $CO_2$  evolution was measured. SBR was expressed as  $\mu\text{g } CO_2\text{-C g}^{-1}\text{soil h}^{-1}$ .

The microbial quotient ( $C_{mic}/C_{org}$ ) was calculated as the ratio of SMBC ( $C_{mic}$ ) to SOC ( $C_{org}$ ), while the metabolic quotient ( $qCO_2$ ) was calculated as the ratio of SBR to SMBC. Such indices are used to describe the microbial biomass contribution to SOC and SBR, respectively (Anderson and Domsch 1989).

The average well color density (AWCD) was determined by using Biolog Eco Micro-plate (Biolog Inc., CA, USA). Biolog ECO micro-plates contain 31 of the most used carbon sources. One control well without a carbon source was used for soil community analysis, and each carbon source was triplicated. The overall color development on the Biolog ECO plates was expressed as AWCD. During the AWCD development, a 10 g of fresh soil was placed into an autoclaved triangular flask with 100 ml of 0.85 % sterilized NaCl solution and then shake on a reciprocal shaker for 30 min and stand for 1 h. A  $10^{-3}$  diluted solution of the soil suspension was made, and a 150  $\mu\text{l}$  aliquot was used to fill in each well. The plates were incubated at 25 °C for 156 h, and the optical density at 590 nm was read every 12 h (Widmer et al. 2001).

All of the experimental procedures and sampling analyses were performed using conventional methods (Jackson et al. 1984). Statistical analysis was performed in Excel with  $F$  test at a significant level  $p=0.05$ . For the effects of SAR on soil nutrients, the degree of freedom was 23 and the value for  $F_{0.05}$  was 2.66. For the effects of SAR on SMBC, SMBN, and SBR, the degree of freedom was 20 and the value for  $F_{0.05}$  was 2.85. For the effects of SAR on AWCD, the degree of freedom was 90 and the value for  $F_{0.05}$  was 3.02. The principal component analysis (PCA) was performed using SPSS.V.15.0.

## Results and discussion

### Impacts of SAR on soil nutrients

Impacts of SAR upon the soil chemical properties at six different pH levels are shown in Table 1. The SOM contents at the end of the experiments ranged from 13.65 to 15.49  $\text{g kg}^{-1}$ . Compared with the control treatment (pH 7.0), about 11.87, 10.68, 11.35, 6.99, 4.31, and 5.97 % of SOM were leached out at the end of the experiments (60 days), respectively, at pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0. These percentages were obtained based on data from Table 1. Such a loss in SOM occurred mainly because of the leaching of dissolved organic matter with SAR. A statistical analysis (i.e.,  $F$  test) showed that variations in SOM contents within different pH levels were significant at  $\alpha=0.05$ . Ling et al. (2007) reported that SOM contents decrease with time when a latosol was leached by SAR. Liu et al. (2009) also found that most dissolved organic matter (DOM) could be lost from lateritic red soil in the early phase of acid rain leaching. Couple studies have shown that the release of organic matter from soil due to acid rain is primarily due to the loss of DOM (Yamada et al. 2002).

Analogous to the case of SOM, significant decreases in TP, TN, TK, and alkali-hydrolyzable N were observed ( $p<0.05$ ). Compared with the control treatment (pH 7.0), about 15.55, 5.12, 10.32, 9.49, 4.52, and 1.71 % of TN were leached out, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0; about 16.65, 14.14, 15.22, 10.64, 6.89, and 7.47 % of TP were leached out, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0; about 12.14, 11.16, 10.03, 9.67, 5.81, and 4.87 % of TK were leached out, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0; and about 21.04, 4.48, 8.62, 5.52, 10.06, and 3.46 % of alkali-hydrolyzable N were leached out, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0. The decreases in SOM, TN, TK, and alkali-hydrolyzable N were not all proportional to the decrease in pH levels, but the maximum decreases were mainly observed at pH 2.5. This was so because the soil pH buffer capacity was destroyed at  $pH \leq 2.5$  (Aitken 1992). As a result, more SOM, TN, TK, and alkali-hydrolyzable N had been leached out from the soil.

