Terrestrial Hints in the Prospection of Microbial Life in Venus' Clouds

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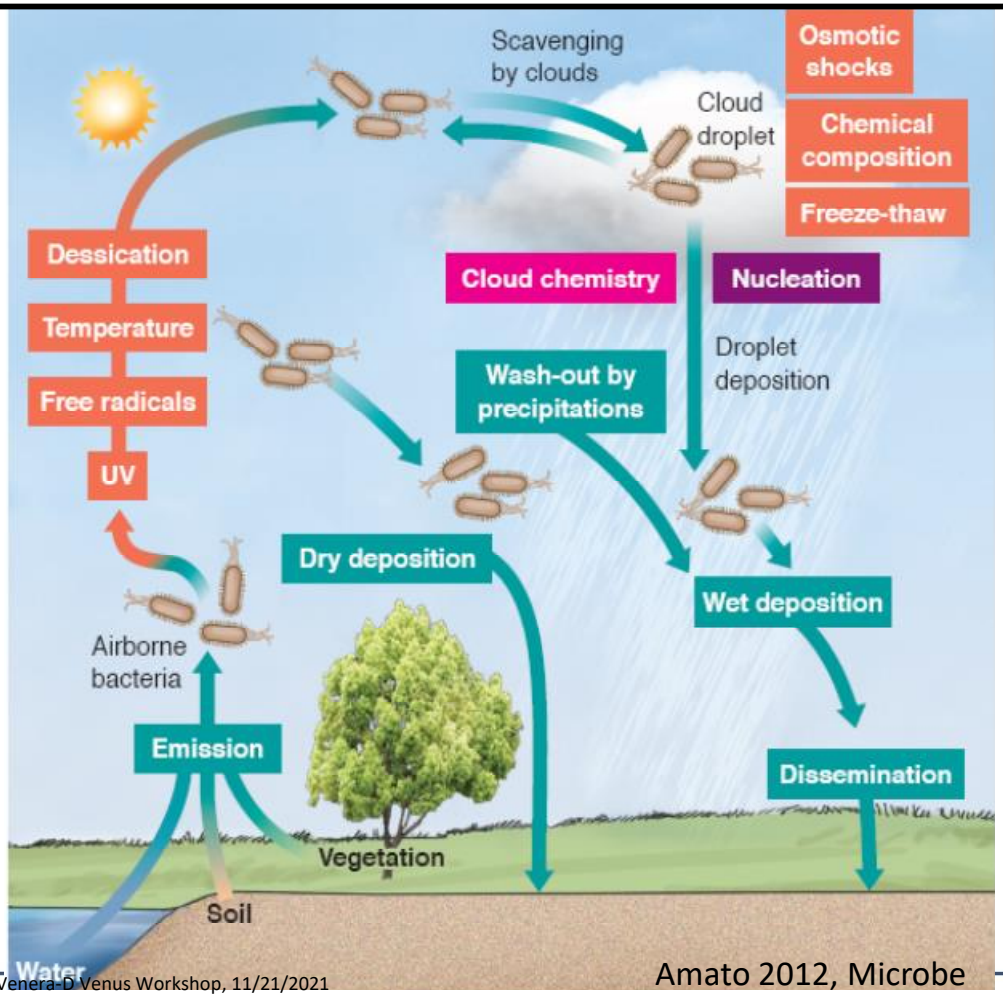
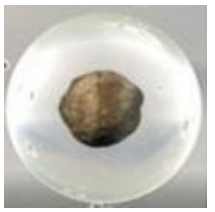
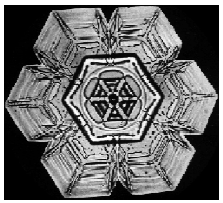
Motivations for investigating microbes in clouds on Earth

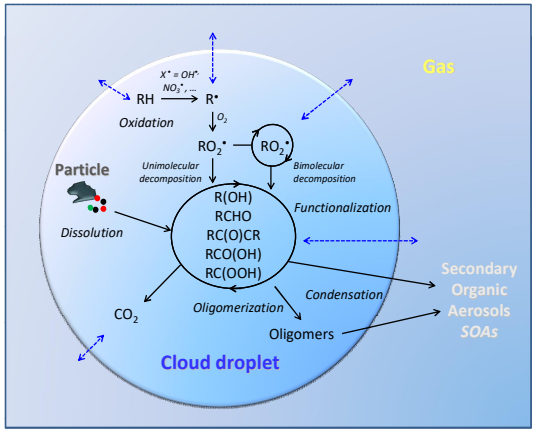
- How atmospheric transport affects microbial ecology? How do this biome functions?
- How the presence of (living) microbes influences atmospheric processes (chemistry, nucleation…)?



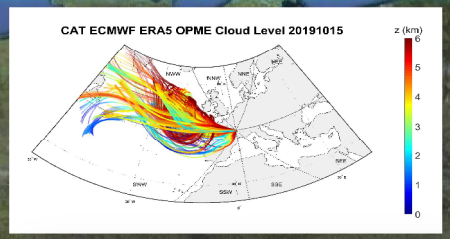
Amato 2012, Microbe

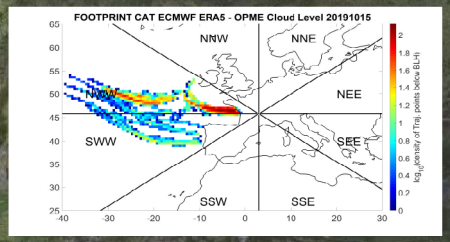


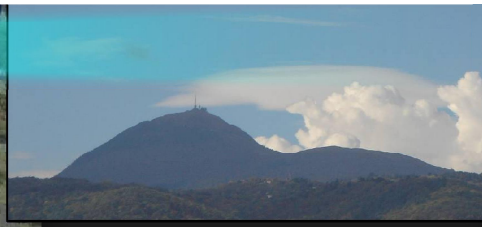


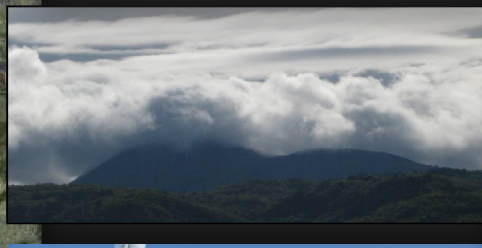


Sampling sites









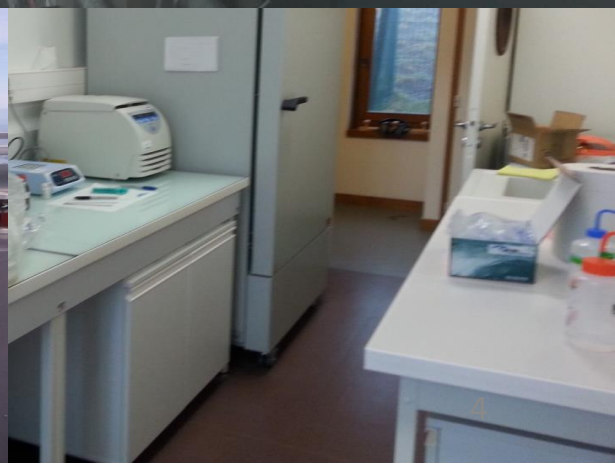
FRANCE

Clermont-ferrand

Puy de Dôme (1465m)

