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

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A R T I C L E   I N F O
Article history:
Received 10 February 2014
Accepted 26 May 2014
Available online 30 July 2014

Keywords:
Biolog™
Metabolic capability
Soil microbial activity
Soil microbial diversity and richness

A B S T R A C T
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 (Ca²⁺, Mg²⁺, K⁺, Na⁺) by leaching with SO₄²⁻ and NO₃⁻ as the accompanying anions, and a decrease in soil pH, thus causing potentially toxic concentrations of Al³⁺ 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 km², 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%.
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\textsubscript{4}\textsuperscript{2-} to NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{4}\textsuperscript{+} 2.67 mg/L, Ca\textsuperscript{2+} 3.37 mg/L, Mg\textsuperscript{2+} 0.33 mg/L, Cl\textsuperscript{-} 14 mg/L, K\textsuperscript{+} 0.79 mg/L, Na\textsuperscript{+} 0.36 mg/L, F\textsuperscript{-} 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\textsuperscript{-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 in the inoculated 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\textsuperscript{′}) as H\textsuperscript{′} = -Σ (Pi × logPi), 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.

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
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).

| 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.
PC 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 different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources. Currently, there are few studies that have used Biolog plates to investigate the different effects on utilization of the three carbon sources.

**Table 2**

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

| PC1 Code | Carbon source | r       | PC2 Code | Carbon source | Carbon source |
|----------|---------------|---------|----------|---------------|---------------|
| Carbohydrates C2 | L-Erythritol | 0.904a | Carbohydrates A3 | d-Galactonic acid γ-lactone | -0.748b |
| G1 | D-L-Lactose | 0.762b | B2 | d-Xylose | 0.599a |
| H1 | DL-a-Glycerol | -0.674a | D2 | d-Mannitol | -0.583a |
| Carboxylic acids E3 | γ-Hydroxybutyric acid | 0.927b | E2 | N-Acetyl-γ-glucosamine | -0.719b |
| Amino acids B4 | L-Asparagine | 0.819b | A4 | L-Arginine | 0.599a |
| D4 | L-Serine | 0.752b | B1 | Pyruvic acid methyl ester | -0.749b |
| E4 | L-Threonine | 0.820b | F3 | Itaconic acid | 0.804b |
| F4 | Glycyl-L-Glutamic acid | 0.878b | G3 | α-Ketobutyric acid | 0.843b |
| Amines E5 | Putrescine | 0.647b | H3 | α-Malic Acid | 0.783b |
| Polymers D1 | Tween 80 | 0.792b | C4 | L-Phenylalanine | 0.652a |
| Miscellaneous F1 | Glycogen | 0.661a | Amine G4 | Phenylethyl-amine | 0.683a |
| D3 | 4-Hydroxy benzoic acid | 0.769b | Miscellaneous C3 | 2-Hydroxy benzoic acid | 0.822b |

* a Significant at 0.05. b Significant at 0.01.

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.

**Financial support**

Agency/institution: 1) Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry; 2) National Natural Science Foundation of China. Program financial support: 1) the Public Welfare Project of the National Scientific Research Institution; 2) the National Science Foundation for Distinguished Young Scholars of China. Project numbers: 1) CAFRIFEEP2008004; 2) 30901149.

**Acknowledgements**

This research was supported by the Public Welfare Project of the National Scientific Research Institution (CAFRIPEEP2008004), and the National Science Foundation for Distinguished Young Scholars of China (30901149).

**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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