Response of Soil Culturable Microbial Abundance to Simulated Nitrogen Deposition in Natural Evergreen Broad-Leaved Forests in the Western China Rain Screen Zone: Postprint

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Date: 2017-03-08T00:00:00+00:00

Abstract

To understand the effects of nitrogen deposition on soil microbial quantities in the natural evergreen broad-leaved forest of the Rainy Screen Area of Western China, a field simulation of N (NH4NO3) deposition was conducted from November 2013 to December 2014 with nitrogen deposition levels of control (CK 0 kg N hm-2a-1), low nitrogen deposition (L 50 kg N hm<-2a-1), medium nitrogen deposition (M 150 kg N hm-2a-1), and high nitrogen deposition (H 300 kg N hm-2a-1) to investigate the effects on culturable microbial quantities in the 0-10 cm and 10-20 cm soil layers of the natural evergreen broad-leaved forest. The results showed that the quantities of bacteria, fungi, and actinomycetes in the 0-10 cm soil layer were all significantly greater than those in the 10-20 cm soil layer, and nitrogen deposition did not alter the original vertical distribution pattern. The L treatment had no significant effect on the total soil microbial quantity in either the 0-10 cm or 10-20 cm soil layers, whereas the M and H treatments significantly reduced the total soil microbial quantity. Nitrogen deposition decreased bacterial quantities in both the 0-10 cm and 10-20 cm soil layers, with the inhibitory effect intensifying as nitrogen deposition amount increased. Nitrogen deposition reduced fungal quantities in the 0-10 cm soil layer, but there was no clear pattern between the magnitude of reduction and nitrogen deposition amount; in the 10-20 cm soil layer, the M and H treatments significantly increased fungal quantities in summer, indicating that appropriate nitrogen deposition could effectively alleviate the nitrogen-limited status of soil fungi in summer. The effect of nitrogen deposition on actinomycete quantities in the 0-10 cm soil layer exhibited a pattern of initial promotion followed by inhibition; the L and M treatments increased actinomycete quantities, while the H treatment decreased them. Nitrogen deposition increased actinomycete quantities in the 10-20 cm soil layer, with the M treatment showing the greatest promotional effect. The effects of nitrogen deposition on soil microbial quantities weakened with increasing soil depth.

Full Text

Preamble

Response of Culturable Soil Microorganisms to Simulated Nitrogen Deposition in a Natural Evergreen Broadleaf Forest in the Rainy Area of Western China

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Abstract

To understand the effects of nitrogen deposition on soil microbial quantities in the Rainy Area of Western China, we conducted a field simulation experiment in a natural evergreen broadleaf forest using four nitrogen deposition levels: control (CK, 0 kg N hm^{-2}), low nitrogen (L, 50 kg N hm^{-2}), medium nitrogen (M, 150 kg N hm^{-2}), and high nitrogen (H, 300 kg N hm^{-2}). The study examined how nitrogen deposition affects culturable soil microorganisms in the 0–10 cm and 10–20 cm soil layers.

The results showed that bacteria, fungi, and actinomycetes were significantly more abundant in the 0–10 cm layer than in the 10–20 cm layer. Nitrogen deposition did not alter this vertical distribution pattern. In the 0–10 cm layer, nitrogen deposition reduced fungal numbers, while in the 10–20 cm layer, it reduced bacterial numbers. The decline showed no clear relationship with nitrogen deposition rate. The L treatment had no significant effect on total microbial numbers, whereas M and H treatments significantly reduced total microbial numbers in both soil layers. The inhibitory effect on bacteria in both layers strengthened with increasing nitrogen deposition.

During summer, M and H treatments significantly increased fungal numbers in the 10–20 cm layer, indicating that moderate nitrogen deposition can effectively alleviate nitrogen limitation for soil fungi in this season. Nitrogen deposition decreased fungal numbers in the 0–10 cm layer, but the decline showed no obvious pattern with deposition rate. In the 10–20 cm layer, nitrogen deposition increased actinomycete numbers, with the greatest increase under the M treatment. In the 0–10 cm layer, nitrogen deposition first increased then decreased actinomycete numbers: L and M treatments increased them while H treatment reduced them. Overall, the effects of nitrogen deposition on soil microbial quantities weakened with increasing soil depth.