Data SIO, NOAA, U.S. Navy, NGA, GEBCO
© 2010 Cnes/Spot Image
Image © 2010 GeoContent
Image © 2010 TerraMetrics
46°18'19.93" N 3°48'47.18" E élév. 1593 ft

Puy de Dôme Mountain Atmospheric Observatory



Samplers

Aerosols (Quartz filter, HiVol PM10)

Clouds (impactors)

Aerosols and clouds (High Flow Rate impingers)

Precipitation (Eigenbrodt automated sampler)

Aerosols (Coriolis)

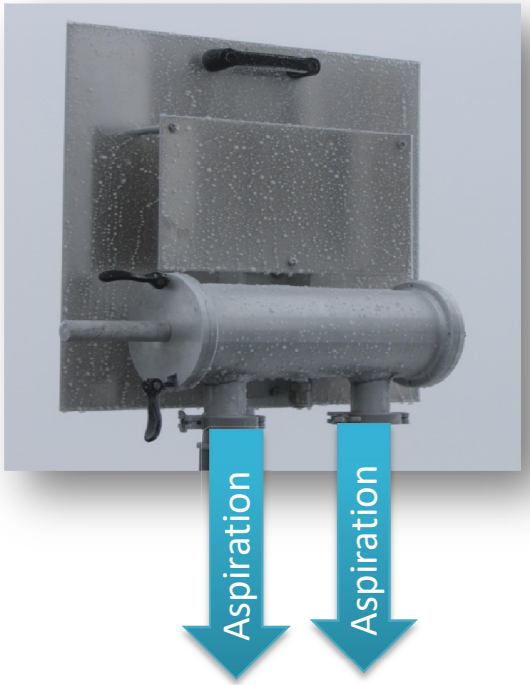
Aerosols (SKC biosamplers)

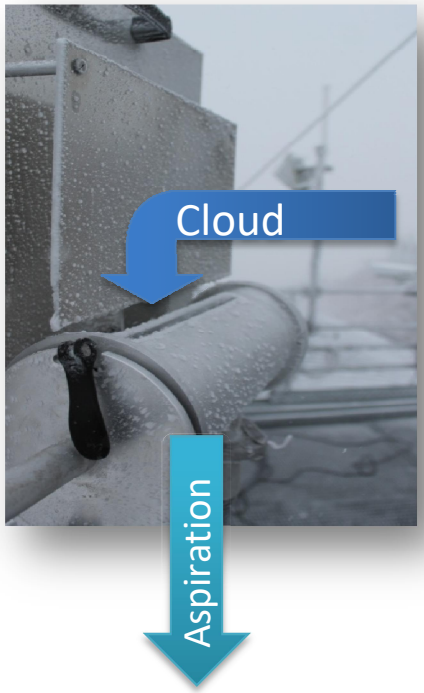
Snow cover

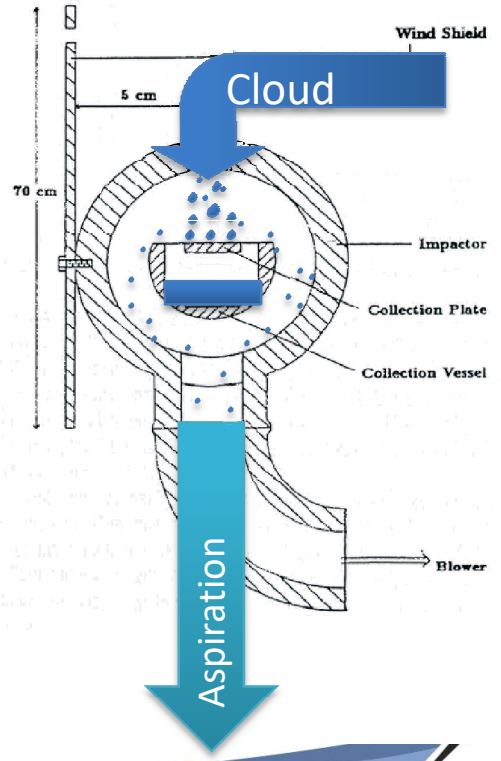
Sampling

Cloud droplet impactors

- Cutt-off diameter ~7 µm (droplets)
-Sampling rate of ~100 mL of water/h @ LWC=0.3g/m^3
- Decontamination by autoclave

