



Impact of simulated acid rain on soil microbial community function in Masson pine seedlings



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ABSTRACT

Background: Accompanying its rapid economic development and population growth, China is the world's third largest acid rain region, following Europe and North America. The effects of acid rain on forest ecosystem were widely researched, including the growth, the nutrient of the leaf and soil, and so on. However, there are few reports about the effects of acid rain on the soil microbial diversity. This study investigated the effects of acid rain on soil microbial community function under potted Masson pine seedlings (*Pinus massoniana* Lamb).

Results: After 7 months of treatment with simulated acid rain, the low acid load treatment (pH 5.5) stimulated soil microbial activity, and increased soil microbial diversity and richness, while the higher levels of acid application (pH 4.5, pH 3.5) resulted in lower soil microbial activity and had no significant effects on soil microbial diversity and richness. Principal component analysis showed that there was clear discrimination in the metabolic capability of the soil microbial community among the simulated acid rain and control treatments.

Conclusion: The results obtained indicated that the higher acid load decreased the soil microbial activity and no effects on soil microbial diversity assessed by Biolog of potted Masson pine seedlings. Simulated acid rain also changed the metabolic capability of the soil microbial community.

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1. Introduction

During the past few decades, the impact of acid deposition has been intensively investigated. Changes in the soil chemical status and the function of the decomposer community may lead to imbalance between nutrient cycling and productivity of an ecosystem [1]. Acid deposition is known to affect soil chemical properties and cause decreases in soil fertility. This is mainly because of the loss of base cations (Ca^{2+} , Mg^{2+} , K^+ , Na^+) by leaching with SO_4^{2-} and NO_3^- as the accompanying anions, and a decrease in soil pH, thus causing potentially toxic concentrations of Al^{3+} and heavy metals in the soil solution [2,3,4]. Organic material deposited on or in the soil is decomposed and mineralized mainly through the activities of microorganisms and soil animals. Therefore, soil microorganisms play a key role in maintaining the fertility of terrestrial habitats, and it can be inferred that factors that alter the rates of microbial processes in

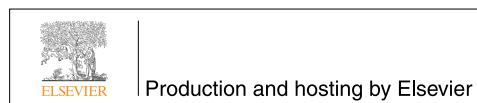
soil may influence forest ecosystem functions such as C sequestration. Acidic loads applied during a short time period, sometimes even in a single load, have been shown to have toxic effects on the soil respiration rate, microbial community structure, and microbial biomass [1,5,6]. Both direct and indirect effects of an increased acid load on the size, composition, and activity of the soil microbes have been reported [1,5,7,8,9,10,11,12,13].

Accompanying its rapid economic development and population growth, China is the world's third largest acid rain region, following Europe and North America [14]. The area affected by serious acid deposition is estimated to exceed one million km^2 , which is about 40% of the territorial area of China. In China, there are three seriously polluted areas: Central China, Southwestern China, and Eastern China. Acid precipitation has been shown to be very harmful to forest productivity, with the direct economic loss of forestry productivity reduced by acid deposition being assessed as 28.4 million Yuan in Jiangsu province [15]. In Sichuan province in southwestern China, the forest area harmed from acid rain is up to 280,000 ha, which is one-third of the whole forestry area in Sichuan; and the dead forest area is 15,000 ha, 6% of the forestry area [16]. Forest productivity is weakened especially of species sensitive to acid precipitation such as the Masson pine (*Pinus massoniana* Lamb), which is the most widespread and important economic species grown in southwestern China. Wu [17] showed that when the precipitation pH was lower than 4.0, the productivity of Masson pine was reduced by 43%.

