



Physiological responses of a young *Picea Sitchensis* stand to long-term nitrogen and sulphur deposition: a lesson from $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ in tree rings

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Recent studies recognised nitrogen deposition (Ndep) as one of the main drivers of the terrestrial carbon sink, together with elevated atmospheric CO_2 . This is particularly true for N-limited temperate and boreal forests, where Ndep can act as a low-level but long-term input of fertiliser. The scientific debate has been mainly focused on the quantitative contribution that Ndep makes towards tree growth and carbon sequestration by forests, while knowledge of underlying physiological mechanisms is still incomplete. In fact, whether Ndep can positively affect tree physiological traits (e.g., CO_2 assimilation, stomatal conductance, intrinsic water use efficiency, WUE_i , and growth), is likely to depend on other factors, such as current N status, deficiency of other nutrients, duration of Ndep exposure and interaction with other pollutants. For instance, simultaneous deposition of sulphur (S) may negate the potential beneficial effects of Ndep and accelerate tree decline.

How trees respond to N fertilization has been extensively investigated by previous studies. However, their relevance is limited because of: i) the short duration of the N fertilization experiment (with few exceptions), coupled with elevated doses applied; ii) the method of fertilization, mostly as direct applications to the soil, which does not completely simulate Ndep. In fact, part of the atmospheric N can be intercepted and retained by the tree canopy, representing a direct addition to plant metabolism.

By directly manipulating Ndep on the canopy, will the effects on tree physiological traits be larger than predicted from studies using soil applications? In the long-term, do we expect physiological adjustments to increasing levels of atmospheric N inputs, in relation to the balance between N, potassium or phosphorus availability? Does combined N and S deposition onto the canopy increase or decrease tree physiological performance?

In order to answer these questions, the effects of simulated wet N and S deposition on tree physiological traits were investigated in a young *Picea Sitchensis* (Bong.) Carr. plantation on an acid peat soil (Deepsyke forest, Scotland, UK). The Deepsyke experiment is unique in providing the opportunity to evaluate the long-term effects of frequent aerial N and S spraying onto a forest canopy for a period of 5 to 8 years.

The adopted approach was based on the measurements of stable carbon ($\delta^{13}\text{C}$), oxygen ($\delta^{18}\text{O}$) and nitrogen ($\delta^{15}\text{N}$) isotope composition in tree rings. We used $\delta^{13}\text{C}$ for assessing changes in WUE_i , while the degree of photosynthetic and stomatal responses to the different treatments were investigated using a conceptual model, combining variations of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$. The differences between canopy vs. soil N applications were evaluated as magnitude of changes in WUE_i and underlying mechanisms involved. Furthermore, physiological responses were also assessed in relation to leaf nutrient status. Finally, changes in tree internal N cycle in relation to canopy nitrogen uptake and the relative contribution to variation of WUE_i were detected by $\delta^{15}\text{N}$ in tree rings.