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Comparative effects of sulfuric and nitric acid rain on litter decomposition and soil microbial community in subtropical plantation of Yangtze River Delta region

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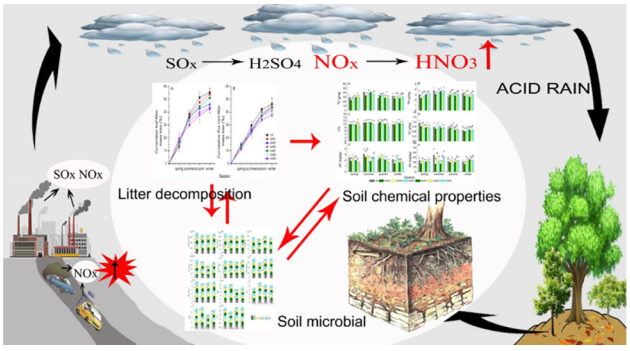
HIGHLIGHTS

- Sulfuric (SAR) and nitric acid rain (NAR) accelerated litter decomposition in the early phase of experiment.
- SAR and NAR depressed litter decomposition and their inhibitory effects were stronger on leaf-litter.
- High intensity NAR inhibited soil microbial more significantly than SAR during the rainy season.
- Gram-negative bacteria and fungi were more sensitive to acid rain, actinomycetes was more sensitive to SAR intensity.

GRAPHICAL ABSTRACT



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ABSTRACT

Acid rain is mainly caused by dissolution of sulfur dioxide and nitrogen oxides in the atmosphere, and has a significant negative effect on ecosystems. The relative composition of acid rain is changing gradually from sulfuric acid rain (SAR) to nitric acid rain (NAR) with the rapidly growing amount of nitrogen deposition. In this study, we investigated the impact of simulated SAR and NAR on litter decomposition and the soil microbial community over four seasons since March 2015. Results first showed that the effects of acid rain on litter decomposition and soil microbial were positive in the early period of the experiment, except for SAR on soil microbes. Second, soil pH with NAR decreased more rapidly with the amount of acid rain increased in summer than with SAR treatments. Only strongly acid rain (both SAR and NAR) was capable of depressing litter decomposition and its inhibitory effect was stronger on leaf than on fine root litter. Meanwhile, NAR had a higher inhibitory effect on litter decomposition than SAR. Third, in summer, autumn and winter, PLFAs were negatively impacted by the increased acidity level resulting from both SAR and NAR. However, higher acidity level of NAR (pH = 2.5) had the strongest inhibitory impact on soil microbial activity, especially in summer. In addition, Gram-negative bacteria (cy19:0) and fungi (18:1ω9) were more sensitive to both SAR and NAR, and actinomycetes was more sensitive to SAR intensity. Finally, soil total carbon, total nitrogen and pH were the most important soil property factors affecting soil microbial activity, and high microbial indices (fungi/bacteria) with high soil pH. Our results suggest that the ratio of SO_4^{2-} to NO_3^- in acid rain is an important factor which could affect litter decomposition and soil microbial in subtropical forest of China.

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1. Introduction

Rapid economic growth and increased energy demand have resulted in severe air pollution problems in China, such as acid rain. Following Northeast America and Central Europe, the southern region of China has become the third region in the world seriously affected by acid rain during the past decades (Singh and Agrawal, 2007; Liang et al., 2016). Acid precipitation has occurred in about 40% of the entire territory, especially in fast developing industrial regions, such as the areas around the Yangtze River Delta (Tu et al., 2005; Wang et al., 2007).

The major sources of acid rain are sulfur dioxide (SO_2) and nitrogen oxides (NO_x) (Huang et al., 2009; Zhao et al., 2008). However, since the late 1990s, total SO_2 emissions have decreased due to the implementation of a series of SO_2 control measures, such as improvements to combustion and pollution control technology, changes to the energy structure (Chan and Yao, 2008). The sulfate ions (SO_4^{2-}) in precipitation have decreased significantly (Lv et al., 2014). Meanwhile, NO_x emissions have increased significantly due to the rapidly growing number of motor vehicles (Zhao et al., 2009). Therefore the relative contribution of nitrate ions (NO^{3-}) to acidification has increased significantly as well. The ratio of SO_4^{2-} to NO^{3-} in precipitation has decreased from 6 in 2003 to 5 in 2014 in Nanjing, China (Tu et al., 2005; Lv et al., 2014). So nitric acid rain (NAR) may gradually substitute for sulfuric acid rain (SAR) in the future, and the forest ecosystem will face a different challenge.

Soil is one of the main components of forest ecosystem and effects on it from acid deposition are important (Reininge et al., 2011), because all plants depend on the soil for their nutrient and water supply (Fujii, 2014). With increasing acid rain input to soil, the soil ecosystem would receive a greater H^+ load, leading to soil acidification, inhibition of litter decomposition, nutrient loss, soil microbial activity reduction, etc. (Fujii, 2014; Liu et al., 2014; Qiu et al., 2015). In addition, Wolters (1991) and Wang et al. (2012) found that the rate of leaf litter decomposition decreased with acid rain (Lv et al., 2014), but the change of litter decomposition rate in fine roots is less studied.

Furthermore, acid rain also directly affects the soil microbial community, which is a sensitive ecological indicator to evaluate soil health (Kumar et al., 2017). For example, simulated acid rain (pH = 3.4) could directly reduce or completely inhibit microbial activity (Bewley and Stotzky, 1983). Bååth and Anderson (2003) showed that the total microbial biomass was positively correlated to soil pH. Nevertheless, most previous studies focused more on the effects of SAR on the soil microbial component (McColl and Firestone, 1991; Esher et al., 1992; Wang et al., 2014; Liu et al., 2014; Xu et al., 2015) than on the effects of NAR (McColl and Firestone, 1991; Lv et al., 2014). Although Lv et al. (2014) reported the effects of both SAR and NAR on soil microbial biomass and enzyme activities, the soil microbial community structure was not examined. Moreover, how NAR indirectly influences soil microbial community through changing litter decomposition and soil chemical properties is not fully understood (Wang et al., 2010; Xu et al., 2015).

To explore the effects of SAR and NAR on a plantation soil ecosystem, we have established a series of mesocosm experiments in a typical artificial forest system in the Yangtze River Delta region of China. Our primary objectives were: (1) to compare the change of litter decomposition in response to SAR and NAR in both leaves and fine roots, (2) to measure the effects of SAR and NAR on the soil microbial community, and (3) to demonstrate the relationships between soil chemical properties, litter decomposition and the soil microbial community under SAR and NAR. Based on the previous studies and reports (Lv et al., 2014; Wang et al., 2012; Xu et al., 2015), we hypothesized that: (1) acid rain would depress the litter decomposition process in leaf and fine root litter and the inhibitory effects would be more significant on leaf litter than on fine root litter, (2) the effects of NAR on litter decomposition and soil microbial community would be stronger than those of SAR.

2. Materials and methods

2.1. Study site

The study site was located in the Tong Shan forest plantation (31°37′ N, 118°51′E), Nanjing, China. The altitude of the Tong Shan forest plantation was from 38 m to 388 m. The research area consists of artificial pure forests, such as *Quercus acutissima* pure forest, *Cunninghamia lanceolata* pure forest and *Phyllostachys edulis* pure forest. It has a subtropical monsoon climate; the annual mean temperature is 16.5 °C. Annual cumulative hours of sunshine are 2199.5 h. The rainy season is from June to August (summer), and the average annual precipitation is 1117.2 mm from 2002 to 2013. The annual average pH value of rainfall is approximately 5.15 (Tu et al., 2005), with an annual acid rain frequency (total events of acid rain/total events of rainfall) of approximately 55.8% (Lv et al., 2014). The experimental plots were located in a *Q. acutissima* plantation. At the time of the study (2015–2016), the trees were about 48 years old. The average tree height of *Q. acutissima* was 13.8 m. The stand density was 425 trees ha^{-1}. The existing amount of litter was 18.04 t hm^{-2}. The soil properties at the study site were measured using the samples collected in the control plots (0–10 cm depth) during the study period, and showed that soil pH, soil total carbon (TC), total nitrogen (TN), total sulfur (TS), available phosphorus (AP), available potassium (AK) were 4.11 ± 0.06, 33.51 ± 6.04 mg g^{-1}, 3.21 ± 0.50 mg g^{-1}, 0.86 ± 0.21 mg g^{-1}, 2.50 ± 0.43 mg kg^{-1} and 38.10 ± 9.59 mg kg^{-1}, respectively.