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The Biolog Microplate method, based on utilization patterns of sole carbon sources, has been widely used in assessing microbial function diversity [18,19,20] since it was introduced by Garland & Mills [21]. The method is simple and economical. In the present study, the Biolog Microplate method was used to detect possible changes in the soil microbial community under simulated acid rain stress. We aimed to understand the effects of acid rain on soil microorganisms under Masson pine and finally provide some scientific approaches to protecting plants from acid rain.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in a greenhouse at the Chinese Academy of Forestry in Beijing, China. Seedlings of 1-year-old Masson pine were supplied by the Chun'an forest farm in Zhejiang province and planted in pots containing soil sampled from Chongqing, which is one of the most seriously affected acid rain areas. The pot dimensions were top diameter 22 cm, bottom diameter 12.5 cm and height 15 cm. The soil characteristics were pH 4.96, organic content 20.5 g/kg, total N 1.18 g/kg, total P 0.453 g/kg, and total K 14 g/kg. The seedlings were planted in pots on November 28, 2011, and acid rain was sprayed once a week for 7 months from 16 February 2012 to 25 September 2012. In the control treatment, acid rain was replaced by deionized water. The mole ratio of SO_4^{2-} to NO_3^- in the acid rain was 5:1, and there were three acid rain treatments with pH 3.5, pH 4.5, and pH 5.5 in addition to the control treatment (CK). Other ion concentrations were NH_4^+ 2.67 mg/L, Ca^{2+} 3.37 mg/L, Mg^{2+} 0.33 mg/L, Cl^- 14 mg/L, K^+ 0.79 mg/L, Na^+ 0.36 mg/L, F^- 0.39 mg/L. Each treatment consisted of 30 pots.

2.2. Sampling and analysis

When the treatment was ended, soil in three pots was sampled randomly from the 30 pots in each treatment to analyze microbial community function. Microbial community function was analyzed by the Biolog system using sole-carbon-source-utilization (Biolog Inc., Hayward, CA). Triplicate 10 g soil samples were suspended in 90 mL of 0.85% sterile NaCl solution and vibrated for 30 min. They were then serially diluted to 10^{-3} . The dilution was inoculated on a Biolog-ECO plate in a dark chamber at a constant temperature of 25°C. After inoculation, the inoculated plates were scanned at 595 nm with a Biolog microplate reader at 24 h intervals for 168 h. The absorbance values for the wells containing carbon sources were designated as vacant against the control well. Overall color development in the Biolog plates was expressed as average well color development (AWCD) [21]. To assess the substrate utilization pattern of the microbial community, the AWCD for three main carbon substrate groups (carbohydrates, carboxylic acids, and amino acids) was also calculated [22]. The richness of the microbial community function was assumed as the total number of wells with an absorbance of over 0.2. Microbial community function diversity was calculated as the Shannon–Wiener diversity index (H') as $H' = -\sum (P_i \times \log P_i)$, where P_i is the proportion of total microbial metabolic capability (blanked absorbance values of well in this study) for a particular carbon source. At this point, we used the absorbance of the microplates at 144 h after the start of the incubation.

2.3. Statistical analyses

One-way analysis of variance was used to determine statistically significant differences in microbial assays among treatments. The least significance difference at a 95% confidence interval (LSD 0.05) was used for multiple comparisons. On the basis of the covariance matrix, principal component analysis (PCA) was used to distinguish the soil microbial community's carbon substrate utilization pattern among the various treatments.

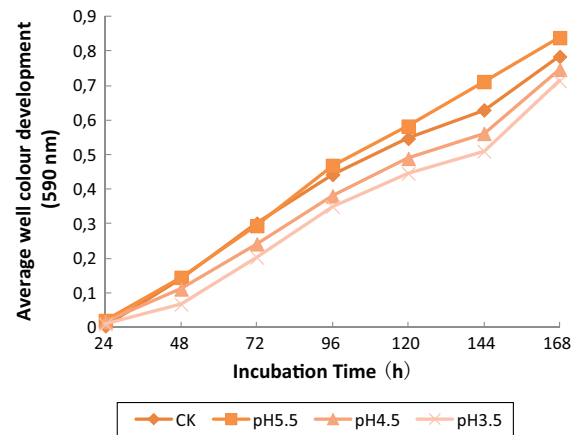


Fig. 1. AWCD in soil microbiology under different acidic treatment.