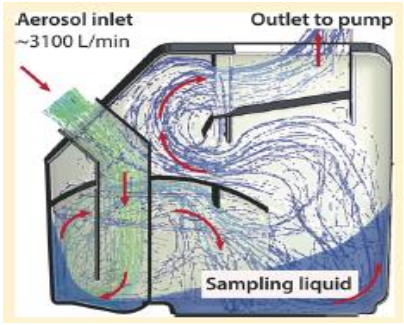




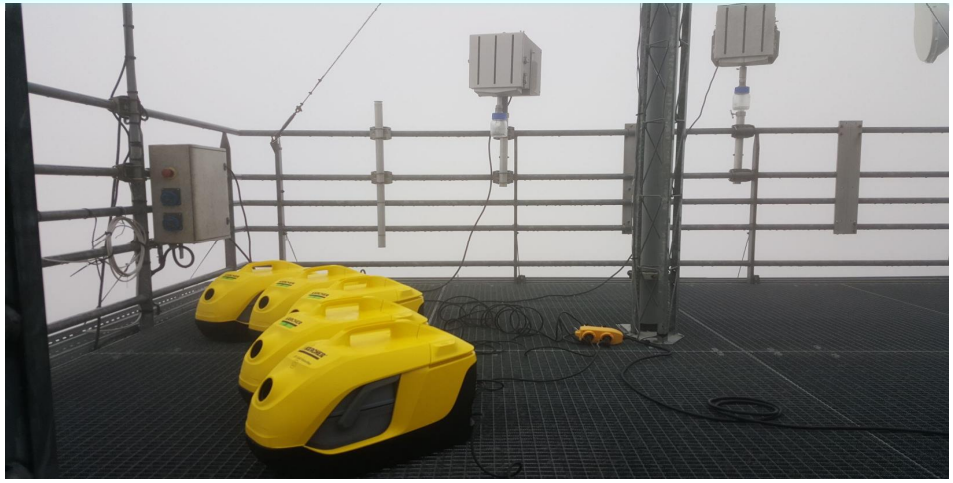


Sampling

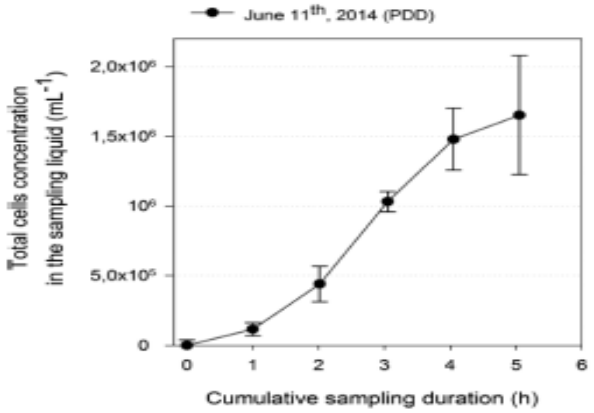
High-Flow rate impinger

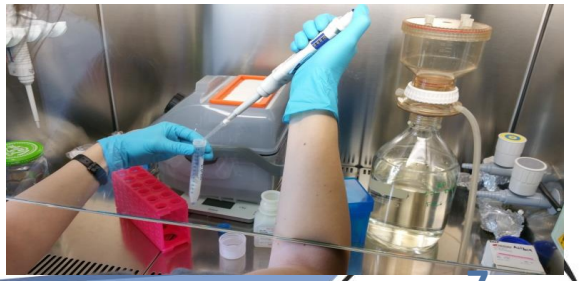


Šantl-Temkiv et al., Environ. Sci. Technol. 2017



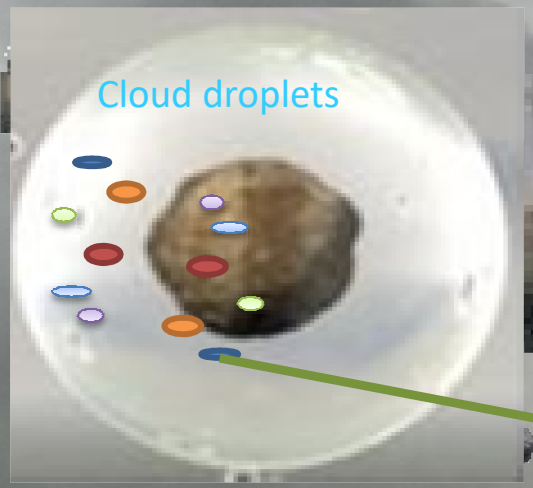
- High air flow : rapid accumulation of material in liquid solution (buffer, fixating agents, etc)
- Suitable for aerosols and clouds
- Decontamination UV





Clouds

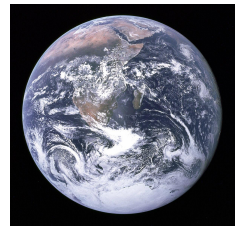
- Water condensed on aerosols
- Droplets or ice crystals (~10 µm diameter)
- ~1 ×10^{5} – 1 ×10^{9} droplets m^{-3} (0.06 – 0.6 g of condensed water m^{-3})
- Typical time scale = 1 h – 1 d
- Earth's radiative budget/ Atmospheric chemistry



- Acidic (pH = 3 - 7) and oxydative environment
- Low conductivity (~1 – 300 µS cm^{-1})
- Ions from gas and solid phases (NO_3^-, SO_4^{2-}, Na^+, NH_4^+, Cl^-, C_{orga})
- Dissolved Organic compounds
- Reactivity and transfers between phases - Photochemistry
- Non-soluble particles

MICROORGANISMS

Investigating microbes in clouds



→ Typical droplet diameter in clouds = ~7 µm

Å~7 x10^8 droplets/mL (or 2.4 x10^8 droplets/m^3 of cloud at LWC= 0,34 g/m^3)

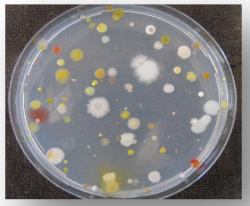
Å~10^4-10^5 cells/mL

➔ 1 cell every 10,000 droplets!

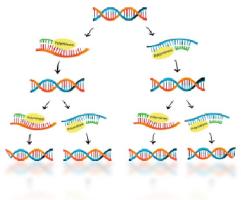
High speed photo of fog droplets
Ben pcc at English Wikipedia

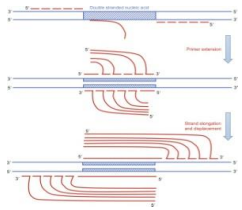
Requirements for investigating microbes in clouds

- **Need for sensitive and specific methods:**
  - Signal amplification: cultures, (q)PCR, MDA
  - Analysis after treatment: flow cytometry/fluorimetry (specific stains), luminescence (ATP)
  - Direct analysis: flow cytometry/fluorimetry (pigments), Mass spec (molecular profiles)

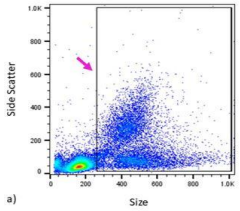


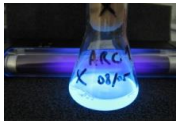
Incubation based methods



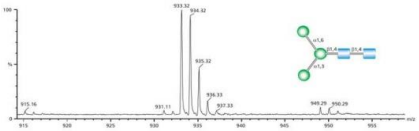


DNA based methods





Light based methods



Molecular based methods

Online *vs* offline analyses

- **Offline**
  - Allow analyses of large sample volumes, preconcentration
    → increased sensitivity
  - Need for accumulating samples → how to keep it intact? What sampler?
  - Require many manipulations and (still) hands...
    → probably not feasible on Venus yet!

Filtration? Impaction?
Electrostatic precipitation?
Impingement?

- **Online**
  - Need for high-throughput analysis (thousands of particles)
  - Highly sensitive direct method required
  - Problems of specificity (false positives)
  - Possibility of derivating signals (add stain, reagent, etc)?

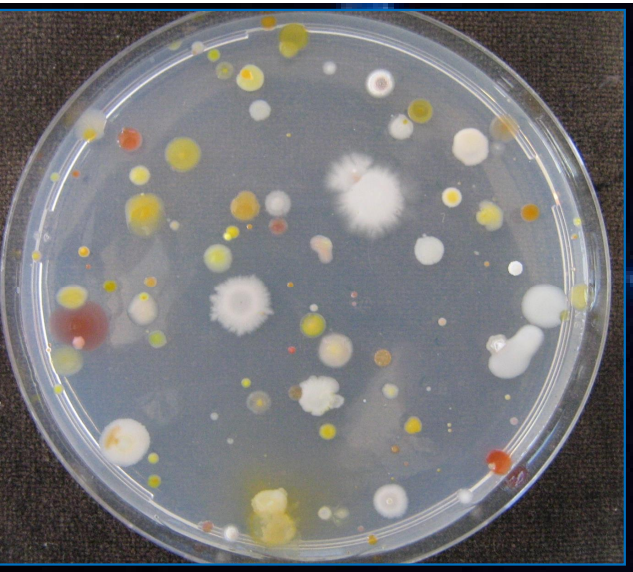
Laser detection (fluorescence)

Airborne particles

liquid suspended particles (flow cytometry)

Aerosol mass spectrometry

- **What are we looking for?** Cells? Molecular Biomarkers? Imprints attesting of biotic processes?



