

Combining multiple isotopes and metagenomic to delineate the role of tree canopy nitrification for European forests along a nitrogen deposition and climate gradient

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Forest canopies influence our climate through carbon, water and energy exchanges with the atmosphere. However, less investigated is whether and how tree canopies change the chemical composition of precipitation, with important implications on forest nutrient cycling. Previously, we provided for the first time isotopic evidence that biological nitrification in tree canopies was responsible for significant changes in the amount of nitrate from rainfall to throughfall across two UK forests (Guerrieri *et al.* 2015) at high nitrogen (N) deposition. This finding strongly suggested that bacteria and/or Archaea species of the phyllosphere were responsible for transforming atmospheric N before it even reaches the soil. Despite microbial epiphytes representing an important component of tree canopies, attention has been mostly directed to their role as pathogens, while we still do not know whether and how they affect nutrient cycling. Our study aims to 1) characterize microbial communities harboured in tree canopies for two of the most dominant species in Europe (*Fagus sylvatica* L. and *Pinus sylvestris* L.) using metagenomic techniques, 2) quantify the functional genes related to nitrification but also to denitrification and N fixation, and finally 3) estimate the NO₃ derived from biological canopy nitrification vs. atmospheric NO₃ by using δ¹⁵N, δ¹⁸O and δ¹⁷O of NO₃ in forest water. We considered i) twelve sites included in the ICP forests long-term European monitoring network, chosen along a climate and nitrogen deposition gradient spanning from Fennoscandia to the Mediterranean and ii) a manipulation experiment where liquid N additions were carried out both above tree canopies and over the soil. We will present results regarding microbial diversity in the phyllosphere, water samples (rainfall and throughfall) and soils over the gradient. Furthermore, we will report differences between the two investigated tree species for the phyllosphere core microbiome in terms of relative abundance of bacterial and Archaea classes and those species related to N cycling. Finally we will assess whether there are differences among species and sites in the number of functional genes related to N cycling and how they are related to the N deposition and/or climate.

Guerrieri R, Vanguelova EI, Michalski G, Heaton THE, Mencuccini M (2015). *Isotopic evidence for the occurrence of biological nitrification and nitrogen deposition processing in forest canopies*. *Global Change and Biology* 21 (12): 4613-4626.