2.2. Experimental treatments

The mesocosm experiments consisted of a complete random block design with seven treatments of 21 discrete plots (0.6 m × 2.0 m), separated from each other by approximately 5 m. Three replicates were used for each treatment, and plots were randomly selected to receive the simulated acid rain (AR) treatments. The AR treatments were established by irrigating the plots with water of deferential pH values: CK (pH 6.6, the local stream water as the control), SAR1 and NAR1 (pH 4.5), SAR2 and NAR2 (pH 3.5), SAR3 and NAR3 (pH 2.5). The stock solution of sulfuric acid rain (SAR) was prepared by mixing 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} HNO_3 at molar ratio of 5:1 that corresponded to the general anion composition of rainfall in Nanjing city (Wang et al., 2007; Lv et al., 2014). The stock solution of nitric acid rain (NAR) was prepared by mixing 0.5 mol L^{-1} H_2SO_4 and 0.5 mol L^{-1} HNO_3 at molar ratio of 1:5. The acid rain solution was applied to each plot twice a month using a sprinkler from March 2015 to February 2016, including four periods: spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). The total amount of simulated acid rain was 62.07 mm the monthly proportions of which were based on the monthly precipitation from 2002 to 2013. The amount applied to each plot was 5.55% of precipitation amount based on the ratios of average monthly precipitation to annual precipitation. The amount of during period (spring, summer, autumn and winter) was 12.79 mm (20.62% of total amounts), 32.35 mm (52.11%), 9.35 mm (15.08%) and 7.57 mm (12.19%).

Leaf and fine root litter decomposition rates were determined using the standard litter-bag technique (Singh and Gupta, 1977). We collected fresh leaf litter and fine roots in November 2014 from the study plantation site. Fine root litter was manually washed free of soil. Prior to the experiment, all leaves and fine roots from each species were air-dried and the air-dry moisture contents measured. 10 g of leaf litters were added to the polyethylene litter-bags (15 cm × 20 cm) of 1 mm mesh size (Sundarapandian and Swamy, 1999) and 3 g fine root litter were also added to the polyethylene litter-bags (10 cm × 10 cm) of 0.1 mm mesh size (Xu et al., 2013). Leaf litter-bags were placed on the forest floor in each plot in February 2015. Fine root litter-bags were placed in the soil at a depth of 0–10 cm (at about 45° angle relative to the soil surface) in each plot using a hoe. Three replicate samples (soil

and litter) per treatment were systematically harvested at the end of each season. Soil samples were passed through a 2 mm sieve to remove leaves, plant roots, gravel and stones. Half of these soil samples were air-dried for subsequent chemical analysis. The other half of these soil samples were then kept in a refrigerator at −20 °C for further phospholipid fatty acid (PLFA) analysis. Soil adhering to leaf and fine root litter samples was carefully removed, and we washed the litter manually with distilled water. All of the litter samples were oven-dried at 60 °C for 24 h to a constant weight for mass loss determination.

2.3. Chemical soil analysis

The collected soil samples were air-dried and the ants and other invertebrates, stones, roots, seeds, coarse organic matter, and other impurities were removed by sieving through a 2 mm mesh. Soil pH was determined at a 1:2.5 soil: solution ratio (in deionized water) by using a PB-10 pH meter (Sartorius GmbH, Göttingen, Germany) after shaking for 1 h (LY/T 1239-1999, 1999). Soil total carbon (TC), total nitrogen (TN) and total sulfur (TS) were determined using an elemental analyzer (Vario EL III, ELementar, Germany) after the soil samples were further ground. Available phosphorus (AP) of soil samples was extracted with ammonium fluoride (NH_4F, 0.03 mol L^{-1}) and hydrochloric acid (HCl, 0.025 mol L^{-1}) (LY/T 1233-1999, 1999; Liu et al., 1996), and measured by UV–Vis spectrophotometer. Available potassium (AK) of soil samples was determined by the extraction with CH_3COONH_4 (1 mol L^{-1}) (LY/T 1236-1999, 1999), and measured by flame photometer.

2.4. Phospholipid fatty acid (PLFA) analysis

The total biomass and the biomass of specific microbial groups as well as the microbial community structure were estimated by phospholipid fatty acid (PLFA) analysis using the procedure described by Guo et al. (2016). The PLFAs were extracted from 3 g fresh soils by adding 15.2 ml Bligh-Dyer solvent [chloroform, methanol, citrate buffer (0.15 M, pH 4.0); 1:2:0.8, v/v/v]. Following the process of soil lipid extraction, silicic acid chromatography and the methylation of polar lipids with methyl nondecanoate (19:0), the lipids were evaporated using a nitrogen evaporator. The separated fatty acids were identified using a gas chromatography (Agilent 6890 N, USA) fitted with a MIDI peak identification system.

Total PLFAs (tPLFAs) concentration (nanomole PLFA per gram soil) was used as an index of the total microbial biomass. The sum of PLFAs characteristic of general bacteria (BAC), Gram positive bacteria (G+), Gram negative bacteria (G−), actinomycetes (ACT), fungi (FUN) and arbuscular mycorrhizal fungi (AMF) was used to determine broad taxonomic microbial groupings (Moore-Kucera and Dick, 2008). The PLFAs of these groups included the following: BAC, sum of i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0, cy19:0, 16:1ω7, 16:1ω9, 18:1ω7, 17:1ω8, 17:1ω9 (Frostegård and Bååth, 1996); G+, sum of i15:0, a15:0, i16:0, i17:0, a17:0 (O'Leary, 1988); G−, sum of cy17:0, cy19:0, 16:1ω7, 16:1ω9, 18:1ω7, 17:1ω8, 17:1ω9 (Moore-Kucera and Dick, 2008); ACT, sum of 10Me16:0, 10Me17:0, 10Me18:0 (Connaughton et al., 2006); FUN, 18:2 ω6 and 18:1ω9 (Federle et al., 1986); AMF, 16:1ω5 (Guo et al., 2016). Lastly, the ratios of fungal to bacterial lipids (F/B), Gram+ bacterial to Gram− bacterial lipids (G+/G−), saturated (S, sum of 14:0, 15:0, 16:0, 17:0, 18:0) to monounsaturated (M, sum of i16:1, i16:1ω11, 16:1ω7, 16:1ω5, 17:1ω9, a17:1, 17:1ω8, 18:1ω9, 18:1ω7, 18:1ω5 and 11Me18:1ω7) PLFAs (S/M) and the ratios of the sum of cyclopropyl PLFAs (C, sum of cy17:0 and cy19:0) to the sum of the monoenoic precursors (P, sum of 16:1ω7 and 18:1ω7), abbreviated as C/P, were used as indicators of physiological or nutritional stress in bacterial communities (Moore-Kucera and Dick, 2008).

2.5. Statistical analyses

We used repeated measures ANOVA to examine the effects of acid rain treatments on litter loss and the soil microbial community. Similarity percentage (SIMPER) was applied to access which taxa were primarily responsible for the observed differences between groups in samples using PAST 3.01. Canonical correspondence analysis (CCA) and redundancy discriminant analysis (RDA) were performed to reveal the relationships between the soil microbial community, litter decomposition and soil chemical properties by using Canoco 5.

3. Results

3.1. Response of soil properties to SAR and NAR

Soil pH was significantly higher without acid rain input than with either SAR or NAR input over four experimental seasons (grey box in Fig. 1). In both SAR and NAR treatment groups, we found a clear declining trend of soil pH with higher acidity input. However, there was no significant difference in soil pH between the same acidity of SAR and NAR inputs. In spring, soil pH decreased significantly with stronger acidity input, such as SAR2, SAR3, NAR2, NAR3, compared to the control CK ($p < 0.05$) (yellow and blue boxes in Fig. 1A). Following summer, we found a similar trend of pH with SAR treatments as we showed in spring. In contrast, there was a significantly lower pH with NAR1 than with CK (green box with diagonal symbol in Fig. 1B). After autumn and winter, soil pH decreased significantly with stronger acidity input (SAR2, SAR3, NAR2, NAR3) compared to CK. However, only NAR1 led to a significantly lower pH compared to CK, not SAR1 (Fig. 1C, D).

Soil nutrients showed different patterns with acid rain over four experimental seasons compared to pH (Fig. 2). After spring acid rain was applied, soil nutrients (TC, TN, TS, AP and AK) with SAR1, TC with SAR2 and TS with SAR3 declined significantly compared to CK ($p < 0.05$). Meanwhile, TS with all NAR treatments and AP with NAR2, 3 were significantly lower than those with CK ($p < 0.05$). However, TC with NAR2 was significantly higher than CK ($p < 0.05$). In summer, TC, TN with SAR1, 2 and NAR2, AP with SAR3, N1 and AK with SAR1, NAR1 were significantly lower than those with CK ($p < 0.05$). In contrast, TC with SAR1, 3 and NAR2, TN with SAR3, AP with SAR2 and AK with NAR3 were significant higher than CK in autumn. In winter, TC, AP and AK with SAR1 declined significantly compared to CK ($p < 0.05$). TC with NAR1, TN with NAR3 and AP with NAR1, 2, 3 showed the same patterns with SAR1.