3. Results

3.1. Average well color development (AWCD)

In all treatments, the AWCD increased with the incubation time (Fig. 1). AWCD reflects soil microbial ability to utilize carbon sources and microbial activity. In comparison to the CK treatment, the low acid load treatment (pH 5.5) increased the source carbon utilization by soil microbes, while the medium and high acid load treatments (pH 4.5 and pH 3.5) decreased soil microbial activity.

The low acid load treatment (pH 5.5) increased soil microbial utilization of all three main carbon sources (carbohydrates, carboxylic acids and amino acids) in the potted Masson pine seedlings. The medium acid load treatment (pH 4.5) had no effect on utilization of carbohydrates, but increased the utilization of carboxylic acids and decreased the utilization of amino acids. In the high acid load treatment (pH 3.5), the ability of soil microbial groups to use carbohydrates was stimulated, but the utilization of amino acids was reduced, and there were no effects on the utilization of carboxylic acids (Fig. 2).

3.2. Microbial community function richness and diversity

Low and medium level acid loads (pH 5.5, pH 4.5, respectively) increased soil microbial community functional richness and Shannon diversity compared with the CK treatment, while the high acid load

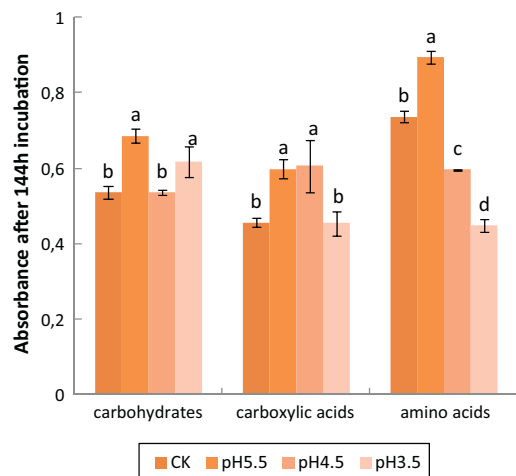


Fig. 2. The effect of acidic treatment on utilization of three main carbon sources of soil under Masson pine.

Table 1
Richness and diversity of soil microbial community under simulated acid rain.

Treatments	Shannon–Wiener diversity index	Richness index
CK	1.35 ± 0.01b	22 ± 0.55b
pH 5.5	1.37 ± 0.01ab	24 ± 0.70a
pH 4.5	1.38 ± 0.01a	24 ± 0.63a
pH 3.5	1.34 ± 0.02ab	22 ± 0.67ab

Average ± SE, with the same letter showed no significant difference between treatments, with different letter means significant difference.

treatment showed no significant inhibition on richness and diversity indices (Table 1).

3.3. Principal component analysis

PCA was conducted to determine the microbial community functions of Masson pine soil under the different simulated acid rain treatments. PCA showed that PC1 and PC2 explained 29.3% and 26.9% of the variances of AWCD, respectively. There was clear discrimination in the metabolic capability of the soil microbial community between the simulated acid rain and CK treatments (Fig. 3).

The carbon sources significantly related to principal components are listed in Table 2. Of 31 carbon sources, 12 were significantly correlated with the PC1 component, including mainly carbohydrates and amino acids, and 13 carbon sources were significantly correlated with the PC2 component, mainly carbohydrates and carboxylic acids.

