Effects of Short-term Nocturnal Warming on Root Exudate Rate and Chemical Composition of Spruce Seedlings in Subalpine Coniferous Forests (Postprint)

Authors: Jiang Zheng, Xiao Juan, Zhang Ziliang, Qiao Mingfeng, What is···, Liu Qing, Yin Huajun

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Abstract

Using spruce seedlings under continuous 7-year infrared radiation warming treatment as the study object, we quantitatively analyzed the effects of warming on root carbon (C) and nitrogen (N) secretion rates and main volatile chemical components. The results showed that: (1) Warming significantly increased the root C secretion rate of spruce seedlings, but had no significant effect on N secretion rate, accompanied by a significant increase in the C:N stoichiometric ratio of root exudates; (2) The response of changes in input content of different chemical components to warming exhibited distinct differences, with the magnitude and direction of response being dependent on chemical component type. Specifically, the contents of sugars, amino acids, and phenolic compounds all significantly increased under warming treatment, whereas the relative contents of esters and ethers significantly decreased; (3) Further analysis revealed that the content of different compounds within the same component also showed differential responses to warming. For instance, warming only led to significant increases in the contents of 2,6-di-tert-butyl-4-methylphenol and 4-tert-butylcalix[4]arene among phenolic compounds (increased by 88.9% and 375.7% compared to the control, respectively), while having no significant effect on the contents of other phenolic compounds. These results demonstrate that warming can induce profound changes in the relative contents of various components of plant root exudates, which holds important theoretical significance for further understanding root exudate inputs under different environmental changes and the specific soil microbial nutrient processes they induce.

Full Text

Preamble

Effects of Short-Term Nighttime Warming on the Rates and Main Chemical Components of Root Exudates Produced by *Picea asperata* Seedlings in Subalpine Coniferous Forests

Jiang Zheng[1],[3], Xiao Juan[1],[3], Zhang Ziliang[1],[2], Qiao Mingfeng , He Wei[1], Liu Qing[1], Yin Huajun[1],[3],* [1]Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization, Chengdu Institute of Biology, Chinese Academy of Sciences [2]Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province [3]University of Chinese Academy of Sciences College of Environmental Science and Engineering, China West Normal University Sichuan Tourism University

*Corresponding author. E-mail: yinhj@cib.ac.cn

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Abstract

While root exudates are widely recognized as crucial drivers of belowground biogeochemical processes, few studies have examined how elevated temperature affects both the rates and chemical composition of root exudation in forest ecosystems. This study investigated the ecological consequences of warming on root exudation by conducting a nighttime warming experiment using infrared heating devices on *Picea asperata* seedlings in a subalpine coniferous forest. Root exudates were collected from intact fine roots of plants in both warmed and control plots using a modified culture-based cuvette system specifically developed for field-based exudate collection. We quantified total organic carbon (TOC) and total nitrogen (TN) concentrations in root exudates and expressed these as C and N exudation rates per unit root biomass. Furthermore, the main chemical components of *P. asperata* root exudates were analyzed using gas chromatography-mass spectrometry (GC-MS).

Our results showed that: (1) Experimental warming significantly increased root C exudation rates (g C g ^{1} root biomass h ^{1}) but had no significant effect on root N exudation rates (g N g ^{1} root biomass h ^{1}), leading to a significant increase in the C:N ratio of root exudates. (2) Warming significantly affected the relative contents of chemical compounds, with the magnitude and direction of response closely related to compound class. Specifically, warming significantly increased the relative contents of sugars, amino acids, and phenolics, while markedly decreasing the relative contents of esters and ethers. (3) Warming had significant effects on the contents of different chemical components within root exudates. For example, the relative contents of two phenolic compounds—2,6-Di-tert-butyl-4-methylphenol and 4-Tert-butylcalix[4]arene—increased by 88.9% and 375.7%, respectively, compared to control plots, while no significant differences were observed for other components.

Collectively, our results suggest that experimental warming can profoundly influence both exudation rates and the relative contents of specific exudate components. This provides a theoretical foundation for improving understanding of soil C-nutrient cycling processes mediated by root exudation inputs in subalpine coniferous forests under environmental change.

Keywords: climate warming; *Picea asperata*; root exudates; chemical components; subalpine coniferous forests

1. Study Area Overview

The study site was located at the Maoxian Mountain Ecosystem Research Station (31°41 N, 103°53 E, 1820 m elevation), Chengdu Institute of Biology, Chinese Academy of Sciences. The region has a warm temperate subalpine monsoon climate with cold, dry winters. Mean annual precipitation is 919.5 mm, mean annual temperature is 8.9°C, and annual sunshine duration is 1139.8 hours. Forest soils are primarily leached brown earth and brown soil with pH 5.8-6.0. The natural vegetation is rich and diverse, forming distinct zones from mountain base to summit including shrubs, deciduous broadleaf forests, subalpine coniferous forests, and alpine meadows.