In addition, significant differences were found between CK and acid rain treatments for TS ($p < 0.01$), AP ($p < 0.05$) in spring, TC, AK in summer ($p < 0.05$) and TN in autumn ($p < 0.05$) (Table 1). There were significant differences between SAR and NAR treatments for TC ($p < 0.001$), TN ($p < 0.01$), AP ($p < 0.05$) in spring, TS ($p < 0.01$), AP ($p < 0.001$) in autumn and AK ($p < 0.05$) in winter. TC with SAR except for winter and NAR in spring and summer showed significant differences among acid rain intensities. However, significant differences for TN among SAR intensities were only found in summer, and among NAR intensities were found in autumn and winter. Statistically significant SAR intensities influencing AP and AK were found in spring, autumn and winter. In contrast, statistically significant influence of NAR intensities on AP and AK were not found in autumn.

3.2. Response of litter decomposition rate to SAR and NAR

The cumulative mass loss increased linearly with seasons in both leaf and fine root litter for all treatments and generally leaf litter had a higher loss than fine root litter (Fig. 3). Surprisingly, we found leaf litter ($p < 0.05$) and fine root litter ($p > 0.05$) both had a higher cumulative mass loss with SAR and NAR than with CK only in spring, indicating that acid rain can promote litter decomposition in the early stage (Table 1). However, this positive impact did not appear in fine root litter

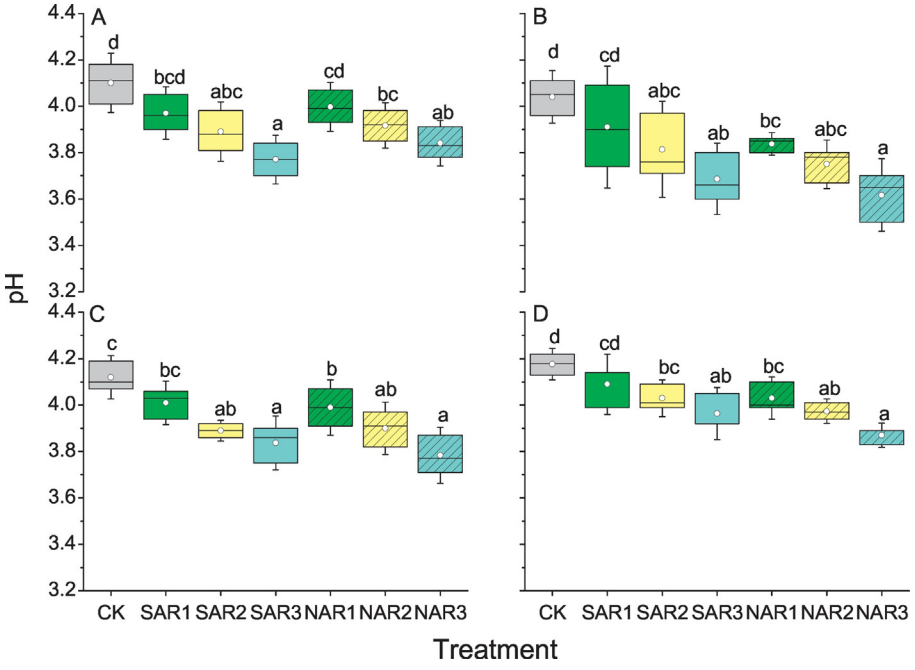

Fig. 1. Changes of the soil pH value at 0–10 cm soil layer (A, spring, B, summer, C, autumn, D, winter) under different simulated acid rain treatments from March 2015 to February 2016. The empty circles within each box represent the average pH value; middle lines within each box represent median pH value, and vertical lines represent interquartile ranges. Those cases in which the effect of treatments is significant ($p < 0.05$) are marked with different letters according to Duncan tests. The experimental treatments are: CK, control; SAR1, pH 4.5, S:N 5:1; SAR2, pH 3.5, S:N 5:1; SAR3, pH 2.5, S:N 5:1; NAR1, pH 4.5, S:N 1:5; NAR2, pH 3.5, S:N 1:5; NAR3, pH 2.5, S:N 1:5.

with stronger acidity levels (SAR3 and NAR3). Consistent significant negative impacts of SAR and NAR on cumulative mass loss were found in summer, in both leaf and fine root litter (Table 1). In leaf litter, the same acidity level of NAR had a higher inhibiting effect on litter decomposition than SAR after summer, however the differences were not significant ($p > 0.05$). In addition, this difference between SAR and NAR was only observed in winter in fine root litter, as we saw a similar inhibition impact of SAR and NAR in summer and autumn (Table 1). Statistically significant acid rain intensities influencing leaf litter decomposition were found, both for SAR and NAR, over four experimental seasons, except for SAR after summer. In contrast, significant difference of fine root litter decomposition among SAR intensities was only found in spring ($p < 0.01$), and significant differences among NAR intensities were found in spring ($p < 0.05$), autumn ($p < 0.01$) and winter ($p < 0.01$).

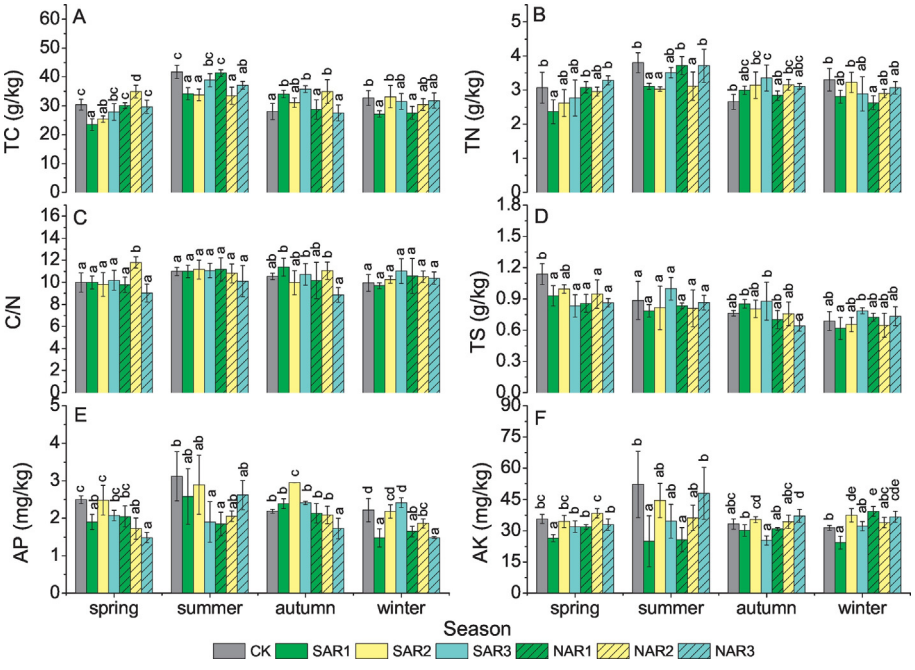

Fig. 2. Effects of acid rain (SAR and NAR) addition on abiotic soil variables from March 2015 to February 2016. Error bars are standard errors of the mean. Different letters indicate significant differences ($p < 0.05$) among different treatments according to Duncan tests. TC, total carbon, TN, total nitrogen, C/N, ratio of TC to TN, TS, total sulfur, AP, available phosphorus, AK, available potassium. The experimental treatments are: CK, control; SAR1, pH 4.5, S:N 5:1; SAR2, pH 3.5, S:N 5:1; SAR3, pH 2.5, S:N 5:1; NAR1, pH 4.5, S:N 1:5; NAR2, pH 3.5, S:N 1:5; NAR3, pH 2.5, S:N 1:5.

Table 1
One-way ANOVA analysis (p values) of the effects of acid rain with different types (T) and intensities (I) addition on litter decompositions and abiotic soil variables from March 2015 to February 2016.

	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
	Leaf-litter				Fine root litter			
AR	0.026*	0.043*	0.001**	0.008**	0.135	0.008**	0.018*	0.033*
T	0.864	0.070	0.219	0.473	0.465	0.278	0.290	0.076
I-SAR	0.005**	0.115	0.001**	0.001**	0.002**	0.266	0.293	0.065
I-NAR	0.037*	0.005**	0.000***	0.001**	0.021*	0.054	0.007**	0.002**
	TC				TN			
AR	0.463	0.021*	0.107	0.214	0.399	0.079	0.013*	0.061
T	0.000***	0.312	0.076	0.664	0.003**	0.102	0.302	0.454
I-SAR	0.018*	0.005**	0.004**	0.096	0.321	0.002**	0.104	0.292
I-NAR	0.033*	0.004**	0.080	0.117	0.513	0.172	0.015*	0.035*
	C/N				TS			
AR	0.876	0.855	0.806	0.391	0.001**	0.675	0.887	0.908
T	0.698	0.374	0.255	0.697	0.517	0.621	0.007**	0.720
I-SAR	0.964	0.986	0.306	0.191	0.019*	0.383	0.556	0.134
I-NAR	0.011*	0.599	0.109	0.858	0.024*	0.895	0.273	0.633
	AP				AK			
AR	0.028*	0.051	0.708	0.127	0.248	0.043*	0.719	0.440
T	0.022*	0.339	0.000***	0.043*	0.074	0.740	0.069	0.037*
I-SAR	0.035*	0.231	0.000***	0.004**	0.007**	0.087	0.004**	0.002**
I-NAR	0.002**	0.023*	0.129	0.006**	0.024*	0.066	0.096	0.012*

***, ** and * indicate significant difference at $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. SAR, sulfuric acid rain; NAR, nitric acid rain; TC, total carbon; TN, total nitrogen; C/N, ratio of TC to TN; TS, total sulfur; AP, available phosphorus; AK, available potassium. AR, the difference between acid rain treatments and control check; T, the difference between SAR and NAR treatments; I-SAR or I-NAR, the difference among acid rain intensities of SAR or NAR treatments, respectively.