4. Discussion

Soil microbial activity has been found to be either reduced [1,8], stimulated [11], or unaffected [5,13] after being subjected to simulated acid rain. The present study showed that a low acid load not only stimulated soil microbial activity (expressed as AWCD) but also increased soil microbial community functional diversity and richness

indices. The stimulation effect can be attributed to a “fertilizer effect” of the low concentration acid precipitation. A study in Dinghushan forests in subtropical China showed that an additional N load improved the growth of Masson pine needles but inhibited the growth of broad-leaved species [23]. A simulated N precipitation experiment in a subtropical forest in South China showed that addition of a medium N level significantly increased the growth of seedlings [24]. S and N are necessary major elements for plant growth, and thus a low amount of S and N in acid rain can act as a fertilizer, increasing soil fertility and plant production, especially in low fertility soils. Through stimulating plant growth, root exudation will increase, and thus acid rain may improve soil microbial activity. Also, appropriate acid rain treatment may increase the growth of some acidophilic microorganisms, with a resulting increase in soil microbial diversity and richness.

However, a “fertilizer effect” of acid rain is likely to be just a short-term phenomenon, because the available ions will be reduced [25], and also high concentrations of acid rain have an inhibitory impact on plants. Li et al. [24] showed that the growth of tree seedlings was gradually inhibited in a high N precipitation treatment, with foliar photosynthesis first increasing and then decreasing with increasing N input. In the present study, soil microbial activity (AWCD) reduced after medium and high acid load treatments. A possible reason for this is that pH is a critical limiting factor for soil microbial growth, which was obviously inhibited at low pH. After eight growing seasons being treated with acid rain, soil respiration of pine and birch was reduced [6] because of decreased root exudation, and because soil microbial communities rely on low molecular weight compounds in root exudation. Trolldenier [26] also showed that changes in root exudation affected soil respiration and changed plant nutrient supply. Reduced root exudation may be caused by lower photosynthesis. Also plant biomass reduction may result in decreased litter conversion into humus, with a consequent reduction in the available carbon source for microbial community utilization, thus decreasing soil microbial activity.

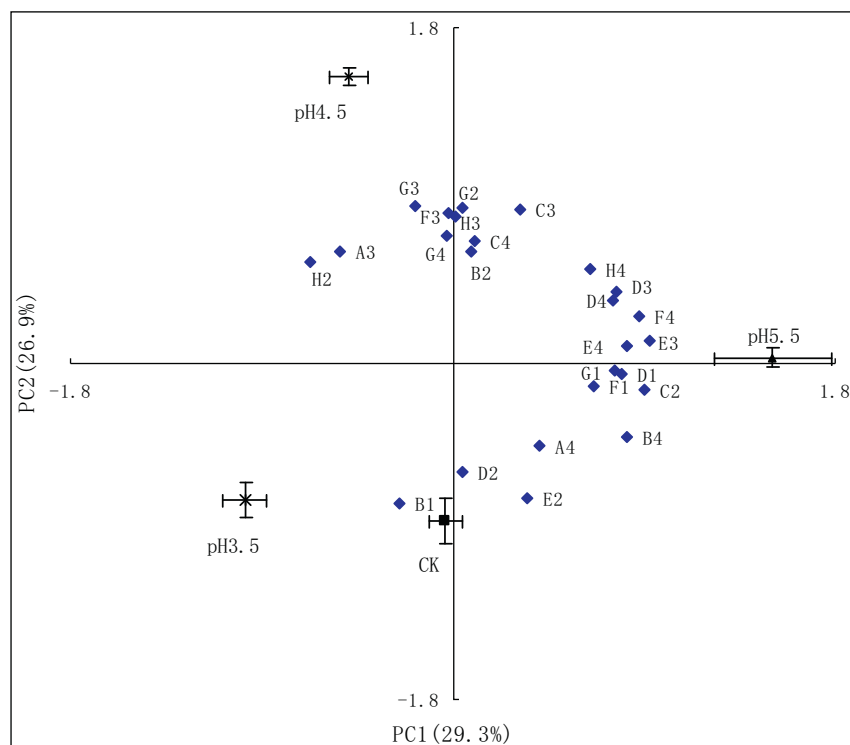


Fig. 3. Principal component analysis of carbon source utilization pattern distinguished between Masson pine soils with different acidic treatments. (The carbon source code same with Table 2).

