Leaf microbes and nitrification in the canopies of European forests: evidence from stable isotopes, meta-barcoding and qPCR

Rossella Guerrieri[1], Anna Barceló[2], Stafania Mattana[1], Joan Calíz[3], David Elustondo[4], Sofie Hellstein[5], Giorgio Matteucci[6], Päivi Merilä[7], Greg Michalski[8], Manuel Nicolas[9], Elena Vanguelova[10], Arne Verstraeten[11], Peter Waldner[12], Mirai Watanabe[13], Josep Peñuelas[1,14], Maurizio Mencuccini[1]

[1] Centre for Ecological Research and Forestry Applications, CREAF, c/o Universidad Autonoma de Barcelona, Edificio C, 08290 Cerdanyola, Barcelona (Spain); [2]Servei de Genòmica i Bioinformàtica, IBB-Parc de Recerca UAB - Mòdul B, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona (Spain); [3]Centre of Advanced Studies of Blanes, CEAB-CSIC, Spanish Council for Scientific Research (Spain); [4]Universidad de Navarra, Laboratorio Integrado de Calidad Ambiental (LICA), Campus Universitario, 31080, Pamplona (Spain);[5] IVL Swedish Environmental Research Institute, P.O. Box 5302, SE-400 14 Göteborg, Sweden; [6]CNR-ISAFOM, Via Patacca, 85, 80056 Ercolano, Napoli, Italy; [7]Natural Resources Institute Finland (Luke), Oulu, P.O. Box 413, 90570 Oulu, Finland; [8]Department of Earth, Atmospheric, and Planetary Sciences, Purdue University, 550 Stadium Mell Drive, West Lafayette, IN 47907, USA; [9]ONF, Office National des Forêts, Departement Recherche et Developpement, Bâtiment B, Boulevard de Constance, F-77300 Fontainebleau, France; [10]Centre for Ecosystem, Society and Biosecurity, Forest Research, Alice Holt, UK;[11]Research Institute for Nature and Forest, Gaverstraat 4, 9500 Geraardsbergen (Belgium);[12]WSL, Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf (Switzerland); [13]National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan;[14] CSIC, Global Ecology Unit CREAF-CSIC-UAB, 08193 Bellaterra, Barcelona (Spain)

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. Recently, we provided for the first time 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 bacteria and/or Archaea species of the phyllosphere are responsible for transforming atmospheric N before it reaches the soil. 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 meta-barcoding techniques, *2)* quantify the functional genes related to nitrification, and 3) estimate 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 forest water. We considered twelve sites included in the European ICP forests monitoring network, chosen along a climate and N deposition gradients, spanning from Fennoscandia to the Mediterranean. Preliminary results indicate differences in bacterial structure and composition across phyllosphere (and between the two species considered), water samples and soil. Moreover, *presence of nitrifying bacteria and Archea* (as assessed through qPCR) and *their activity* (as derived from $\delta^{18}O$ and $\delta^{17}O$) were detected across all the samples, regardless the level of N deposition.