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Nitrification in tree canopies of European forests: evidence from oxygen isotopes in nitrate and microbial analyses in rainfall and throughfall water.

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There is mounting evidence demonstrating that fluxes and chemical composition of precipitation is substantially changed after passing through tree canopies, particularly in the case of atmospheric nitrogen (N) compounds, with important implications on forest N cycling. However, the processes underpinning those changes – beyond the leaf retention and/or leaching of N compounds - have been less investigated. In a previous study we provided isotopic evidence that biological nitrification in tree canopies was responsible for significant changes in the amount of NO_3^- from rainfall to throughfall across two UK forests at high nitrogen (N) deposition. This finding strongly suggested that forest canopies are not just passive filters for precipitation water and dissolved nutrients, and that the microbial life hidden within them can be responsible for transforming atmospheric N before it reaches the soil. We extended the isotopic approach at the

European scale, and combined it to next-generation sequence analyses with the aim of elucidating canopy nitrification and identify phyllosphere microbes responsible for it. Specifically, in this study we: 1) estimated the relative contribution of NO_3^- derived from biological canopy nitrification vs. atmospheric deposition by using $\delta^{18}O$ and $\delta^{17}O$ of NO_3^- in rainfall and throughfall water; 2) quantified the functional genes related to nitrification, and finally 3) characterized the microbial communities harboured in tree canopies (i.e., phyllosphere) and in the underlying soils for two dominant tree species in Europe (Fagus sylvatica L. and Pinus sylvestris L.) using metabarcoding techniques. We considered twelve sites included in the European ICP Forests monitoring network, chosen along climate and N deposition gradients, spanning from Fennoscandia to the Mediterranean. We will show that presence of nitrifying microbes (as assessed through qPCR) and their activity (as derived from $\delta^{18}O$ and $\delta^{17}O$) were detected in the tree canopies across most of the sites, and that canopy nitrification was significantly correlated with atmospheric N deposition. Finally, we will discuss differences in microbial community structure and composition across phyllosphere (and between the two tree species considered), water and soil samples in the investigated forests. Our study demonstrates the potential of integrating stable isotopes with microbial analyses to advance our understanding on canopy-atmosphere interactions and their contribution to N cycling.