The atmosphere as an inoculator of a functional phyllosphere microbiota

Frédéric Mathonat[1], Anne-Catherine Lehours[2], Antoine Vergne[2], Barbara Ervens[1], and Pierre Amato[1]

[1] Institut de Chimie de Clermont-Ferrand, CNRS, Universite Clermont Auvergne, Clermont-Ferrand, France [2] Laboratoire Microorganismes: Génome et Environnement, CNRS, Université Clermont Auvergne, Clermont-Ferrand, France

e-mail : frederic.mathonat@uca.fr

Context and objectives

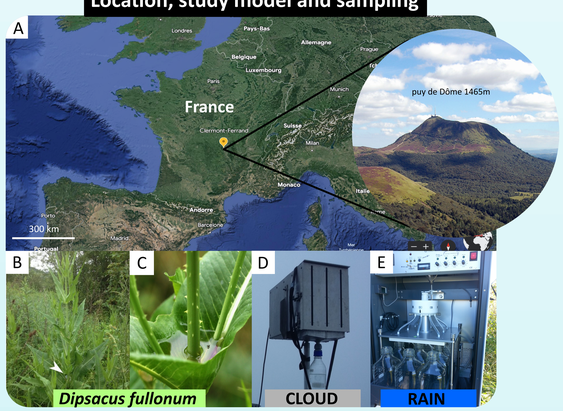
- The aerial part of plants (phyllosphere) are in constant interaction with the atmosphere.
- Some plants, like *Dipsacus fullonum* have specialized structures to collect rainwater, the phytotelms.
- Rainfall supplies these reservoirs with water and inoculates them with a particular microbiota

Objective : Compare structural and functional microbial diversity in the phyllosphere and the atmosphere.

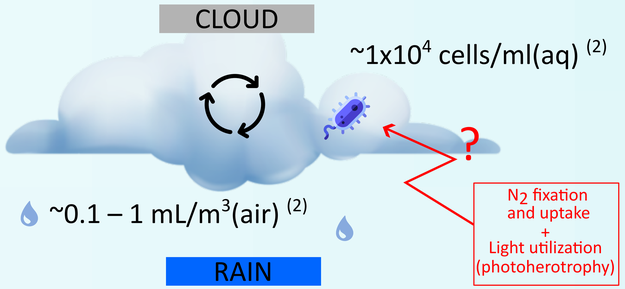
Experimental design

- DNA extraction
  - qPCR (rRNA 16S, *nifH*, *pufM* gene) → **analyses of functional genes**
  - MiSeq sequencing (rRNA 16S gene) → **analysis of the diversity**
- HPLC → **quantification of the pigment (bacteriochlorophyll *a*)**
- Microorganism
  - Diazotroph (capable of fixing atmospheric nitrogen (N_2)) => *nifH* gene
  - Anoxygenic Phototrophic Bacteria (APB) => *pufM* gene / bacteriochlorophyll *a*
- Aerosol particle

Location, study model and sampling



(A) : Map of sampling locations / puy de Dôme mountain
(B/C) : *Dispacus fullonum* / Phytotelm
(D) : Cloud droplet impactor
(E) : Automated rain collector



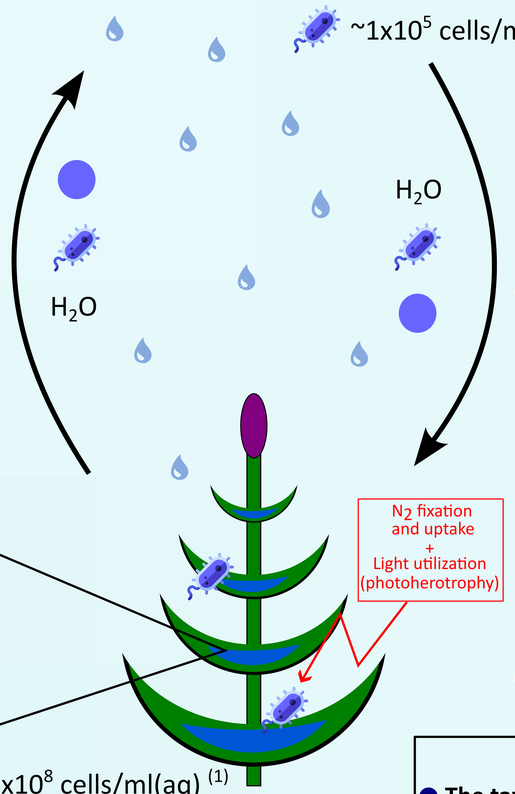
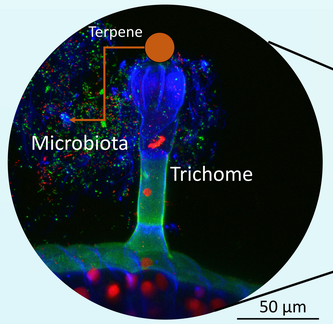


Figure 1 : Trichomes, plant structure inside the phytotelm. [1]

Trichomes, glandular hair-like structures found in the phytotelms at high density.