2. Experimental Materials and Design

2.1 Plant Material and Soil Preparation

Experimental plants were 2-year-old *Picea asperata* seedlings of uniform height, basal diameter, and growth status, obtained from the Miyaluo Forest Farm nursery. In May, seedlings were transplanted into experimental plots and allowed to acclimate. The experimental soil was forest surface soil collected near the Maoxian research station. Using the "guest soil method," the top 0-30 cm of soil in the experimental area was completely replaced with the test soil, which had initial physicochemical properties of 61 g/kg soil organic carbon and 4.0 g/kg total nitrogen.

2.2 Warming Treatment Setup

The experiment employed a paired-plot design with 2 m × 2 m plots. Each pair consisted of one warmed plot and one control plot. In warmed plots, infrared radiators (HS-2420, Kalglo Electronics Inc., Bethlehem, PA, USA) were suspended 1.5 m above ground level to provide continuous nighttime warming from 19:00 to 7:00. Warming began in June and continued throughout the experimental period. To avoid shading effects from the infrared heaters, dummy heaters of identical size were suspended at the same height in control plots. To minimize edge effects, control and warmed plots were spaced 3 m apart, and heaters were repositioned every two weeks to ensure uniform heating. We regularly removed weeds and monitored soil moisture, watering as needed to maintain consistent soil moisture across all plots.

3. Environmental Factor Monitoring

In June, automated temperature monitoring devices were installed in the experimental site. Air temperature at 20 cm height (level with seedling tops) was measured using sensors (Campbell AR5, Avalon, USA) positioned at plot centers, with small plastic plates suspended above the sensors to block direct infrared radiation. Soil temperature sensors at 0 and 5 cm depths were also installed in both warmed and control plots. All sensors automatically recorded data at 1-hour intervals stored in data loggers.

4. Root Exudate Collection and Analysis

4.1 Collection Method

During the peak growing season (July–August), root exudates were collected using an improved static in-situ collection device. In each plot, one healthy spruce seedling was randomly selected and an undamaged fine root (<2 mm diameter, 20–30 cm length) was carefully excavated along its growth direction. The root was gently rinsed three times with nutrient solution free of C and N elements (0.1 mmol/L KH PO , 0.2 mmol/L K SO , 0.2 mmol/L MgSO ·7H O, 0.3 mmol/L CaCl ·2H O) to remove surface minerals and adsorbed soil particles. The cleaned fine root was then transferred to a syringe filled with acid-washed glass wool and glass sand, sealed with plastic wrap and aluminum foil. The nutrient solution was injected as a buffer, and the assembly was buried in soil for 6 hours. Exudates were collected by vacuum filtration into brown bottles. This process was repeated three times per sample. Root-free devices served as blanks. After collection, fine roots were harvested, brought to the laboratory, and dried at 65°C to constant weight to determine root dry mass for calculating exudation rates.

4.2 Carbon and Nitrogen Analysis

Total organic carbon (TOC) and total nitrogen (TN) concentrations in root exudates were measured using a Multi-N/C 2100 analyzer (Analytik Jena AG, Germany). Exudation rates were calculated by subtracting blank values, then dividing by root dry mass and collection time, expressed as g C g ^{1} root biomass h ^{1} and g N g ^{1} root biomass h ^{1}.

4.3 Total Sugar Analysis

Total sugar content was determined using the anthrone colorimetric method. Collected exudate solutions were diluted to the appropriate concentration range, placed in clean test tubes, and heated in a boiling water bath for 15 minutes after adding anthrone reagent. After rapid cooling in an ice bath, absorbance was measured at 620 nm. Sugar concentrations were determined from a glucose standard curve, and exudation rates were expressed as total sugar content per unit root dry mass per unit time.

4.4 Amino Acid Analysis

Amino acid concentrations were measured using a Varioskan Flash microplate reader (Thermo, USA) with fluorescence detection. o-Phthalaldehyde and -mercaptoethanol were used as derivatization reagents, and glycine was used for standard curves. Exudation rates were expressed as amino acid content per unit root dry mass per unit time.

4.5 Chemical Component Analysis (GC-MS)

In situ collected root exudates were continuously extracted with ethyl acetate. The combined extracts were concentrated using a rotary evaporator, redissolved in ethyl acetate, and filtered through 0.22 m membranes. GC-MS analysis was performed on an HP-5 MS column (30 m × 0.25 mm × 0.25 m) with helium carrier gas. The temperature program started at 50°C for 1 min, increased at 8°C/min to 250°C, held for 10 min, then to 280°C for 3 min. The injector temperature was 250°C and transfer line temperature was 280°C. Mass spectra were scanned from 40–700 amu. Compounds were identified by matching against mass spectral libraries using forward and reverse match factors, with relative percentages calculated by peak area normalization.

4.6 Statistical Analysis

Data were analyzed using SPSS 17.0 software. Differences in TOC, TN, total sugar, and amino acid exudation rates and volatile substance contents between warmed and control plots were tested using independent-samples t-tests. All figures were generated using Origin 8.0 and Microsoft Excel 2010.

5. Results

5.1 Warming Effects on Temperature

Throughout the experimental period, the mean daily soil temperature in warmed plots increased by 3.88°C compared to control plots, while mean air temperature increased by 1.83°C [Figure 1: see original paper].