3.3. Phospholipid fatty acid (PLFAs) analyses

The microbial biomass values obtained from the 0–10 cm soil layer at the end of each season with different experimentally simulated acid rain treatments are shown in Fig. 4. Both SAR and NAR had a negative impact on total PLFAs (tPLFAs) in all seasons, except for spring. However, the significant difference ($p < 0.05$) between acid rain treatments and CK was only found in summer (Table 2). In contrast, weaker acidity level in SAR (SAR1) had a significant impact on decreasing tPLFAs in both spring and summer, whereas, stronger acidity level in NAR (NAR3) caused a significant decline tPLFAs after summer (Fig. 4A). In addition, the statistically significant difference ($p < 0.01$) of tPLFAs between SAR and NAR was found in spring (Table 2). Soil bacteria (BAC), which is the total of gram-positive bacteria (G+) and gram-negative bacteria (G−), was negatively impacted by SAR in both spring and summer, especially by SAR1, which had a significant impact on decreasing BAC in summer ($p < 0.05$). A similar negative relationship was found on NAR and BAC only in summer, when NAR3 had a significant influence on decreasing BAC in summer compared to NAR1 and NAR2 (Fig. 4B). Importantly, such correlation was different in G+ and G− separately. We found a consistent similar negative relation between SAR/NAR and G−, especially in spring and summer (Fig. 4C). However, G+ was significantly positively impacted by NAR2, 3 in spring ($p < 0.05$), which caused high ratio of G+/G− (Fig. 4J). Also, there was a significant difference shown in autumn and winter, as SAR3 significantly decreased G+ in autumn and winter, NAR1 and NAR3 significantly influenced G+ in winter (Fig. 3D). SAR1 and SAR3 had a significant impact on decreasing ACT in summer and NAR3 decreased ACT significantly in both autumn and winter (Fig. 4E).

Similar impacts of SAR/NAR were found on FUN and AMF. Weaker acidity level in SAR (SAR1) had a significant impact on decreasing soil FUN and soil AMF in summer, and stronger acidity level in NAR

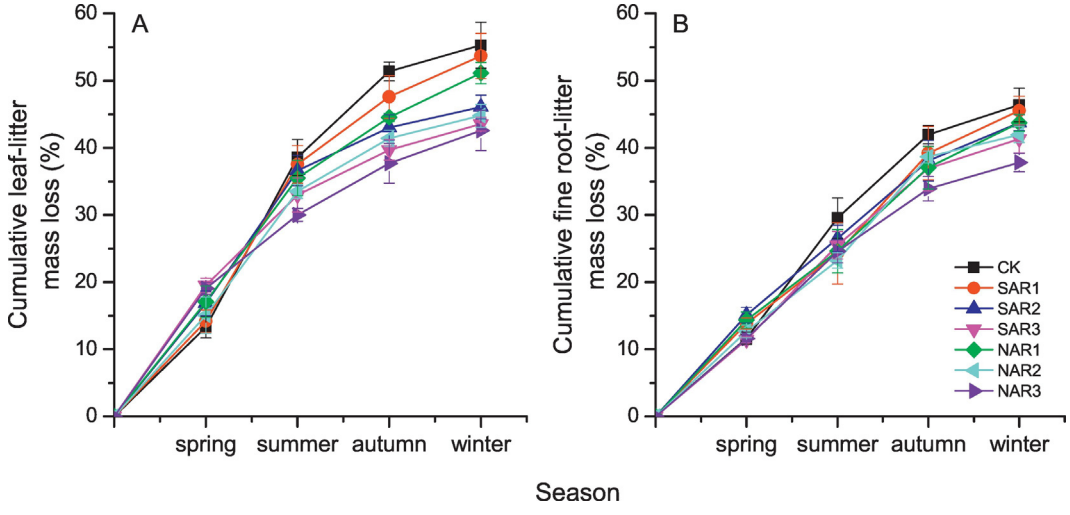

Fig. 3. Changes in cumulative mass losses of the two litter types (A, leaf-litter, B, fine root-litter) during litter decomposition under different simulated acid rain treatments from March 2015 to February 2016. Error bars are standard errors of the mean. The experimental treatments are: CK, control; SAR1, pH 4.5, S:N 5:1; SAR2, pH 3.5, S:N 5:1; SAR3, pH 2.5, S:N 5:1; NAR1, pH 4.5, S:N 1:5; NAR2, pH 3.5, S:N 1:5; NAR3, pH 2.5, S:N 1:5.

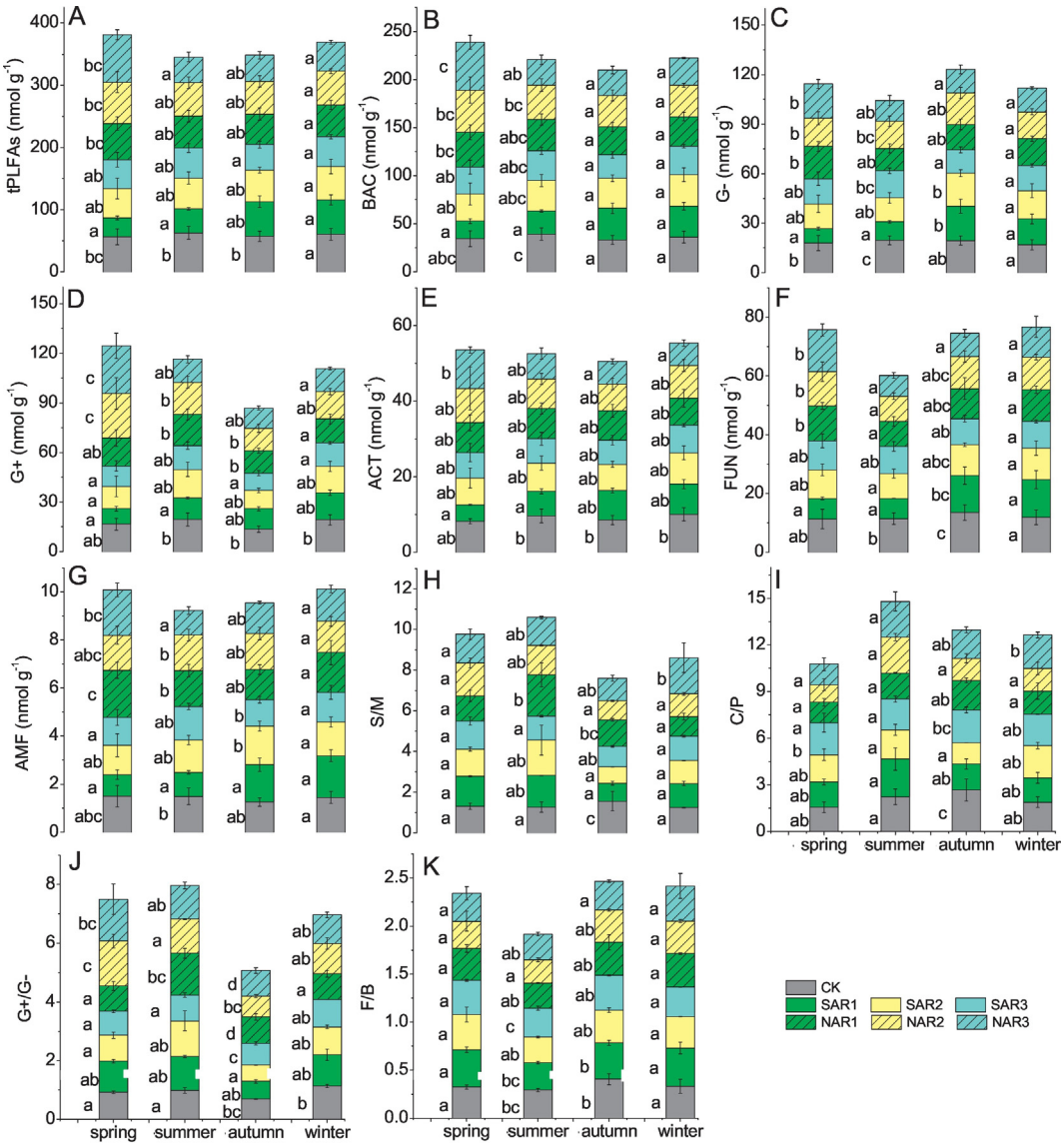

Fig. 4. Effects of acid rain (SAR and NAR) addition on total PLFAs (tPLFAs), composition of PLFAs [bacteria (BAC), gram-positive bacteria (G+), gram-negative bacteria (G−), actinomycetes (ACT), fungi (FUN), arbuscular mycorrhizal fungi (AMF)] and microbial stress indicators [saturated/monounsaturated (S/M), cyclopropyl fatty acids/monoenoic precursors (C/P), G+/G−, fungal/bacteria (F/B)] from March 2015 to February 2016. Error bars are standard errors of the mean. Different letters indicate significant differences ($p < 0.05$) among different treatments according to Duncan tests. The experimental treatments are: CK, control; SAR1, pH 4.5, S:N 5:1; SAR2, pH 3.5, S:N 5:1; SAR3, pH 2.5, S:N 5:1; NAR1, pH 4.5, S:N 1:5; NAR2, pH 3.5, S:N 1:5; NAR3, pH 2.5, S:N 1:5.