Microbial culture of cloud water at 15°C
(R2A agar medium)

Cyanobacterium sp.

Chloroidium saccharophilum (Gree
Algae)

Enrichment cultures of cloud water
(BG11 and MWC media)

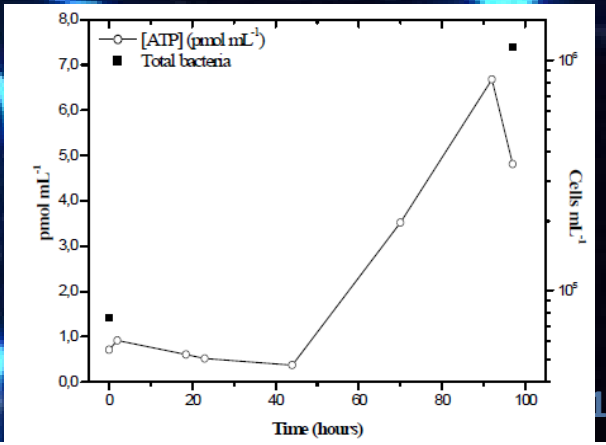
© Kevin Dillon, Rutgers Univ.

Epifluorescence microscopy of cloud water (DAPI staining x945)

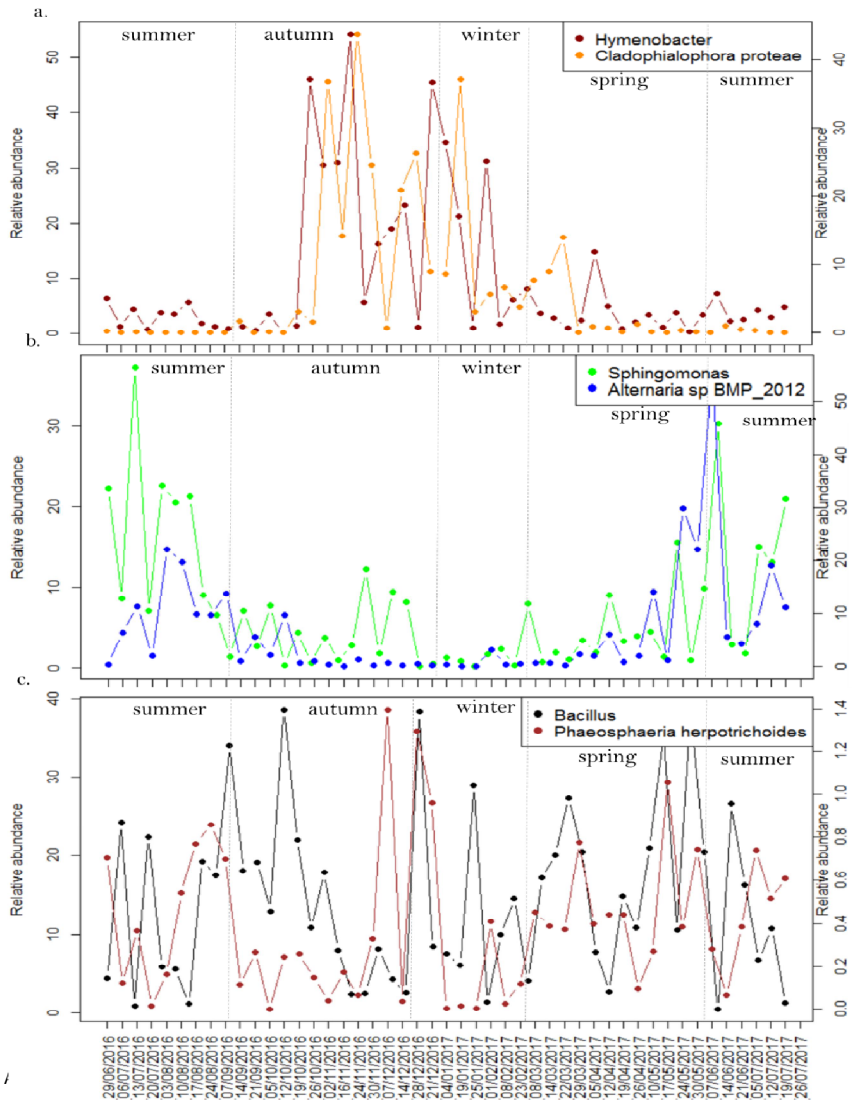
-~10^3-10^5 total cells /mL (flow cytometry)
- High diversity

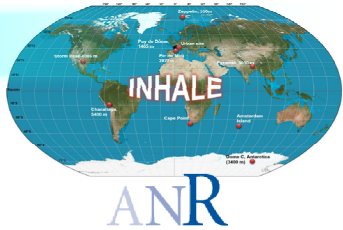
- < 1% cultivable (R2A agar, 5°C and 17°C)

- Intact cloud water incubated



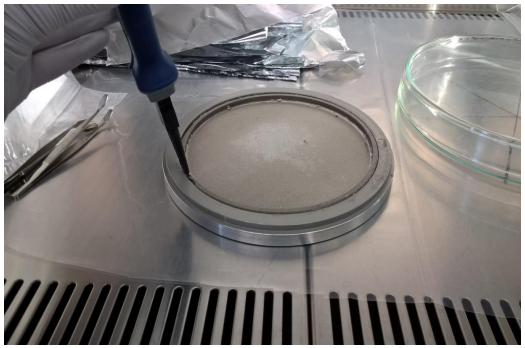
Bacteria dynamics in aerosols





Temporal variability of bacteria in aerosols
(1-week resolution; 1.5 yr) (16S rRNA gene amplicon sequencing and qPCR)

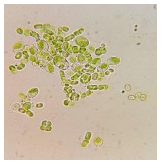
Tignat-Perrier et al., SciTotEnv 2020



Microbial assemblages in the atmosphere more or less mirror the microbial diversity at the surface (landscape/region/continental scale)