Keywords: simulated nitrogen deposition; culturable microorganisms; Rainy Area of Western China; natural evergreen broadleaf forest

Introduction

Nitrogen deposition refers to the process by which nitrogen is removed from the atmosphere and deposited onto the Earth' s surface through rainfall or dust. Together with biological nitrogen fixation and nitrogen mineralization, it represents one of the three major sources of nitrogen in ecosystems and constitutes a critical component of the global nitrogen biogeochemical cycle. Since the industrial revolution, the production and use of chemical nitrogen fertilizers, combustion of fossil fuels, and rapid development of animal husbandry have led to continuously elevated concentrations of NOx ($NO + NO_2$) and NH_3 in the atmosphere. Consequently, anthropogenic atmospheric nitrogen deposition has become a significant component of the global nitrogen biogeochemical cycle.

As both a nutrient source and acid source, dramatic changes in atmospheric nitrogen deposition severely affect the productivity and stability of terrestrial and aquatic ecosystems, making it a scientific issue of widespread public and scientific concern. North America, Western Europe, and East Asia have become the world' s three major nitrogen deposition hotspots. In China, the lowest critical loads for nitrogen deposition (<10 kg hm^{-2}) occur in the western Tibetan Plateau and Alxa Plateau, while the highest (>40 kg hm^{-2}) are found in the Northeast Plain, middle-lower Yangtze Plain, and Sichuan Basin.

Forest soil microbial activity is an important aspect of soil properties, and the abundance of three major microbial groups (bacteria, fungi, and actinomycetes) serves as a key indicator of soil microbial activity levels. Excessive nitrogen input can alter soil microbial communities. Wallenstein et al. found that bacteria in Harvard Forest decreased with increasing nitrogen application rates. Xue et al. observed that nitrogen deposition promoted total soil microbial numbers and that actinomycete abundance positively correlated with nitrogen deposition levels, though the effect shifted to inhibition beyond certain nitrogen application rates. Other studies have shown that excessive nitrogen deposition does not significantly affect bacterial numbers, while fungal numbers negatively correlate with nitrogen deposition throughout the process. Due to differences in experimental forest types and detection methods, research results vary considerably. Most studies suggest nitrogen deposition inhibits fungal numbers, though some indicate promotional effects, possibly related to soil acidification 程度和 nitrogen deposition levels. Overall, the effects of nitrogen deposition on forest soil microbial numbers remain highly uncertain.

The Rainy Area of Western China, located on the western edge of the Sichuan Basin, forms a narrow rainy zone due to the influence of the Qionglai Mountains' topography. This region receives abundant precipitation, making it a natural laboratory for studying nitrogen deposition. Research indicates that total nitrogen deposition in this area reaches 95 kg N hm^{-2} annually, with an increasing trend. Continuous nitrogen deposition will inevitably affect the structure and function of forest ecosystems in this region. To understand whether and how nitrogen deposition affects soil microbial numbers in evergreen broadleaf forests, we conducted a simulated atmospheric nitrogen deposition experiment in a natural evergreen broadleaf forest. This study helps elucidate how soil microorganisms in this region respond to nitrogen deposition under global environmental change, providing theoretical basis for understanding material cycling and management of forest ecosystems in the Rainy Area of Western China.

1. Study Area Overview

The study site is located in Bifengxia Scenic Area, Yucheng District, Ya' an City, Sichuan Province (102°90 E, 29°40 N) at an elevation of 977.62 m. The region has a subtropical humid monsoon mountain climate with mild and humid conditions. The annual average temperature is 16.2°C, with $ 10°Caccumulatedtemperatureof5231°C.Annualprecipitationaverages1772.2mm,evaporationis1039.6mm,
The frost-free period lasts 304 days.

The experimental area has rich plant species diversity with complex community structure. Dominant tree species include *Schima superba*, *Pittosporum tobira*, *Symplocos botryantha*, *Lithocarpus hancei*, *Cinnamomum cassia*, *Camellia japonica*, *Eurya japonica*, *Acer davidii*, *Machilus lichuanensis*, *Machilus pingii*, *Lithocarpus megalophyllus*, and *Rhus succedanea*. Soil type is primarily yellow soil with thickness >60 cm. The 0–20 cm soil layer has total nitrogen content of (1.14$±0.13)g/kg,nitratenitrogenof(17.17±1.54)g/kg,ammoniumnitrogenof(6.87±0.56)mg/kg,andpHof6.2

2. Experimental Design

In March, we selected representative natural evergreen broadleaf forest plots in Bifengxia, Yucheng District, Ya' an City. Three 3 m × 3 m sample plots were established with 5 m buffers between them. Based on the local nitrogen deposition rate of 95 kg N hm^{-2} and its increasing trend, we established four treatments: control (CK, 0 kg N hm^{-2}), low nitrogen (L, 50 kg N hm^{-2}), medium nitrogen (M, 150 kg N hm^{-2}), and high nitrogen (H, 300 kg N hm^{-2}). Nitrogen was applied as NH_4NO_3 solution using a handheld sprayer at 50 cm height above the forest floor. Control plots received equal amounts of water to minimize effects from added water on forest biogeochemical cycles.