(NAR3) caused a significant decline FUN in summer and AMF in summer and autumn (Fig. 4F,G). The differences between acid rain treatments and CK for BAC ($p < 0.05$), G− ($p < 0.01$), ACT ($p < 0.05$) and FUN ($p < 0.01$) were in consistent with tPLFAs, as the same as between SAR and NAR (Table 2). In contrast, G+ and ACT declined significantly compared to CK in winter ($p < 0.05$), and the differences between SAR and NAR for G+ was found both in spring ($p < 0.01$) and autumn ($p < 0.05$). Meanwhile, acid rain intensity also led to different effects on soil microbial activity. In summer, tPLFAs, BAC, G−, FUN with SAR and G−, FUN with NAR, respectively, showed significant differences among acid rain intensities ($p < 0.05$) by one-way ANOVA (Table 2).

The ratio of S/M, C/P, G+/G− and F/B, which are soil microbial stress indicators, showed different trends with acidity level gradients. There were no significant differences of S/M, C/P and F/B between CK and the three acidity levels in SAR and NAR in both spring and summer, except for a significant increase in S/M with NAR1 and a significant decrease in F/B with NAR2 in summer (Fig. 4H,I,K). In autumn, we started to see a decline in both S/M and C/P, especially with SAR1, SAR2 and NAR2, NAR3. In spring, summer and autumn, G+/G− with NAR were mostly higher than that with SAR, especially in autumn with NAR1,3 (Fig. 3J). In winter, we found non-significant differences between G+/G−, F/B with both SAR and NAR intensities, except for G+/G− with NAR1. However, we found a clear significant increase of S/M and C/P with NAR 3 (Fig. 4H, I).

3.4. Changes in the PLFA biomarkers

The SIMPER analysis identified five biomarkers that would be most affected by acid rain: cy19:0, 18:1ω9, i15:0, i16:0, 10Me16:0 (Table 3). The five most influential biomarkers accounted for 49.06–73.18% of the dissimilarities between the acid rain treatments over four experimental seasons (Table 3). In the early stage of simulate acid rain, G− (cy19:0) was more sensitive to SAR, while G+ (i15:0, i16:0) was more sensitive to NAR. In summer and autumn, G− (cy19:0) and Fun (18:1ω9) were more sensitive to both SAR and NAR. However, ACT (10Me16:0) was most sensitive to NAR in winter. In addition, the contribution of ACT (10Me16:0) was increased with the SAR intensity

Table 2
One-way ANOVA analysis (p values) of the effects of acid rain with different types (T) and intensities (I) addition on soil microbial variables from March 2015 to February 2016.

	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
	tPLFAs				BAC			
AR	0.860	0.012*	0.124	0.068	0.956	0.028*	0.250	0.061
T	0.001**	0.463	0.746	0.591	0.001**	0.352	0.962	0.625
I-SAR	0.156	0.041*	0.108	0.447	0.172	0.037*	0.114	0.534
I-NAR	0.265	0.120	0.253	0.145	0.259	0.147	0.341	0.181
	G−				G+			
AR	0.543	0.006**	0.335	0.446	0.791	0.160	0.245	0.010*
T	0.005**	0.943	0.267	0.745	0.001**	0.129	0.024*	0.585
I-SAR	0.114	0.010*	0.077	0.878	0.252	0.129	0.132	0.171
I-NAR	0.639	0.060*	0.152	0.482	0.118	0.206	0.742	0.096
	ACT				FUN			
AR	0.733	0.015*	0.063	0.024*	0.777	0.002**	0.043*	0.460
T	0.037*	0.307	0.900	0.414	0.003**	0.818	0.417	0.875
I-SAR	0.148	0.129	0.118	0.204	0.227	0.033*	0.100	0.311
I-NAR	0.800	0.231	0.196	0.080	0.540	0.036*	0.151	0.871
	AMF							
AR	0.838	0.266	0.432	0.991				
T	0.001**	0.487	0.541	0.879				
I-SAR	0.354	0.094	0.060	0.189				
I-NAR	0.286	0.138	0.378	0.524				

** and * indicate significant difference at $p < 0.01$ and $p < 0.05$, respectively. SAR, sulfuric acid rain; NAR, nitric acid rain; tPLFAs, total PLFAs; BAC, bacteria; G+, gram-positive bacteria; G−, gram-negative bacteria; ACT, actinomycetes; FUN, fungi; AMF, arbuscular mycorrhizal fungi; AR, the difference between acid rain treatments and control check; T, the difference between SAR and NAR treatments; I-SAR or I-NAR, the difference among acid rain intensities of SAR or NAR treatments, respectively.

increased in summer, autumn and winter, and ACT was most sensitive to NAR3 in summer and winter.

3.5. Linking soil microbial community and litter decomposition to soil chemical properties

A correlation between the soil chemical properties, litter decompositions and microbial biomarkers from summer to winter was discerned by CCA analysis as shown in Fig. 5. According to the CCA results, axes 1 and axes 2 explain 31.28% and 8.52% of the variance, respectively. Among the soil properties, soil TN, TC and pH were the most important factors affecting soil microbial activity. FUN (18:1ω9, 18:2ω6), G− (17:1ω8, 16:1ω9). 16:1ω5 (AMF), a17:0 (G+) showed positive correlation with the soil pH, G+ (i16:0, i15:0, a15:0, i17:0), ACT (10Me16:0, 10Me17:0) and cy17:0 (G−) showed positive correlation with the TN and TC, and cy19:0 showed positive correlation with AP and TS.

In the RDA of microbial indices of PLFA profiles and litter decomposition with soil properties as the explanatory variables axes 1accounted for 52.47% of the variation in the dataset, with 7.32% of the variation accounted for by axes 2 (Fig. 6). Both the first canonical axis ($F = 14.4$, $p = 0.006$) and the sum of all canonical axes ($F = 3.0$, $p = 0.006$) were significant by the Monte Carlo permutation tests. Fine

Table 3
The top five PLFA biomarkers that primarily contribute to differences in microbial community between CK and acid rain treatments from March 2015 to February 2016 calculated by similarity percentage (SIMPER).