On the other hand, two distinct patterns of the available P (AP) concentrations were observed. The AP gradually decreased by 1.50, 8.32, 4.28, and 3.71 %, respectively, at pH 3.5, 4.0, 4.5, and 5.0, but dramatically increased by 49.18 and 12.71 %, respectively, at pH 2.5 and 3.0 (Table 1). Ling et al. (2007) also found that at pH 2.5–3.5, the AP finally increases at the end of the experiments. A possible explanation of this phenomenon could be because the high concentration of hydrogen ion ( $H^+$ ) in acid rain destroyed the fixing mechanism of AP in soil and released a large amount of dissolved AP. The available K decreased slightly from pH 3.0 to 5.0 and then sharply by 52.60 % at pH 2.5 (Table 1). A similar result was also reported by Zhang et al. (2007), whose study shows a

**Table 1** Effects of SAR on the soil organic matter and nutrients. The same letter after the values within the same column indicates no statistical significance between the values

pH	Organic matter (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	TP (g kg <sup>-1</sup> )	TK (g kg <sup>-1</sup> )	Akali-hydrolyzable N (mg kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )
2.5	13.65±1.90c	0.68±0.18b	0.84±0.24c	18.50±0.95d	55.08±5.29c	173.59±5.21a	54.10±4.58c
3.0	13.84±2.96c	0.77±0.07a	0.87±0.12c	18.71±1.98d	57.55±10.22b	131.16±7.21b	106.63±11.53b
3.5	13.73±1.25c	0.73±0.13ab	0.86±0.10c	18.95±1.98c	59.83±8.43b	114.61±9.29c	111.03±10.03a
4.0	14.41±1.96b	0.73±0.16ab	0.90±0.12b	19.02±2.15c	58.12±7.96b	106.67±10.40d	113.88±9.34a
4.5	14.82±0.83b	0.77±0.09a	0.94±0.20b	19.84±2.21b	60.96±7.60b	111.38±8.52c	110.86±7.94a
5.0	14.56±0.76b	0.80±0.16a	0.93±0.16b	20.03±2.16b	54.99±6.60c	112.04±9.21c	112.81±10.32a
7.0	15.49±1.06a	0.81±0.10a	1.01±0.19a	21.07±2.30a	66.67±3.57a	116.36±9.71c	114.14±11.01a

linear increase in effluent K<sup>+</sup> concentration at the SAR pH < 3.0 after 21 days leaching. This result strongly indicated that the K<sup>+</sup> was dramatically displaced by H<sup>+</sup> when the SAR pH was pH ≤ 2.5.

**Impacts of SAR on SMBC, SMBN, SBR, and AWCD**

As a key driving force, soil microorganism community governs the fixation and mineralization of SOC and N and thereby directly affecting the SOC and N cycles. Impacts of SAR on SMBC, SMBN, and SBR are shown in Table 2. Compared with pH 7.0, the SMBC content decreased by 48.46, 41.91, 24.64, 16.88, 23.67, and 25.09 %, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0. Similarly, SMBN decreased by 34.34, 35.89, 37.03, 37.93, 54.81, and 62.07, respectively, at the pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0. The decreases in both of SMBC and SMBN at different pH levels were significant at *p* < 0.05. Results showed that acid rain decreased the activity of soil microbes.

Unlike the cases of SMBC and SMBN, the SBR content increased by 3.34, 5.36, 3.45, and 2.33 %, respectively, at pH levels of 3.5, 4.0, 4.5, and 5.0, indicating that soil acidify to some degree could promote the release of CO<sub>2</sub>. The decreases of SBR by 8.73 and 3.23 %, respectively, at pH 2.5 and 3.0 at *p* < 0.05 indicated that the SAR with pH ≤ 3.0 would cause obvious harms to SBR. This was consistent with the results reported by Ouyang et al. (2005) and Sitaula et al. (1995).

The ratio of CO<sub>2</sub> released from soil respiration to SMBC is called qCO<sub>2</sub> (soil metabolic quotient), which measures the respiration intensity per unit soil microorganism and is sensitive to the ecological factor of soil degradation. Our study showed that the qCO<sub>2</sub> increased with SAR leaching in the following order: pH 2.5 > pH 3.0 > pH 3.5 > pH 4.0 > pH 4.5 > pH 5.0 > pH 7.0. The increase in qCO<sub>2</sub> with decreasing pH revealed that soil microorganisms were threatened by acid rain.

Soil microbial biomass C/N ratio can reflect structural information of microbial community. We found that the highest SMBC/SMBN was 8.11 at pH 2.5 and the lowest was 5.95 at pH 7.0, and the SMBC/SMBN increased significantly in the following order: pH 4.0 > pH 2.5 > pH 3.5 > pH 3.0 > pH 4.5 > pH 5.0 > pH 7.0. In general, the C/N ratio is low in bacteria and high in fungus, so the increase in SMBC/SMBN suggested perhaps the population of fungus in the soil was increased while the population of bacteria in the soil was declined after leaching with SAR.