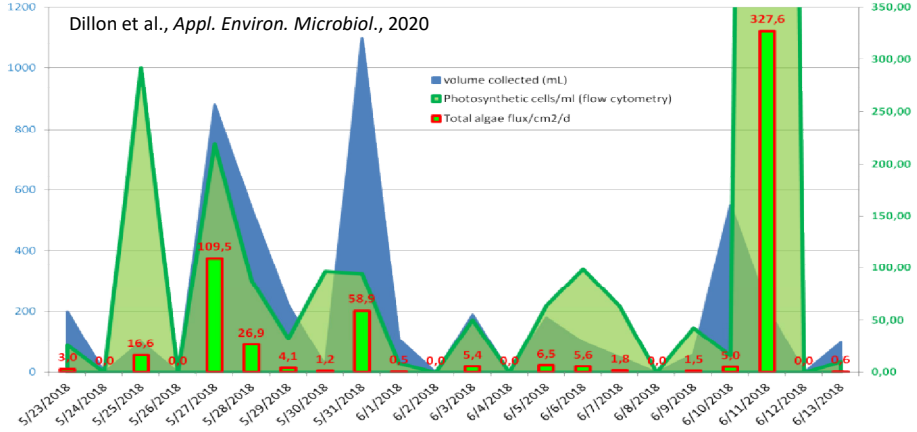
Monitoring of microbes of interest in precipitation

Inputs of photosynthetic microorganisms to surface ecosystems



Wet deposition flux ~ 1 – 350 photosynthetic cells/cm2/d

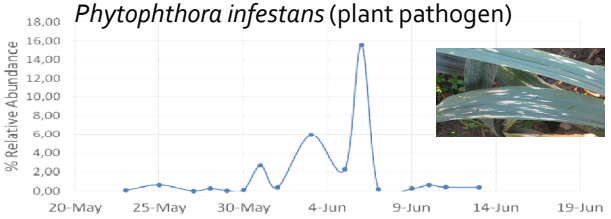
Flow cytometry, cultures and amplicon sequencing

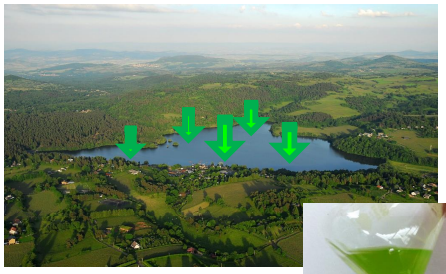


Aydat Lake = 65 ha (=6.5 x10^9 cm2)

➔ **~10^9 -10^{12} photosynthetic cells enter the lake with precipitation/day**

Phytophthora infestans (plant pathogen)





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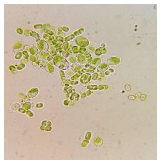

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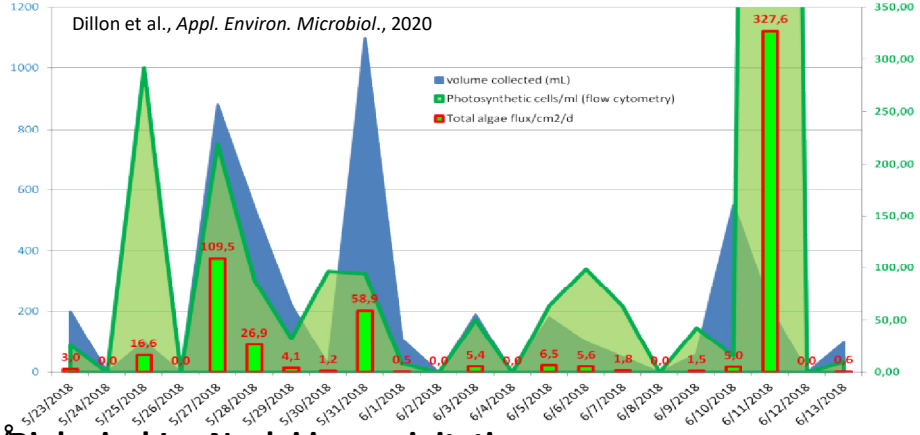

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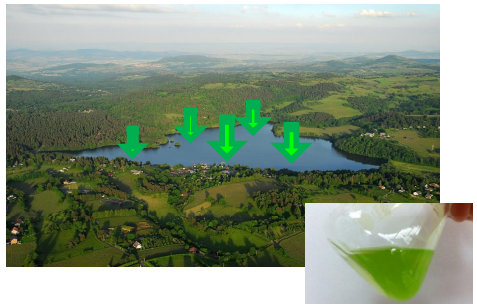
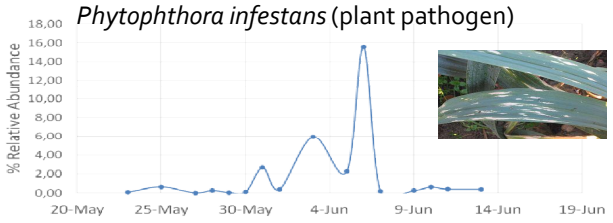
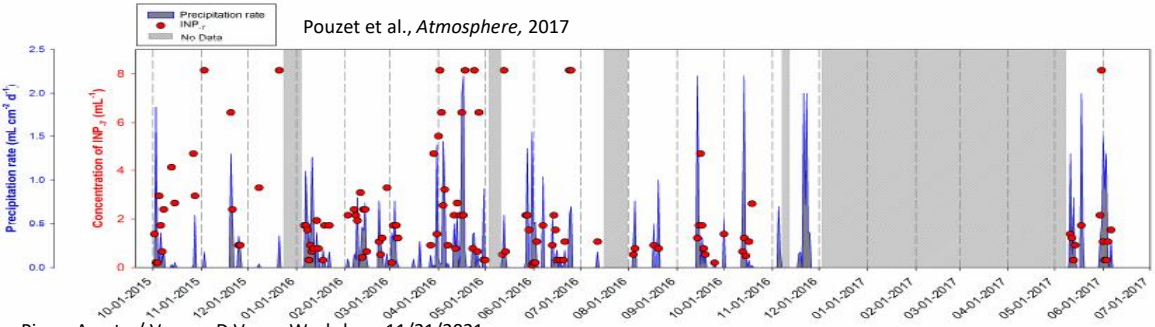
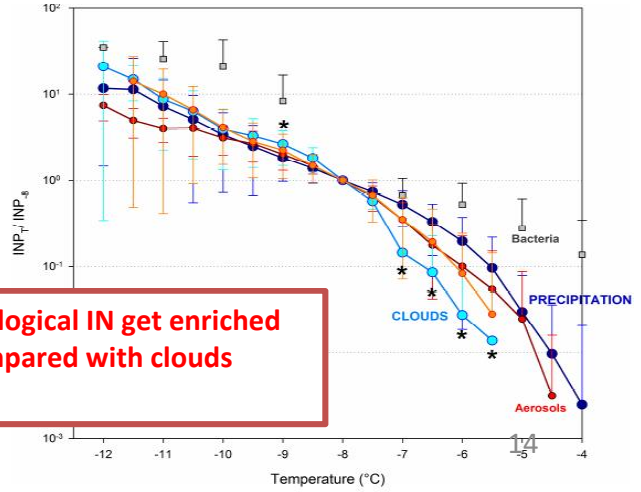




Biological Ice Nuclei in precipitation

1.5 yr of systematic measurements of INA in precipitation





The most active biological IN get enriched in precipitation compared with clouds (bioprecipitation)

Bacteria along the water cycle from clouds to precipitation

ÅBacteria community profiling in coordinated sampling of clouds and precipitation