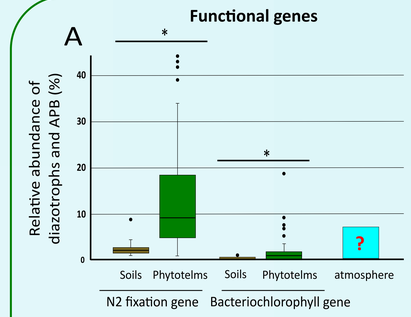


Confocal microscopy photograph after staining

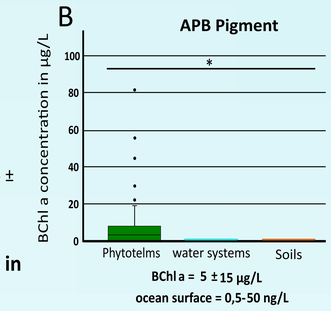
★ **Microbial hot spot around trichomes**

★ **Possible regulation of microbiota by the plant via terpenes toward diazotrophs/APB.**

Figure 2 : Diazotrophy and anoxygenic phototrophy [1]



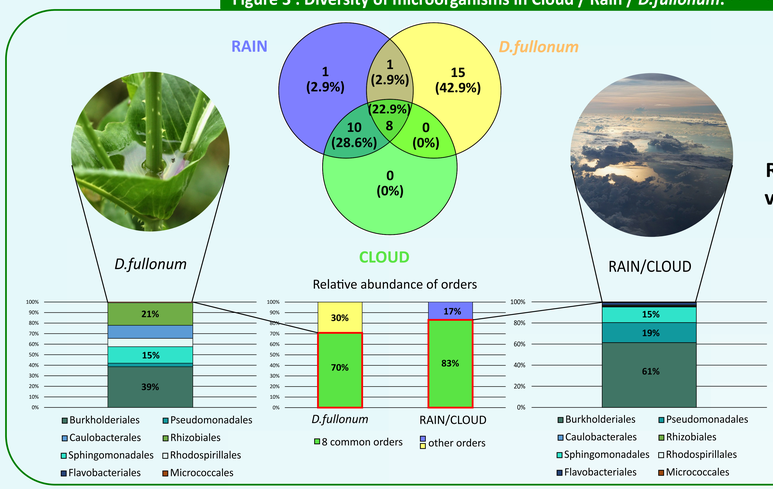
Diazotrophs represent about 10-20% of the total microbial population, but can reach up to 40% in phytotelms (A).



Bacteriochlorophyll *a* , the photosynthetic pigment typical of APB (5 ± 15 µg/L), which is 1 000 times higher than in other aquatic systems (B). In addition, APB can also be nitrogen fixers.

★ **High population of diazotrophs and/or APB in phytotelms of *D.fullonum*.**

Figure 3 : Diversity of microorganisms in Cloud / Rain / *D.fullonum*. [1] and [2]



★ **Burkholderiales, Pseudomonadales, Sphingomonadales and Rhizobiales represent the vast majority of microbial diversity in both the phyllopshere and the atmosphere**

★ **These orders are known to include diazotrophs and/or APB**

Outcomes

- **The taxonomic microbial diversities in *D. fullonum* phytotelms and in the atmosphere are highly similar.**
- **In phytotelms, trichomes likely stimulate certain populations of ABP and/or diazotrophic bacteria**

Perspectives : Is the plant microbiota and its functions beneficial for the plant ? Are functions of phototrophy and diazotrophy advantageous for the survival of microorganisms in the atmosphere ?

(1) A. Vergne, E. Giraud, A. Camuel, C. Bardot, H. Billard, C. Bouquet, B.Corbara, D. Gully, F.Mathonat, C. Jeanthon, I. Mary, J. C. Caissard, A.C. Lehours. 2023. The Pharaoh's snakes of the common Teasel : New insights into Francis Darwin's

(2) R. Péguilhan, L. Besaury, F. Rossi, F. Enault, J.L. Baray, L. Deguillaume, P. Amato. 2021. Rainfalls sprinkle cloud bacterial diversity while scavenging biomass, FEMS Microbiology Ecology, Volume 97, Issue 11, fiab144