The ratio of SMBC to SOM reflected the conversion efficiency and cycling speed of biological activity in organic carbon transformation. Our study showed that the SMBC/OC ratio significantly decreased with acid rain leaching and exhibited in the following order: pH 7.0 > pH 4.0 > pH 3.5 > pH 4.5 > pH 5.0 > pH 3.0 > pH 2.5. The highest ratio was 4.71 and the lowest ratio was 2.76. These results suggested that acid accumulation in the soil could retard the cycling of organic carbon.

Correlation analysis of soil microorganism function and soil chemical property indicated that SMBC had the

**Table 2** Effects of SAR on soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN), and soil basal respiration (SBR) at different pH levels

pH	SMBC (g kg <sup>-1</sup> )	SBR (ugCO <sub>2</sub> Cg <sup>-1</sup> )	qCO <sub>2</sub>	SMBN (g kg <sup>-1</sup> )	SMBC/SMBN	SMBC/OC
2.5	37.63±1.06d	42.55±3.67b	1.13	4.65±0.35d	8.11	2.76
3.0	42.41±1.72d	45.11±2.40b	1.06	5.54±0.37c	7.67	3.04
3.5	55.02±3.45c	48.18±0.27ab	0.88	7.61±0.46b	7.96	4.01
4.0	60.68±4.54b	49.12±2.83a	0.81	7.86±0.36b	8.27	4.21
4.5	55.73±7.98c	48.23±0.54ab	0.85	7.72±0.30b	7.24	3.76
5.0	54.69±0.85c	47.71±1.07ab	0.87	8.05±0.47b	6.81	3.75
7.0	73.01±3.83a	46.62±2.72ab	0.64	12.26±0.52a	5.95	4.71



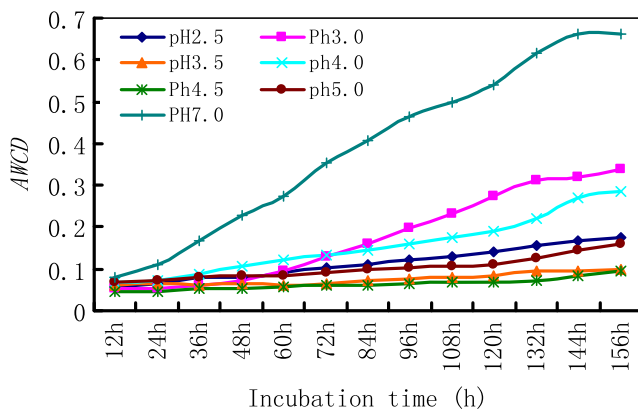
remarkable positive relationship with SOM ( $R^2=0.849$ ), TN ( $R^2=0.665$ ), TP ( $R^2=0.829$ ), and SMBN ( $R^2=0.967$ ). This proved that under the acid condition, the change of soil nutrient content was the major factor affecting soil microbial biomass. In addition, SBR had the remarkable positive relationship with SMBC/SOM, indicating that the difference of soil organic carbon conversion speed caused by acid accumulation was the major reason for the emission of  $\text{CO}_2$ .

The AWCD in the Biolog EcoPlate was showed in Fig. 1. All the data generally followed a same pattern: the test wells developed color gradually increased with incubation time but varied at different SAR pH levels. The AWCD at pH 7.0 was the highest among all treatments at 144 h. The AWCD decreased by 74.89, 51.53, 85.41, 59.17, 87.27, and 78.08 %, respectively, at the pH 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 at  $p < 0.05$ . Result suggested that the SAR had a negative effect on soil bacterial community metabolic function.

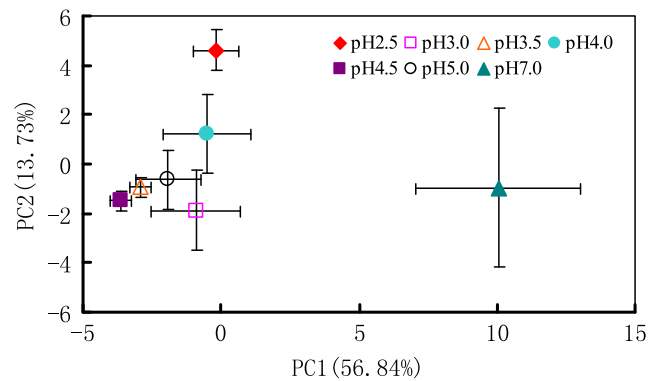
### Utilization of carbon source under SAR stress

There are 31 groups of different carbon sources in Biolog Eco Plate, 9 groups of carboxylic acids, 7 groups of carbohydrates, 6 groups of amino acids, 4 groups of polymers, 2 groups of amines/amides, and 3 groups of miscellaneous. The differences of microbial communities among the 31 groups of carbon sources can be detected from the carbon source use patterns. A principal component analysis (PCA) plot showed that the first four components explain 92.01 % variance of different treatments and the 1st and 2nd components explained 56.84 and 13.73 % variance, respectively (Fig. 2).