- Wet deposition flux 10^7-10^9 bacteria cells/m²/h

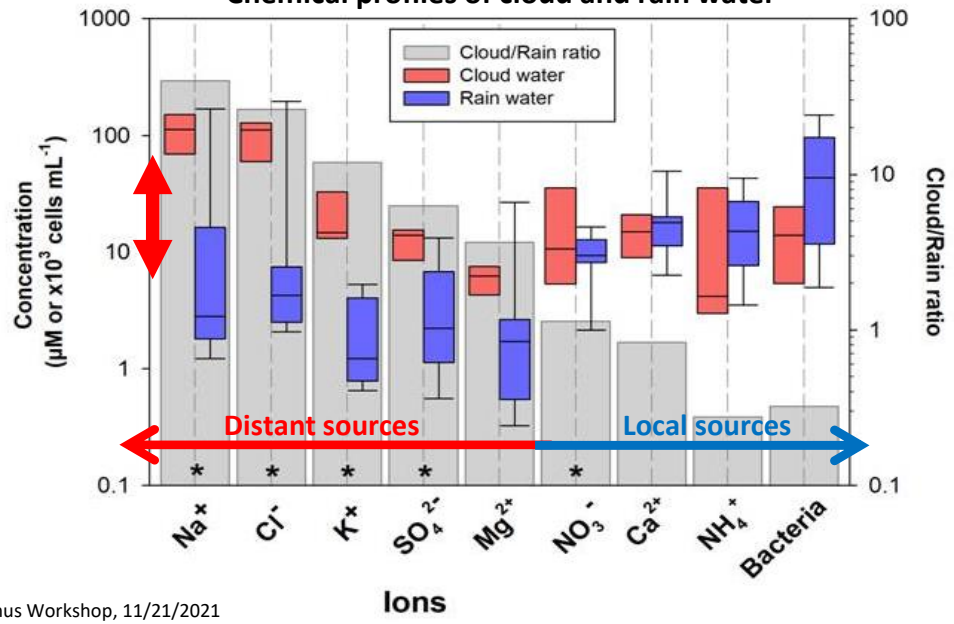
Certain bacteria get enriched/depleted in precipitation versus their source cloud, as do ions:

➔ Contribution of local vs distant sources

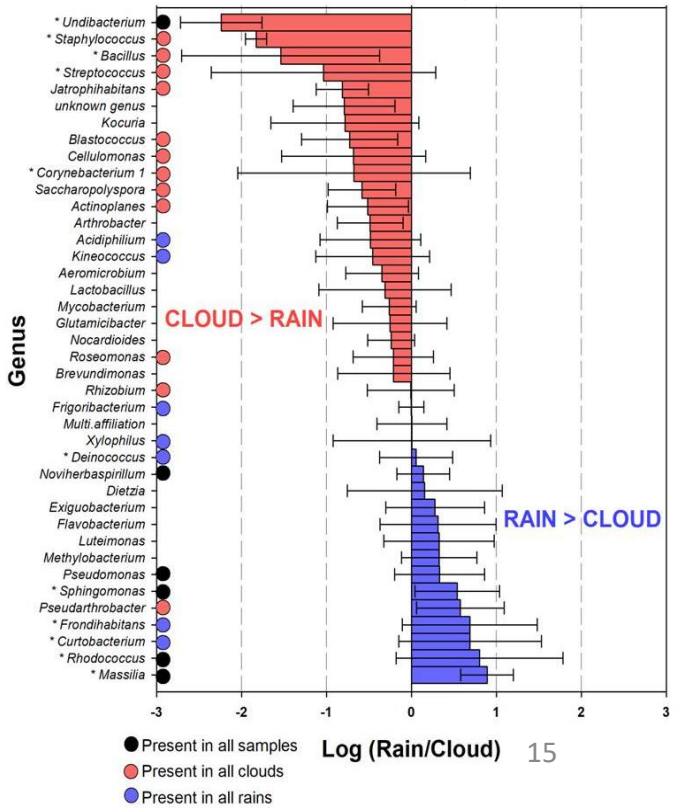
➔ Partitioning possibly related with biological traits (spore-forming, INA, surfactants, ...)

Péguilhan et al, FEMS Microbiol. Ecol. 2021

Chemical profiles of cloud and rain water



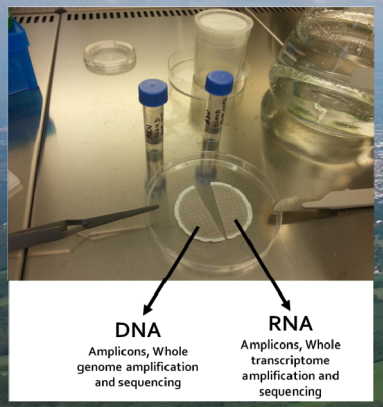
Distribution of bacterial species (16S rRNA gene amplicons)