Season	Treat ments	Fatty acid, contribution (%)					
		1	2	3	4	5	Sum
Spring	SAR1	cy19:0 (17.32)	18:1ω9 (11.65)	i16:0 (10.31)	18:1ω7 (10.00)	i15:0 (9.92)	59.20
	SAR2	cy19:0 (16.75)	i15:0 (12.24)	i16:0 (10.97)	18:1ω9 (10.55)	18:1ω7 (9.75)	60.26
	SAR3	i15:0 (12.61)	cy19:0 (11.81)	18:1ω9 (11.21)	10Me16:0 (10.25)	18:1ω7 (9.98	55.86
	NAR1	i15:0 (12.12)	18:1ω7 (9.91)	18:1ω9 (9.86)	cy19:0 (8.74)	i16:0 (8.43)	49.06
	NAR2	i16:0 (26.66)	16:1ω7 (8.78)	i15:0 (8.37)	18:1ω7 (7.15)	10Me16:0 (6.75)	57.71
	NAR3	i15:0 (21.00)	i16:0 (13.25)	18:1ω9 (9.73)	a15:0 (8.78)	18:1ω7 (6.73)	59.49
Summer	SAR1	cy19:0 (19.09)	18:1ω9 (12.63)	i15:0 (10.81)	10Me16:0 (10.22)	i16:0 (8.66)	61.41
	SAR2	cy19:0 (21.85)	18:1ω9 (12.96)	10Me16:0 (11.56)	i15:0 (10.61)	i16:0 (9.86)	66.84
	SAR3	10Me16:0 (15.57)	cy19:0 (13.94)	i16:0 (12.64)	i15:0 (11.67)	18:2ω6 (8.67)	62.49
	NAR1	cy19:0 (27.06)	18:1ω9 (11.98)	10Me16:0 (10.49)	i15:0 (9.98)	i16:0 (7.88)	67.39
	NAR2	cy19:0 (18.82)	18:1ω9 (13.87)	10Me16:0 (11.00)	i15:0 (10.48)	i16:0 (8.19)	62.36
	NAR3	cy19:0 (19.04)	18:1ω9 (13.79)	10Me16:0 (10.64)	i15:0 (10.37)	i16:0 (8.40)	62.24
Autumn	SAR1	18:1ω9 (18.13)	cy19:0 (16.43)	18:1ω7 (15.32)	10Me16:0 (6.10)	16:1ω7 (6.07)	62.05
	SAR2	cy19:0 (19.68)	18:1ω9 (14.26)	18:1ω7 (13.91)	10Me16:0 (8.41)	i16:0 (7.87)	64.13
	SAR3	cy19:0 (25.54)	18:1ω9 (20.22)	10Me16:0 (8.58)	18:2ω6 (7.26)	i16:0 (5.68)	67.28
	NAR1	cy19:0 (27.99)	18:1ω9 (20.91)	18:2ω6 (8.55)	10Me16:0 (7.30)	18:1ω7 (5.41)	70.16
	NAR2	cy19:0 (23.32)	18:1ω9 (15.42)	18:1ω7 (10.40)	i15:0 (7.84)	10Me16:0 (7.65)	64.63
	NAR3	cy19:0 (28.45)	18:1ω9 (22.61)	18:2ω6 (8.63)	10Me16:0 (7.84)	18:1ω7 (5.65)	73.18
Winter	SAR1	cy19:0 (21.48)	18:2ω6 (14.03)	18:1ω9 (11.46)	10Me16:0 (11.06)	i15:0 (8.27)	66.30
	SAR2	cy19:0 (15.64)	10Me16:0 (14.93)	18:1ω9 (14.70)	i15:0 (11.96)	i16:0 (8.59)	65.82
	SAR3	10Me16:0 (16.21)	18:1ω9 (14.77)	i15:0 (12.83)	cy19:0 (10.62)	i16:0 (8.70)	63.13
	NAR1	10Me16:0 (15.78)	cy19:0 (13.46)	i16:0 (11.18)	i15:0 (10.78)	18:1ω9 (10.10)	61.30
	NAR2	10Me16:0 (15.24)	cy19:0 (13.41)	18:1ω9 (12.94)	i15:0 (10.39)	i16:0 (9.60)	61.58
	NAR3	10Me16:0 (16.75)	i15:0 (14.04)	18:1ω9 (13.99)	i16:0 (11.09)	cy19:0 (7.66)	63.53

The numbers with bracket represent percentage of contribution of differences in microbial community. The experimental treatments are: CK, control; SAR1, pH 4.5, S:N 5:1; SAR2, pH 3.5, S:N 5:1; SAR3, pH 2.5, S:N 5:1; NAR1, pH 4.5, S:N 1:5; NAR2, pH 3.5, S:N 1:5; NAR3, pH 2.5, S:N 1:5.

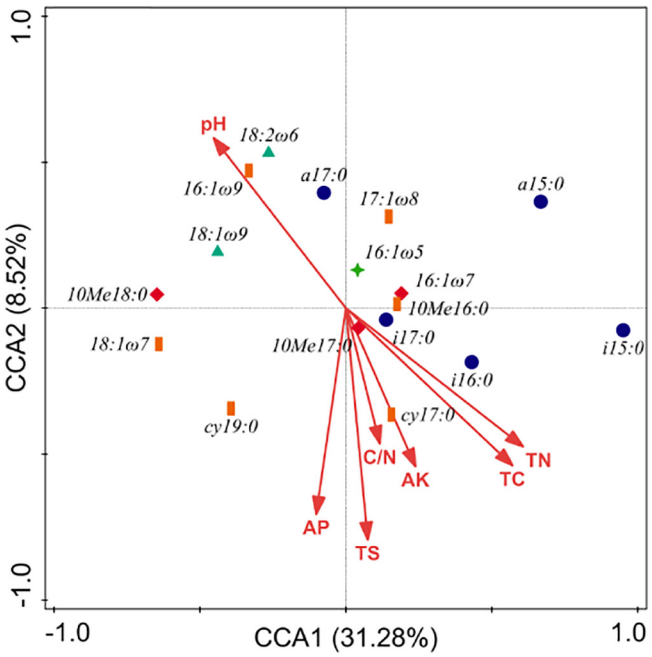

Fig. 5. Canonical correspondence analysis (CCA) reveals the influence of soil properties on soil PLFA biomarkers under sulfuric acid rain (SAR) or nitric acid rain (NAR) addition from summer to winter (June 2015 to February 2016). The mazarine blue circles, orange boxes, red diamonds, blue up-triangles and green stars present gram-positive bacteria (G+), gram-negative bacteria (G−), actinomycetes (ACT), fungi (FUN) and arbuscular mycorrhizal fungi (AMF) biomarkers, respectively. Soil properties (red line): pH, soil pH; TC, total carbon; TN, total nitrogen; C/N, ratio of TC to TN; TS, total sulfur; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

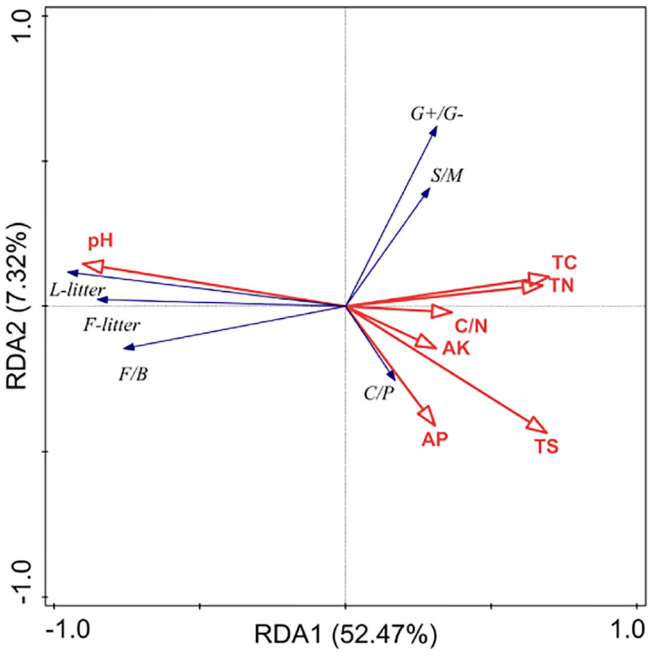

Fig. 6. Redundancy analysis (RDA) of soil microbial stress indices and litter decompositions constrained by soil chemical properties under sulfuric acid rain (SAR) or nitric acid rain (NAR) from summer to winter (June 2015 to February 2016). The angle and length of the blue/red arrows indicate the direction and strength of the relationship of microbial stress indices, litter decomposition with the ordination scores in soil properties. Soil microbial stress indices: saturated/monounsaturated (S/M), cyclopropyl fatty acids/monoenoic precursors (C/P), gram-positive bacteria/gram-negative bacteria (G+/G−), fungal/bacteria (F/B) (blue lines). Litter decomposition: leaf (L-litter) and fine root (F-litter) litter decomposition (blue lines). Soil properties (red line): pH, soil pH; TC, total carbon; TN, total nitrogen; C/N, ratio of TC to TN; TS, total sulfur; AP, available phosphorus; AK, available potassium. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

root litter and leaf litter decomposition had a consistent positive correlation with soil pH. The soil microbial indices showed inconsistent responses to soil properties. High F/B ratio with high soil pH was found at the left-hand end of the ordination plots. In addition, the patterns at the right-hand end of the ordination plots correlated positively with soil nutrients and S/M, C/P and G+/G−.

4. Discussion

Acid rain may trigger both direct and indirect effects on soil microbial communities (El-Tarabily et al., 2008; Wang et al., 2014; Xu et al., 2015). There is ample evidence that acid rain alters soil properties by decreasing soil pH or by altering the quality of organic carbon sources directly, such as litter decomposition and fine root turnover, and these influences affect the soil nutrient pool, along with associated changes in micro-environmental factors (Hines et al., 2006; El-Tarabily et al., 2008; Wakelin et al., 2008). These changes may lead to shifts in the soil microbial biomass (Esher et al., 1992; Lv et al., 2014; Liu et al., 2014). In this study, litter decomposition was reduced under different acid rain treatments after summer acid rain increased, which is consistent with our first hypothesis (Wolters, 1991; Cha et al., 2013). This maybe because the functions of decomposer communities and substrate quality, such as the secretion of exoenzymes and soil nitrogen availability, were depressed after exposure to acid rain (Dangles et al., 2004; Waldrop et al., 2004; Wang et al., 2010; Zhou et al., 2015). However, at the beginning of simulated acid rain in spring, the litter decomposed more rapidly with weak acidity (pH = 4.5) than with CK (Lee and Weber, 1983; Neuvonen and Suomela, 1990; Wang et al., 2012). This may be due to the greater S and N in acid rain, which acted as a fertilizer and promoted the decomposer activity. In our study, we found that the cumulative fine root mass loss of *Q. acutissima* increased more slowly than that of leaf litter and the inhibitory effect of acid rain treatments on fine root decomposition was lower that on leaf litter. This maybe because belowground litter experiences different environmental conditions compared to aboveground litter (Solly et al., 2014) and the function of decomposer communities in the humus layer, such as fungus, were more vulnerable to impact by the acid rain than those in soil layer (Pennanen, 2001). The impacts of acid rain on litter decomposition in previous studies mainly focused on a single type of acid rain, such as SAR (Wolters, 1991; Cha et al., 2013; Zhou et al., 2015). By contrast, the effects of NAR were largely neglected. Consistent with our second hypothesis, the inhibitory effects of NAR on litter decomposition were stronger than SAR starting with summer. Thus, the negative effects of NO_3^- on litter decomposition were more significant than those of SO_4^{2-}. This is perhaps because the ability of exchange with hydroxyl groups (OH^-) of SO_4^{2-} was better than with those of NO_3^-, and because SO_4^{2-} is more easily absorbed by soil particles (Christ et al., 1995; Lv et al., 2014).