5.2 Effects on Root Exudate C and N Rates

Warming significantly increased *Picea asperata* root C exudation rates by 43.89% ($P < 0.05$) but had no significant effect on N exudation rates ($P > 0.05$). Consequently, the C:N ratio of root exudates increased significantly by 28.36% ($P < 0.05$) [Figure 2: see original paper].

5.3 Effects on Total Sugar and Amino Acid Exudation

Warming significantly promoted total sugar exudation rates, which increased by 38.6% to 10.57 g g^{1} root biomass h^{1} ($P < 0.05$). Amino acid exudation rates showed a similar response, increasing by 43.16% to 2.14 g g^{1} root biomass h^{1} ($P < 0.05$) [Figure 3: see original paper].

5.4 Effects on Volatile Chemical Components

Warming significantly affected the relative contents of volatile components in root exudates. Phenolic compounds, the most abundant volatile fraction, increased significantly by 89.1% ($P < 0.01$). In contrast, esters and ethers decreased significantly by 59.5% ($P < 0.01$) and 51.4% ($P < 0.05$), respectively. Ketones, hydrocarbons, steroids, amides, and aldehydes showed no significant differences between treatments .

5.5 Changes in Specific Compound Contents

Further analysis revealed significant changes in individual compounds. Among phenolics, 2,6-Di-tert-butyl-4-methylphenol and 4-Tert-butylcalix[4]arene increased by 88.9% ($P < 0.05$) and 375.7% ($P < 0.01$), respectively. In the ester class, monobutyl phthalate, dehydropregnenolone acetate, and butyl citrate decreased by 79.4% ($P < 0.01$), 48.1% ($P < 0.01$), and 67.4% ($P < 0.05$), respectively. The main ether component, heptadecyloxy-2-(7-···)-2H-··· , decreased by 51.9% ($P < 0.05$). Some compounds within classes showed no significant overall differences, but individual components varied significantly .

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6. Discussion

6.1 Warming Effects on Carbon Allocation to Root Exudates

Our results demonstrate that short-term nighttime warming significantly enhanced *Picea asperata* root C exudation rates, consistent with previous studies showing temperature effects on root growth and physiology [13–14]. This likely reflects a physiological adjustment where plants increase C allocation to roots to enhance soil microbial enzyme activity and nutrient transformation, meeting increased nutrient demands under warming [15]. Similar results have been validated in previous research on this species [27]. The lack of significant change in

N exudation may reflect N limitation in this subalpine coniferous forest ecosystem, where plants restrict N loss as a physiological adaptation under warming [29]. The significant increase in C:N ratio of root exudates under warming will profoundly influence soil nutrient cycling and microbial community composition [30].

6.2 Effects on Chemical Composition and Content

Root exudates contain diverse chemical components including sugars, amino acids, phenolics, esters, and other volatile compounds [5]. Our study detected significant differential responses among chemical classes to warming. As common low-molecular-weight compounds, sugars and amino acids both increased significantly, providing additional C and energy for microbial growth [32] and supporting previous findings of enhanced rhizosphere microbial activity under warming [33].

Phenolic compounds, the dominant volatile fraction, increased significantly under warming. However, responses varied among specific compounds—only 2,6-Di-tert-butyl-4-methylphenol and 4-Tert-butylcalix[4]arene showed substantial increases, while other phenolics were unaffected. This selectivity suggests plants can adjust exudate composition in response to environmental change, potentially influencing specific microbial functions [17, 35]. The significant decrease in esters and ethers indicates warming-induced shifts in metabolic pathways or selective utilization by rhizosphere microbes [37].

6.3 Mechanisms and Ecological Implications

Root exudation represents an adaptive strategy shaped by plant evolution and environmental conditions [34]. Warming may enhance root exudation to stimulate microbial activity and nutrient cycling to support plant growth [27]. However, the specific mechanisms driving compositional changes remain unclear due to methodological limitations. Different exudate components have distinct ecological functions—organic acids can chelate heavy metals [32], while phenolics exhibit strong allelopathic effects that can inhibit root growth and microbial activity [39–42]. The observed compositional shifts under warming will likely have important consequences for soil nutrient cycling and forest ecosystem function in subalpine regions.

6.4 Study Limitations

This study has several limitations. First, using seedlings may not fully represent mature tree responses due to differences in growth, morphology, and physiology. Second, our chemical analysis may not capture the complete exudate profile due to methodological constraints. Long-term field studies on mature trees are needed to validate these findings. Future research should focus on coupling exudate compositional changes with soil biogeochemical cycling processes to better understand belowground responses to climate change in subalpine coniferous forests.

7. Conclusions

Our main findings are: (1) Warming significantly increased *Picea asperata* root C exudation rates and the C:N ratio of exudates; (2) Warming significantly altered the relative contents of specific chemical components and individual compounds, with sugars, amino acids, and phenolics increasing while esters and ethers decreased. These results provide a theoretical basis for understanding how climate change affects root-mediated soil processes in subalpine forests, though caution is needed when extrapolating from seedlings to ecosystem scales.

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