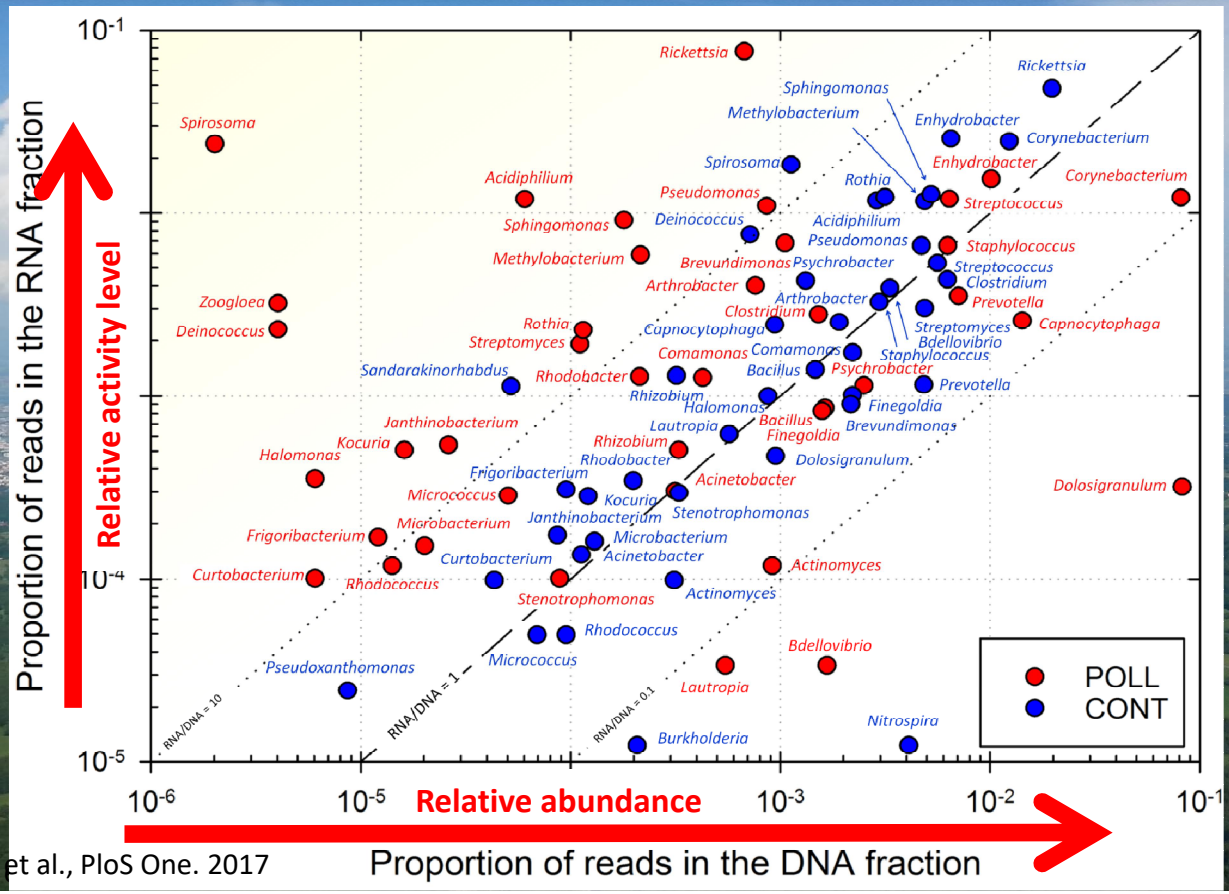


What taxa are metabolically in clouds?

16S rRNA (gene) amplicon sequencing

- Abundant active: (*Rickettsia*), *Sphingomonas* , *Pseudomonas*, *Methylobacterium*, *Enhydrobacter*, *Acidiphilum*
- Rare active: *Deinococcus*, *Spirosoma*
- Abundant not active: *Capnocytophaga*, *Dolosigranulum*





Amato et al., PloS One. 2017

Functioning of cloud communities (metatranscriptomics)

Multiple Displacement Amplification of whole metagenomes and metatranscriptomes extracted from fresh cloud water sampled in RNA Later

➔ **Metabolic functioning in bacteria is largely oriented toward the fight against oxidants, cold, osmotic shocks**

Amato et al., SciRep 2019

- **Transcription/translation activities**: maintenance of metabolic activity

- **Oxidative stress response and detoxification of free radicals and their precursors: enzymes** (catalase, SOD, etc) **and ROS scavengers** (glutathione etc)
- **Lipid modification in membranes** (cold)
- **Osmoprotectants/cryoprotectants synthesis** (trehalose, glutathione, glycine betaine...)

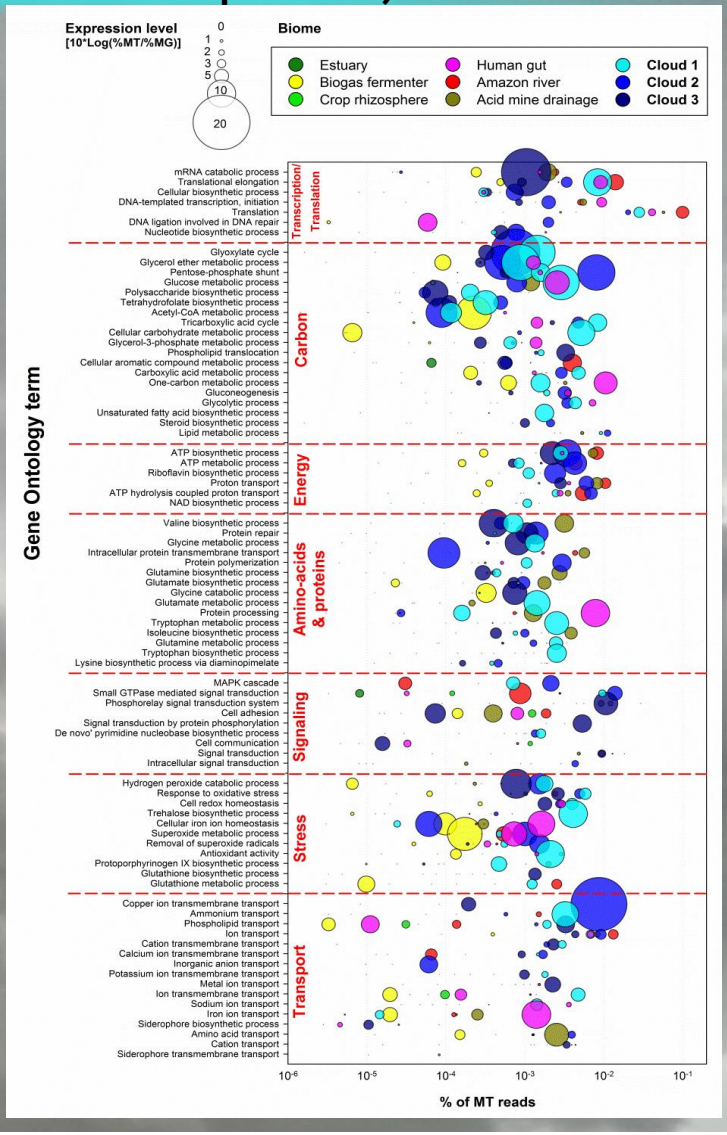
- **Energy demanding processes fueled by central metabolism pathways involved in stress response** (glyoxylate, pentose phosphate, favoring NADPH); production of energy by oxidative phosphorylation

-Probable **utilization of C1 compounds (formate, formaldehyde, methanol, methane) and ammonium**, among others (acetate, succinate)

- **Elevated binding and transmembrane transports,** including siderophores (iron)

- **Elevated activity of transduction/signaling by phosphorylation level transducers (« sensing organs » in microorganisms)**

- **Synthesis of polysaccharides** (EPS, biosurfactants,...)