Our study further revealed the main carbon resources used by soil microbes included the carbohydrates group (Table 3) such as  $\beta$ -methyl-D-glucoside (0.973), D, L- $\alpha$ -glycerol phosphate (0.969), and D-mannitol (0.917) and the carboxylic acids group such as D-xylose (0.885), D-cellobiose (0.614), and 2-hydroxybenzoic acid (0.872). The numbers in the parenthesis were the correlation coefficients. Xu et al. (2007) found that in a tea plantation, the main carbon resources used



**Fig. 1** Effects of SAR on soil average well color development (AWCD) at different pH levels



**Fig. 2** Principal component analysis of diverse utilization of carbon sources

by soil microbes were mainly carbohydrates and carboxylic acid. Results were similar with those of this study. Although

**Table 3** Main substrates of carbon source with high coefficients of determination for PC1 and PC2 in principal component analysis (PCA) of diversity patterns for each site of upper layer

PC1	$R^2$
Carbohydrates	
$\beta$ -methyl-D-glucoside	0.973
L-asparagine	0.978
Tween 40	0.970
i-Erythritol	0.982
Tween 80	0.970
D-mannitol	0.917
$\alpha$ -cyclodextrin	0.703
N-acetyl-D-glucosamine	0.975
Glycogen	0.704
$\alpha$ -D-lactose	0.943
Putrescine	0.820
Carboxylic acids	
Pyruvic acid methyl ester	0.765
D-galacturonic acid	0.673
$\gamma$ -hydroxybutyric acid	0.961
D-glucosaminic acid	0.970
Itaconic acid	0.838
$\alpha$ -ketobutyric acid	0.640
Phenylethylamine	0.823
D,L- $\alpha$ -glycerol phosphate	0.969
Amino acids	
L-arginine	0.837
L-phenylalanine	0.681
L-serine	0.841
L-threonine	0.892
Glycyl-L-glutamic acid	0.649
PC2	
Carbohydrates	
D-xylose	0.885
D-cellobiose	0.614
Carboxylic acids	
2-Hydroxy benzoic acid	0.872

the acid rain decreased soil microbial parameters such as SMBC, SMBN, and AWCD, the main carbon resources used by soil microbes were not changed.

## Summary

Laboratory experiment was performed by leaching the soil columns with the SAR at pH levels of 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 over a 60-day period. The significant decreases in organic matter, TP, TN, and TK were observed after leaching with SAR although the sequence of such decreases may not be the same as the sequence of pH decrease. The maximum decreases in soil nutrient and organic matter were found at pH 2.5, showing that the high concentration of H<sup>+</sup> (or low pH) in acid rain would do great harm to soil quality by reducing nutrients through leaching. Fortunately, such a pH was not very common in the real world and typical pH of acid rain resulted from anthropogenic activities are in a range of 3.5–5.0 (Menz and Seip 2004).

Effect of acid rain on soil microbial function is an increasing agricultural and environmental concern. We found that the contents of SMBC, SMBN, and SBR decreased after leaching with SAR, suggesting that acid rain decreased the activity of soil microbes on C/N transfer. The decrease in AWCD also suggested that the effect on soil bacterial community metabolic function by SAR was negative. On the other hand, acid rain seemed to contribute to the emission of CO<sub>2</sub> to some degree as SBR increased slightly from pH 7.0 to 4.0. Correlation analysis further revealed that the decrease of soil nutrient was the major reason for the change of soil microbial functions. The PCA plot showed the main carbon source used by the bacteria were carbohydrates and carboxylic acids.

Overall, our study demonstrated that the influence of acid rain to soil ecosystem is mainly due to its changes on soil chemical and biological processes, which in turn resulted in degeneration of soil fertility, but the specific underlying mechanism of action is still unknown. As little attention has been given to quantify the impacts of the SAR pH levels on soil microbial function in the past, attempts to locate data in the published literature to compare with our finding were not so successful. In addition, our study about the acid rain in field experiment was limited as the real acid rain in nature was a long period. For a fully understanding of soil microbial community structure under SAR, further study is warranted to investigate the impacts of acid rain on latosol under field conditions.

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