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## Research article

## Effects of simulated atmospheric nitrogen deposition on foliar chemistry and physiology of hybrid poplar seedlings

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## ABSTRACT

During recent decades, the southern and eastern regions of Asia have experienced high levels of atmospheric N deposition. Excess N deposition is predicted to influence tree growth and species composition in the regions, but visual or physiological assessments alone are not sufficient to determine the real effects of atmospheric N deposition. In this study, we simulated atmospheric wet deposition of inorganic N by spraying a  $\text{NO}_3^-$  solution ( $20 \text{ mmol}\cdot\text{L}^{-1}$ ) or a mixture of  $\text{NO}_3^-$  ( $20 \text{ mmol}\cdot\text{L}^{-1}$ ) plus  $\text{NO}_2^-$  ( $100$  or  $300 \mu\text{mol}\cdot\text{L}^{-1}$ ) on leaves of hybrid poplar (*Populus alba* × *Populus berolinensis*) seedlings and examined morphoanatomical traits and physiological processes. Leaves of seedlings sprayed with single or mixed N solutions developed marginal necrosis, curling, and small cracks on the adaxial surface. The silicon (Si)-rich crystals were larger (about 100% increase in crystal diameter compared to untreated seedlings) on the adaxial leaf surface, with a significant positive correlation between the atomic percentage of N and Si on the crystal areas of the surface. Leaves were sensitive to  $\text{NO}_2^-$  compared with  $\text{NO}_3^-$  even at a low concentration; water content, dry mass, and photochemical variables significantly declined and dark respiration increased only in leaves treated with mixed N form. Mixed N foliar applications significantly increased leaf concentrations of the free amino acids Glu, Gln, and Asn and organic acids oxaloacetic acid and citric acid. Besides, mixed N treatment stimulated leaf transamination, as indicated by significant increases in Ala and Asp concentrations and activities of glutamic oxalacetic transaminase and glutamic pyruvic transaminase. However, mixed N applications led to declines in leaf concentrations of putrescine (by 65%,  $p = 0.01$ ) and spermine (by 53%,  $p = 0.01$ ). A higher proportion of  $\text{NO}_2^-$  ( $300 \mu\text{mol}\cdot\text{L}^{-1}$ ) in mixed N solution was inhibitory to key N-metabolic enzymes and N translocation via the phloem. Our results showed that wet deposition of airborne N pollutants modified surface properties and induced additional detrimental effects related to N-compound foliar absorption. Furthermore, our findings indicate that detoxification of reactive N is apparently related to N assimilation and export from the treated leaves via the phloem.

## 1. Introduction

During the past two decades (1995–2015), atmospheric reactive N species and their deposition have increased continuously at a global scale (Stevens et al., 2018). In some regions of Asia, atmospheric inorganic N is mainly wet-deposited in mixed N forms (Liu et al., 2011). A dominant deposition N form in the regions of Japan and China was  $\text{NO}_3^-$ -N, and  $\text{NO}_2^-$ -N accounted for a small fraction of total N deposition (Hayashi et al., 2007; Chen et al., 2019). In previous

simulations of inorganic N wet deposition,  $\text{NO}_3^-$  or  $\text{NH}_4\text{NO}_3$  solutions of different concentrations have been often used (Liao et al., 2010; Mao et al., 2018); however, less emphasis has been placed on  $\text{NO}_2^-$  or a mixture of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . A mixed N species may be more realistic for simulating atmospheric inorganic N deposition.

Atmospheric N deposition can influence plant growth either via leaves or roots (Bourgeois et al., 2019). Root-originating impacts are indirectly through N deposition-mediated soil acidification and changes in soil microbial diversity (Liu et al., 2011). Foliar N deposition directly

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