Table 2
Substrates with high correlation coefficients for PC1 and PC2 in PCA analysis of diversity patterns for each treatment.

PC1			PC2		
Code	Carbon source	r	Code	Carbon source	r
Carbohydrates			Carbohydrates		
C2	l-Erythritol	0.904 ^a	A3	D-Galactonic acid γ -lactone	-0.748 ^b
G1	D-Cellobiose	0.762 ^b	B2	D-Xylose	0.599 ^a
H2	D,L- α -Glycerol	-0.674 ^a	D2	D-Mannitol	-0.583 ^a
Carboxylic acids			Carboxylic acids		
E3	γ -Hydroxybutyric acid	0.927 ^b	E2	N-Acetyl-D-glucosamine	-0.719 ^b
Amino acids			Carboxylic acids		
B4	l-Asparagine	0.819 ^b	A4	l-Arginine	0.599 ^a
D4	l-Serine	0.752 ^b	B1	Pyruvic acid methyl ester	-0.749 ^b
E4	l-Threonine	0.820 ^b	F3	Itaconic acid	0.804 ^b
F4	Glycyl-l-Glutamic acid	0.878 ^b	G3	α -Ketobutyric acid	0.843 ^b
Amines			Amino acids		
H4	Putrescine	0.647 ^a	H3	D-Malic Acid	0.783 ^b
Polymers			Amino acids		
D1	Tween 80	0.792 ^b	C4	l-Phenylalanine	0.652 ^a
F1	Glycogen	0.661 ^a	Amine		
Miscellaneous			G4		
D3	4-Hydroxy benzoic acid	0.769 ^b	Phenylethyl-amine		
			Miscellaneous		
			C3		
			2-Hydroxy benzoic acid		
			0.822 ^b		

^a Significant at 0.05.

^b Significant at 0.01.

PCA showed that simulated acid rain changed the utilization diversity of soil microbial carbon sources (carbohydrates, carboxylic acids and amino acids) by Masson pine seedlings. The low acid load treatment (pH 5.5) simulated soil microbial utilization of the three carbon sources, while the medium and high acid load treatments had different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the effect of acid rain on soil microbial communities. Pennanen et al. [1] found that acid rain only slightly changed bacterial carbon source utilization through using Biolog-GN technology, while phospholipid fatty acid (PLFA) analysis showed that acid rain changed soil microbial community structure. In another study by Pennanen et al. [11], PCA of Biolog showed no discrimination between acid rain treatment and a control treatment. The reasons for the different results were complicated, and included the treatment period, species, and the soil sampling procedure used. In the present study, seedlings were used rather than older trees as in Pennanen's studies, because seedlings are more sensitive to environmental stress. A pot experiment was used in the present study, and the sampling soil with roots spreading all through the pots could be regarded as rhizosphere soil, while in Pennanen's studies humus was used. Acid rain affected the soil microbial community mainly through root exudation. The effect of acid rain on rhizosphere soil has already been shown.

The soil microbial community plays a very important role in nutrient cycles, so the effect of acid rain on soil microbial communities deserves investigation. All methods have their own limits and uncertainty, and therefore, different approaches such as PLFA analysis, Biolog and molecular technology should be used to better investigate the effect of acid rain on soil microbial communities. Soil respiration and soil enzyme studies are also needed.

5. Concluding remarks

Our study showed that simulated acid rain changed soil microbial community function. The lower acid load stimulated soil microbial activity and diversity assessed by Biolog of potted Masson pine seedlings. While the higher acid load decreased the soil microbial activity and no effects on soil microbial diversity. Simulated acid rain also changed the metabolic capability of the soil microbial community.

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Author contribution

Proposed the theoretical frame: ZC, HS; Conceived and designed the experiments: ZC, LW; Contributed reagents/materials/analysis tools: ZC, HS; Wrote the paper: LW, ZC; Performed the experiments: LW, ZC, JW; Analyzed the data: LW, ZC, P-YZ.

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