3. Soil Sample Collection and Preparation

Soil samples were collected in July, October 2013, and January, April, July 2014. Within each plot, five sampling points were randomly established. After removing litter, soil cores were taken from 0–10 cm and 10–20 cm layers. Visible plant and animal residues and roots were removed, and soil samples from the same layer within each treatment were mixed uniformly. Samples were collected in sterile bags, transported to the laboratory, and immediately stored at 4°C.

4. Measurement Methods

The numbers of culturable bacteria, fungi, and actinomycetes were determined using the dilution plate method. Bacteria were cultured on beef extract peptone medium, fungi on Martin' s medium, and actinomycetes on Gause' s No. 1 medium. Results are expressed as colony-forming units per gram of dry soil (cfu/g). All chemical analyses were performed with three replicates. In this study, total soil microbial numbers represent the sum of these three culturable microbial groups.

5. Data Analysis

Data were analyzed using SPSS 20.0 software. One-way ANOVA was used to test the effects of nitrogen treatment, sampling time, and soil layer on soil microbial numbers. The least significant difference (LSD) method was used for multiple comparisons. Significance level was set at $\alpha = 0.05$. Figures were prepared using Excel 2007, with data presented as means.

6. Results

6.1 Effects of Simulated Nitrogen Deposition on Total Microbial Numbers

All treatments showed clear seasonal dynamics in total soil microbial numbers. The 0-10 cm layer had greater microbial numbers than the 10-20 cm layer, and nitrogen deposition did not change this vertical distribution pattern. In both layers, microbial numbers peaked in autumn. Nitrogen deposition did not alter the seasonal dynamics of total microbial numbers, and responses were generally consistent across soil layers.

The L treatment had no significant effect on total microbial numbers in either layer ($P > 0.05$). However, M and H treatments significantly reduced total microbial numbers in the 0-10 cm layer by 9.67% and 41.59%, respectively ($P < 0.05$), and in the 10-20 cm layer by 49.36% and 49.30%, respectively ($P < 0.05$).

Repeated measures ANOVA showed that nitrogen treatment, sampling time, and soil layer all had significant effects on total microbial numbers ($P < 0.001$), as did their interactions (Table 1).

6.2 Effects of Simulated Nitrogen Deposition on Bacterial Numbers

Bacterial numbers showed clear seasonal dynamics, with greater abundance in the 0-10 cm layer than in the 10-20 cm layer. Nitrogen deposition did not alter this vertical distribution pattern. Both layers exhibited peak bacterial numbers in autumn, and nitrogen deposition did not change this seasonal dynamic. However, responses to nitrogen deposition differed between soil layers.

All nitrogen treatments significantly reduced bacterial numbers in the 0–10 cm layer. Compared with CK, L, M, and H treatments decreased bacterial numbers by 12.92%, 50.11%, and 53.15%, respectively ($P < 0.05$). The inhibitory effect intensified with increasing nitrogen deposition. In the 10–20 cm layer, only M and H treatments significantly reduced bacterial numbers by 11.24% and 53.54%, respectively ($P < 0.05$).

[Figure 2: see original paper]

6.3 Effects of Simulated Nitrogen Deposition on Fungal Numbers

Fungal numbers showed clear seasonal dynamics, with greater abundance in the 0–10 cm layer than in the 10–20 cm layer. Nitrogen deposition did not change this vertical distribution pattern, though M and H treatments somewhat altered seasonal dynamics. Both layers typically peaked in autumn, but in M and H treatment plots, summer fungal numbers in the 10–20 cm layer shifted from a seasonal low to a peak.

Responses to nitrogen deposition differed between soil layers. Nitrogen deposition significantly reduced fungal numbers in the 0–10 cm layer, but the decline showed no clear relationship with nitrogen deposition rate. In the 10–20 cm layer, nitrogen deposition increased fungal numbers, with M and H treatments raising them by 18.11% and 8.36%, respectively, during summer, indicating that moderate nitrogen deposition can effectively alleviate nitrogen limitation for soil fungi.

