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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.

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 $A1^{3+}$ 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^{-1} 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 $H' = -\Sigma\ (Pi \times \log Pi)$, where Pi 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.

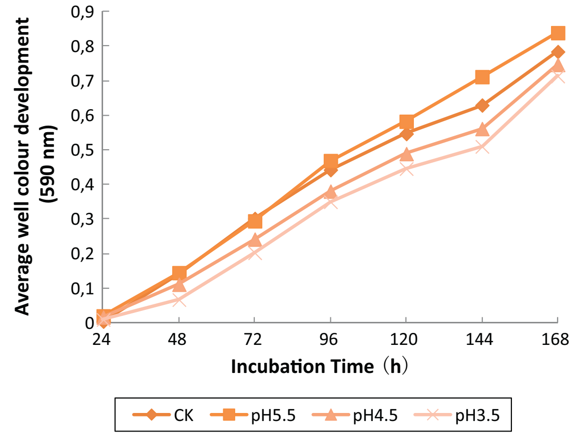

Fig. 1. AWCD in soil microbiology under different acidic treatment.

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

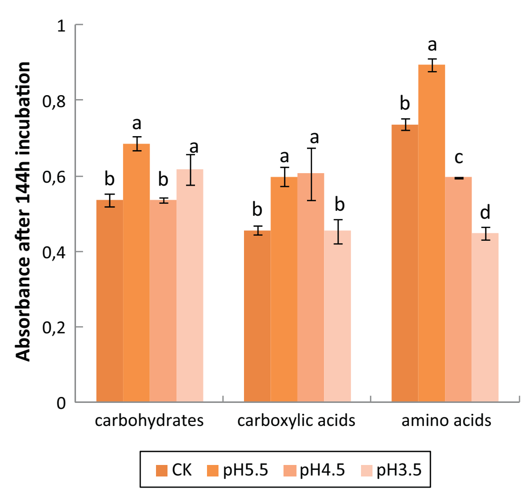

Fig. 2. The effect of acidic treatment on utilization of three main carbon sources of soil under Masson pine.