Litter decomposition is affected by the acid rain and thus may have a strong effect on nutrient dynamics in the soil and also influence the soil microbial community (Kaspari et al., 2008). We found that the total PLFAs with NAR treatments were higher than those of CK and increased with pH decreased only in the beginning of the study. This may be because the N in acid rain acts as a fertilizer, increasing soil fertility and plant production. Thus nitric acid rain increased soil microbial biomass (Enowashu et al., 2009; Ham et al., 2010). Compared to NAR, the impacts of SAR on the soil microbial community were insignificant, which is possibly because of the promoting effect of soil fertilizer S and N was lower than the inhibiting effect of acidity. This is consistent with the above results of the effect of acid rain on litter decomposition. With the acid rain increasing in summer, the SAR and NAR both inhibited the soil microbial community. However lower acidity of SAR (pH = 4.5) had a significantly greater inhibitory effect on microbial than the same acidity level of NAR. In contrast, only the NAR treatments

with stronger acidity (pH = 2.5) had a significant negative impact on soil microbial compared to CK. Such results agreed with those Lv et al. (2014). Under the lower levels of acidity (pH = 4.5), nitrate fertilizer would accelerate the growth of plant and then impact the soil properties although the soil pH with NAR was lower than with SAR. But the situation in which the acceleration by nitrate fertilizer was stronger than inhibition by low pH was negated at higher acidity (pH = 2.5) (Xu et al., 2015). More importantly, we did not see much difference in the impact of SAR and NAR at the later stage of the experiment, because of the lower amount of H^+ input to the soil with less precipitation in autumn and winter. This suggested that the rainy season, with large H^+ input, resulted in a decrease in soil microbial activity, but that soil microbial would recover after the rainy season.

The general PLFAs biomarker group may present non-significant values, when comparing all soil treatments, but there may be a different balance of biomarker PLFAs (Francisco et al., 2016). After spring, G + biomarkers i15:0 and i16:0 strongly increased with NAR compared to SAR, which might suggest that G + played an important role on accelerating the decomposition of litter. The effects of acid rain on G − (cy19:0) and Fun (18:1ω9) were increased with the amount of acid rain increasing in summer. In contrast, the change of biomarkers with NAR showed no difference among acid rain intensity, whereas ACT (10Me16:0) was more sensitive to SAR intensity. The amount of acid rain in autumn and winter decreased with the precipitation decreased, which led to less difference of PLFA biomass among acid rain treatments. However, we found that the contributions of biomarker 18:1ω9 with SAR and 18:2ω6 with NAR increased in autumn compared to summer. After winter, the effects of both SAR and NAR on cy19:0 decreased compared to summer and autumn, especially 10Me16:0 became to the main contributors to the difference in PLFA biomass between NAR and CK treatments. The differential responses of PLFA biomarkers to different acid rain types suggested that changing acid rain from SAR to NAR would lead to the differential response of the soil microbial community.

Acid rain deposition altered soil properties by decreasing soil pH and by affecting the soil nutrient pool. It is widely accepted that pH has a significant effect on the composition of microbial communities (Sun et al., 2015; Xiong et al., 2016). In our study, both SAR and NAR significantly decreased the soil pH, and microbial indices F/B ratio usually decreased with decreasing soil pH, which is consistent with Xiong et al. (2016). Litter decomposition also showed positive correlation with soil pH (Xu et al., 2015) and F/B. Therefore, F/B could be indicators of acid rain deposition. Soil organic matter can provide energy for soil microorganisms, which is the main factor that affects the soil microbial biomass (Yao et al., 2016). Högberg et al. (2007) and Pollierer et al. (2015) reported that the C/N ratio useful as a predictor of microbial community composition as pH. We found that some G +, ACT and G − biomarkers showed positive correlation with the TN and TC.

It should be noted that we just simulated acid rain over four seasons of one year. Meanwhile, culture-dependent methods are considered capable of detecting more taxa than with PLFA analysis (Xiong et al., 2016). Our future mesocosm experiment work should use high throughput sequencing methods for studying effects of SAR and NAR on soil microbial communities after three or more years.

5. Conclusion

After a four-season mesocosm experiment, SAR and NAR have led to changes in litter decomposition and the soil microbial community of a plantation ecosystem in the Yangtze River Delta region of China. In the early period of simulated acid rain, both SAR and NAR accelerated the process of litter decomposition, and only NAR accelerated the soil microorganism activity, especially G + (i15:0 and i16:0). With the amount of acid rain increasing in summer, both the SAR and NAR treatments inhibited the soil microbial activity and litter decomposition, and the inhibitory effects on leaf litter decomposition were higher than on fine root litter decomposition. In addition, the inhibitory effects of NAR on litter decomposition were more significant than those of SAR following summer, and the inhibitory effects of high intensity NAR (pH = 2.5) on soil microbial were more significant than SAR in summer (rain season). G − (cy19:0) and Fun (18:1ω9) were more sensitive to both SAR and NAR, whereas ACT was more sensitive to SAR intensity. A significantly decreasing SO_4^{2-} concentration, as well as a significant increasing NO_3^- concentration will be observed in the future acid rain trend in subtropical forests as a result of rapid economic growth (Lv et al., 2014). In summary, we suggest that the change of acid rain types from SAR to NAR in the future might complicates the ongoing challenge of ecosystem stability and increase the risks to the ecosystem functioning.

Acknowledgements

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References

Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biol. Biochem. 35, 955–963.
Bewley, R.J.F., Stotzky, G., 1983. Simulated acid rain (H_2SO_4) and microbial activity in soil. Soil Biol. Biochem. 15, 425–429.
Cha, S., Lim, S.M., Amirasheba, B., Shim, J.K., 2013. The effect of simulated acid rain on microbial community structure in decomposing leaf litter. J. Ecol. Environ. 36, 223–233.
Chan, C.K., Yao, X., 2008. Air pollution in mega cities in China. Atmos. Environ. 42, 1–42.
Christ, M., Zhang, Y., Likens, G.E., Driscoll, C.T., 1995. Nitrogen retention capacity of a northern hardwood forest soil under ammonium sulfate additions. Ecol. Appl. 5, 802–812.
Connaughton, S., Collins, G., O'Flaherty, V., 2006. Development of microbial community structure and activity in a high-rate anaerobic bioreactor at 18 °C. Water Res. 40, 1009–1017.
Dangles, O., Gessner, M.O., Guérold, F., Chauvet, E., 2004. Impacts of stream acidification on litter breakdown: implications for assessing ecosystem functioning. J. Appl. Ecol. 41, 365–378.
Enowashu, E., Poll, C., Lamersdorf, N., Kandeler, E., 2009. Microbial biomass and enzyme activities under reduced nitrogen deposition in a spruce forest soil. Appl. Soil Ecol. 43, 11–21.
El-Tarabily, K.A., Nassar, A.H., Sivasithamparam, K., 2008. Promotion of growth of bean (*Phaseolus vulgaris L.*) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of Micromonospora endolithica. Appl. Soil Ecol. 39, 161–171.
Esher, R.J., Marx, D.H., Ursic, S.J., Baker, R.L., Brown, L.R., Coleman, D.C., 1992. Simulated acid rain effects on fine roots, ectomycorrhizae, microorganisms, and invertebrates in pine forests of the southern United States. Water Air Soil Pollut. 61, 269–278.
Federle, T.W., Dobbins, D.C., Thornton-Manning, J.R., Jones, D.D., 1986. Microbial biomass, activity, and community structure in subsurface soils. Ground Water 24, 365–374.
Francisco, R., Stone, D., Creamer, R.E., Sousa, J.P., Morais, P.V., 2016. European scale analysis of phospholipid fatty acid composition of soils to establish operating ranges. Appl. Soil Ecol. 97, 49–60.
Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65.
Fujii, K., 2014. Soil acidification and adaptations of plants and microorganisms in Bornean tropical forests. Ecol. Res. 29, 371–381.
Guo, X., Chen, H.Y., Meng, M., Biswas, S.R., Ye, L., Zhang, J., 2016. Effects of land use change on the composition of soil microbial communities in a managed subtropical forest. For. Ecol. Manag. 373, 93–99.
Ham, Y.S., Kobori, H., Kang, J.H., Kim, J.H., 2010. Ammonium nitrogen deposition as a dominant source of nitrogen in a forested watershed experiencing acid rain in central Japan. Water Air Soil Pollut. 212, 337–344.
Hines, J., Megonigal, J.P., Denno, R.F., 2006. Nutrient subsidies to belowground microbes impact aboveground food web interactions. Ecology 87, 1542–1555.
Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150, 590–601.
Huang, D.Y., Xu, Y.G., Peng, P.A., Zhang, H.H., Lan, J.B., 2009. Chemical composition and seasonal variation of acid deposition in Guangzhou, South China: comparison with precipitation in other major Chinese cities. Environ. Pollut. 157, 35–41.
Kaspari, M., Garcia, M.N., Harms, K.E., Santana, M., Wright, S.J., Yavitt, J.B., 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. Ecol. Lett. 11, 35–43.
Kumar, U., Shahid, M., Tripathi, R., Mohanty, S., Kumar, A., Bhattacharyya, P., Jambhulkar, N.N., 2017. Variation of functional diversity of soil microbial community in sub-

humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecol. Indic. 73, 536–543.
Lee, J.J., Weber, D.E., 1983. Effects of sulfuric acid rain on decomposition rate and chemical element content of hardwood leaf litter. Can. J. Bot. 61, 872–879.
Liang, G., Hui, D., Wu, X., Wu, J., Liu, J., Zhou, G., Zhang, D., 2016. Effects of simulated acid rain on soil respiration and its components in a subtropical mixed conifer and broad-leaf forest in southern China. Environ. Sci. 18, 246–255.
Liu, G.S., Jiang, N.H., Zhang, L.D., Liu, Z.L., 1996. Soil Physical and Chemical Analysis and Description of Soil Profiles. Vol. 24. China Standard Methods Press, Beijing, China, p. 266 (in Chinese).
Liu, X., Zhou, J., Li, W., Xu, J., Brookes, P.C., 2014. The combined effects of urea application and simulated acid rain on soil acidification and microbial community structure. Environ. Sci. Pollut. Res. Int. 21, 6623–6631.
Lv, Y., Wang, C., Jia, Y., Wang, W., Ma, X., Du, J., Tian, X., 2014. Effects of sulfuric, nitric, and mixed acid rain on litter decomposition, soil microbial biomass, and enzyme activities in subtropical forests of China. Appl. Soil Ecol. 79, 1–9.
LY/T 1233-1999, 1999. Determination of Available Phosphorus in Forest Soil China (in Chinese).
LY/T 1236-1999, 1999. Determination of Available Potassium in Forest Soil China (in Chinese).
LY/T 1239-1999, 1999. Determination of pH Value in Forest Soil China (in Chinese).
McColl, J.G., Firestone, M.K., 1991. Soil chemical and microbial effects of simulated acid rain on clover and soft chess. Water Air Soil Pollut. 60, 301–313.
Moore-Kucera, J., Dick, R.P., 2008. PLFA profiling of microbial community structure and seasonal shifts in soils of a Douglas-fir chronosequence. Microb. Ecol. 55, 500–511.
Neuvonen, S., Suomela, J., 1990. The effect of simulated acid rain on pine needle and birch leaf litter decomposition. J. Appl. Ecol. 857-872.
O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. Bioscience 38, 328–336.
Pennanen, T., 2001. Microbial communities in boreal coniferous forest humus exposed to heavy metals and changes in soil pH-a summary of the use of phospholipid fatty acids, Biolog® and 3H-thymidine incorporation methods in field studies. Geoderma 100, 91–126.
Pollierer, M.M., Ferlian, O., Scheu, S., 2015. Temporal dynamics and variation with forest type of phospholipid fatty acids in litter and soil of temperate forests across regions. Soil Biol. Biochem. 91, 248–257.
Qiu, Q., Wu, J., Liang, G., Liu, J., Chu, G., Zhou, G., Zhang, D., 2015. Effects of simulated acid rain on soil and soil solution chemistry in a monsoon evergreen broad-leaved forest in southern China. Environ. Monit. Assess. 187, 1–13.
Reininge, R.D., Fiala, P., Samek, T., 2011. Acidification of forest soils in the Hrubý Jeseník region. Soil Water Res. 6, 83–90.
Singh, A., Agrawal, M., 2007. Acid rain and its ecological consequences. J. Environ. Biol. 29, 15.
Singh, J.S., Gupta, S.R., 1977. Plant decomposition and soil respiration in terrestrial ecosystems. Bot. Rev. 43, 449–528.
Solly, E.F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., Schrumpf, M., 2014. Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. Plant Soil 382, 203–218.
Sun, M., Xiao, T., Ning, Z., Xiao, E., Sun, W., 2015. Microbial community analysis in rice paddy soils irrigated by acid mine drainage contaminated water. Appl. Microbial. Biot. 99, 2911–2922.
Sundarapandian, S.M., Swamy, P.S., 1999. Litter production and leaf-litter decomposition of selected tree species in tropical forests at Kodayar in the Western Ghats, India. For. Ecol. Manag. 123, 231–244.
Tu, J., Wang, H., Zhang, Z., Jin, X., Li, W., 2005. Trends in chemical composition of precipitation in Nanjing, China, during 1992–2003. Atmos. Res. 73, 283–298.
Wakelin, S.A., Macdonald, L.M., Rogers, S.L., Gregg, A.L., Bolger, T.P., Baldock, J.A., 2008. Habitat selective factors influencing the structural composition and functional capacity of microbial communities in agricultural soils. Soil Biol. Biochem. 40, 803–813.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol. Appl. 14, 1172–1177.
Wang, C., Guo, P., Han, G., Feng, X., Zhang, P., Tian, X., 2010. Effect of simulated acid rain on the litter decomposition of *Quercus acutissima* and *Pinus massoniana* in forest soil microcosms and the relationship with soil enzyme activities. Sci. Total Environ. 408, 2706–2713.
Wang, L., Chen, Z., Shang, H., Wang, J., Zhang, P.Y., 2014. Impact of simulated acid rain on soil microbial community function in Masson pine seedlings. Electron. J. Biotechnol. 17, 199–203.
Wang, R.L., Staehelin, C., Dayan, F.E., Song, Y.Y., Su, Y.J., Zeng, R.S., 2012. Simulated acid rain accelerates litter decomposition and enhances the allelopathic potential of the invasive plant *Wedelia trilobata* (creeping daisy). Weed Sci. 60, 462–467.
Wang, T., Jiang, F., Li, S., Liu, Q., 2007. Trends in air pollution during 1996–2003 and cross-border transport in city clusters over the Yangtze River Delta region of China. Terr. Atmos. Ocean. Sci. 18, 995.
Wolters, V., 1991. Effects of acid rain on leaf-litter decomposition in a beech forest on calcareous soil. Biol. Fertil. Soils 11, 151–156.
Xiong, Q., Pan, K., Zhang, L., Wang, Y., Li, W., He, X., Luo, H., 2016. Warming and nitrogen deposition are interactive in shaping surface soil microbial communities near the alpine timberline zone on the eastern Qinghai–Tibet Plateau, southwestern China. Appl. Soil Ecol. 101, 72–83.
Xu, H.Q., Zhang, J.E., Ouyang, Y., Lin, L., Quan, G.M., Zhao, B.L., Yu, J.Y., 2015. Effects of simulated acid rain on microbial characteristics in a lateritic red soil. Environ. Sci. Pollut. Res. Int. 22, 18260–18266.
Xu, W., Liu, J., Liu, X., Li, K., Zhang, D., Yan, J., 2013. Fine root production, turnover, and decomposition in a fast-growth Eucalyptus urophylla plantation in southern China. J. Soils Sed. 13, 1150–1160.
Yao, Z., Xing, J., Gu, H., Wang, H., Wu, J., Xu, J., Brookes, P.C., 2016. Development of microbial community structure in vegetable-growing soils from open-field to plastic-greenhouse cultivation based on the PLFA analysis. J. Soils Sediments 1–9.
Zhao, Y., Duan, L., Xing, J., Larssen, T., Nielsen, C.P., Hao, J., 2009. Soil acidification in China: is controlling SO_2 emissions enough? Environ. Sci. Technol. 43, 8021–8026.
Zhao, Y., Wang, S., Duan, L., Lei, Y., Cao, P., Hao, J., 2008. Primary air pollutant emissions of coal-fired power plants in China: current status and future prediction. Atmos. Environ. 42, 8442–8452.
Zhou, Y., Clark, M., Su, J., Xiao, C., 2015. Litter decomposition and soil microbial community composition in three Korean pine (*Pinus koraiensis*) forests along an altitudinal gradient. Plant Soil 386, 171–183.