[Figure 3: see original paper]

6.4 Effects of Simulated Nitrogen Deposition on Actinomycetes

Actinomycete numbers showed clear seasonal dynamics, with greater abundance in the 0–10 cm layer than in the 10–20 cm layer. Nitrogen deposition did not alter this vertical distribution pattern, and both layers peaked in autumn. Responses differed between soil layers and nitrogen treatments.

In the 0–10 cm layer, nitrogen deposition caused nonlinear changes: L and M treatments increased actinomycete numbers by 18.89% and 32.54%, respectively, while H treatment decreased them by 16.53% ($P < 0.05$). In the 10–20 cm layer, all nitrogen treatments increased actinomycete numbers, with M treatment showing the strongest promotion (42.92% increase, $P < 0.05$).

[Figure 4: see original paper]

7. Discussion

Soil microbial abundance is an important biological indicator of soil quality. Nitrogen deposition directly affects soil microbial communities by altering substrate quality, stoichiometric ratios, and environmental conditions, thereby influencing microbial biomass and community composition. Bacteria, fungi, and actinomycetes are the main components of soil microorganisms.

The 0–10 cm layer had significantly greater numbers of bacteria, fungi, and actinomycetes than the 10–20 cm layer, consistent with findings by Gong et al. and Yang. This is because the surface layer has well-developed root systems whose exudates and detritus provide abundant energy sources for microbial growth and reproduction. Nitrogen deposition could not change this vertical distribution pattern.

All treatments showed peak microbial numbers in autumn, consistent with Gong et al. Autumn represents the end of the growing season when litter decomposition releases substantial nutrients after a period of decomposition, combined with favorable hydrothermal conditions that allow microbial numbers to peak. In summer, actinomycete numbers were significantly lower than in other seasons because drought-tolerant actinomycetes are not adapted to moist conditions. Additionally, high summer precipitation reduces soil aeration, limiting microbial activity.

Nitrogen deposition significantly reduced bacterial numbers in both layers, similar to Liu et al. This may occur for two reasons: first, nitrogen deposition reduces belowground carbon allocation; second, it creates high osmotic stress and alters soil physicochemical properties, changing microbial substrate utilization patterns. We also found that nitrogen deposition promoted accumulation of soil organic carbon and inorganic nitrogen in this forest, altering the soil carbon and nitrogen pools and other properties, thus reducing bacterial numbers.

Soil fungi are heterotrophic organisms that use organic matter as a carbon source and play important roles in soil carbon cycling. Nitrogen deposition reduced fungal numbers in the 0–10 cm layer, likely related to decreased abundance of ectomycorrhizal fungi in nitrogen-rich environments. Kjøller et al. found that nitrogen deposition reduces ectomycorrhizal fungal fruiting body productivity, decreasing their abundance. The response of fungi in the 10–20 cm layer was more complex, possibly due to lower root density and different fungal community structures.

Actinomycetes can decompose compounds that most fungi and bacteria cannot and participate in the decomposition of recalcitrant organic matter. In the 0–10 cm layer, actinomycete numbers showed nonlinear responses to nitrogen deposition, with L and M treatments increasing and H treatment decreasing numbers. This aligns with Xue et al. In the 10–20 cm layer, all nitrogen treatments promoted actinomycete numbers, with M treatment showing the strongest effect. These differences between soil layers may relate to varying nitrogen concentrations and actinomycete community composition. Eisenlord et al. found that some Actinomycetales species are positively affected by nitrogen deposition, while lignin-degrading Streptosporangiaceae are negatively affected.

8. Conclusions

Nitrogen deposition significantly affected soil microbial numbers in the natural evergreen broadleaf forest of the Rainy Area of Western China. The 0–10 cm layer had greater numbers of bacteria, fungi, and actinomycetes than the 10–20 cm layer, and nitrogen deposition did not alter this vertical distribution pattern. Nitrogen deposition reduced bacterial numbers in both layers, with inhibition strengthening as deposition increased. It reduced fungal numbers in the 0–10 cm layer (with no clear dose-response relationship) but increased them in the 10–20 cm layer during summer. Low and medium nitrogen deposition increased actinomycete numbers in the 0–10 cm layer, while high nitrogen deposition decreased them. All nitrogen treatments increased actinomycete numbers in the 10–20 cm layer, with medium nitrogen showing the strongest promotion.

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Note: Figure translations are in progress. See original paper for figures.