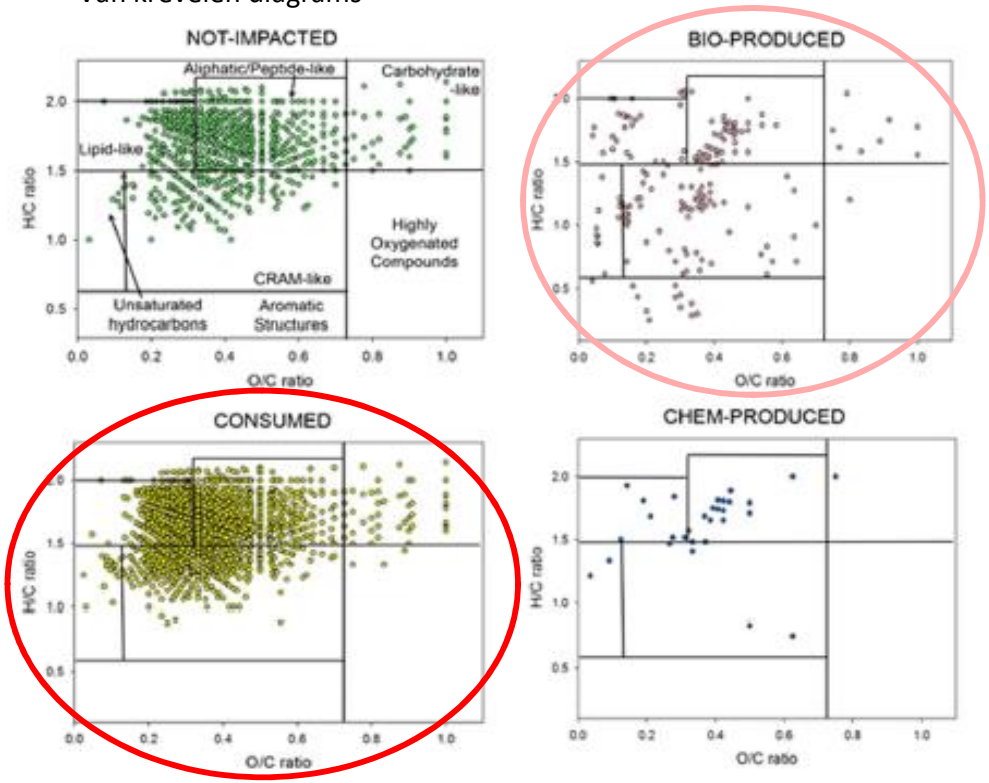
Chemical imprint of biological activity on cloud water chemical composition

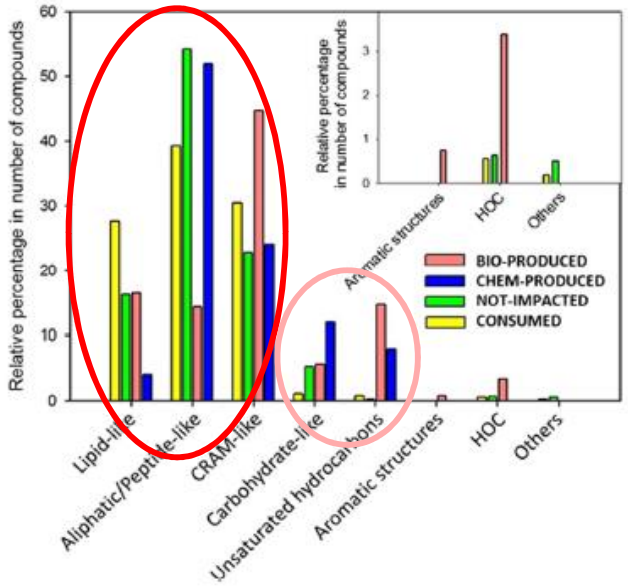
Chemical imprint by FT-ICR-MS

Bianco et al, SciRep 2019

Bulk incubations of cloud water incubated w and w/o endogeneous microorganisms (60h @15°C)

Van krevelen diagrams



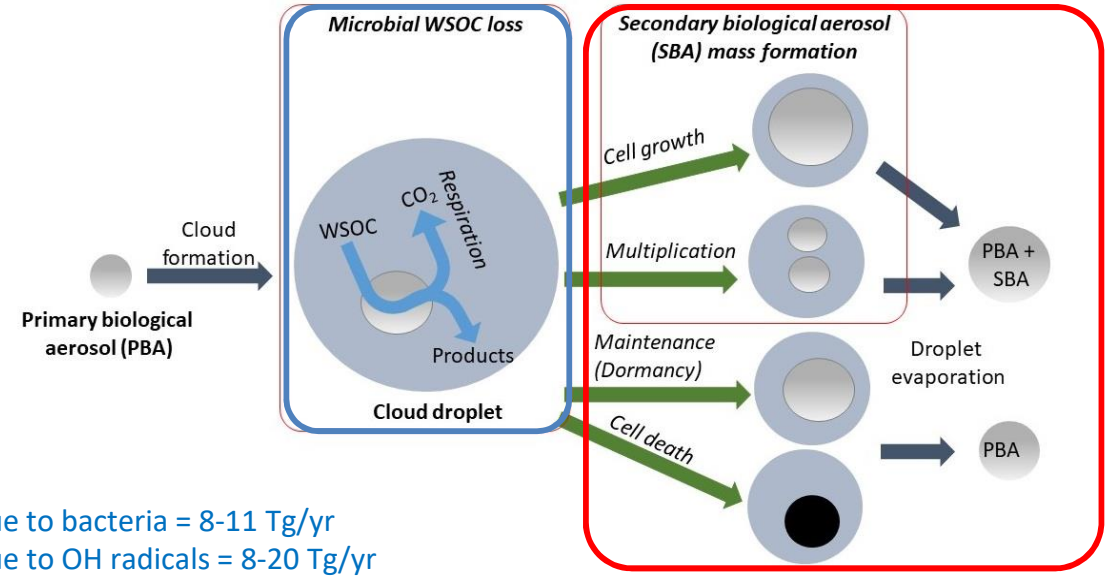


CRAM = Carboxylic-rich alicyclic molecules
HOCs = highly oxygenated compounds

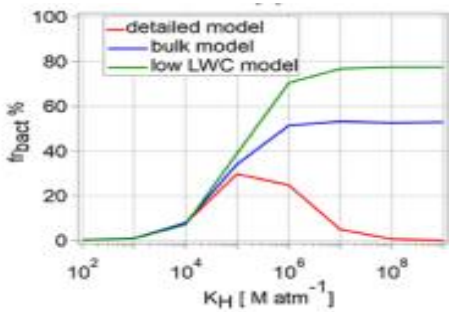
Microbial activity in cloud water (incubated as bulk) influences chemical composition

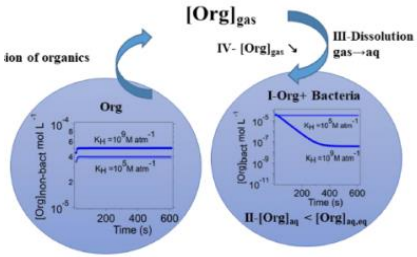
Modeling: bacteria multiplication and carbon consumption in clouds

➔ Total « Secondary Biological Aerosols » production = 3.7 Tg/yr
➔ **Production is equivalent to emissions (SBA ~ PBA)**
➔ **< 1 cellular division expectable during cloud's lifetime**



➔ Total WSOC loss due to bacteria = 8-11 Tg/yr
➔ Total WSOC loss due to OH radicals = 8-20 Tg/yr
➔ **Equivalent theoretical loss of WSOC by bacteria as by OH radicals**
➔ **Mostly important for compounds with average solubility and volatility (formate, acetate, etc) for efficient tranfers between droplets**





Ervens & Amato , Atmos. Chem. Phys. 2020
Khaled et al., Atmos. Chem. Phys. 2020

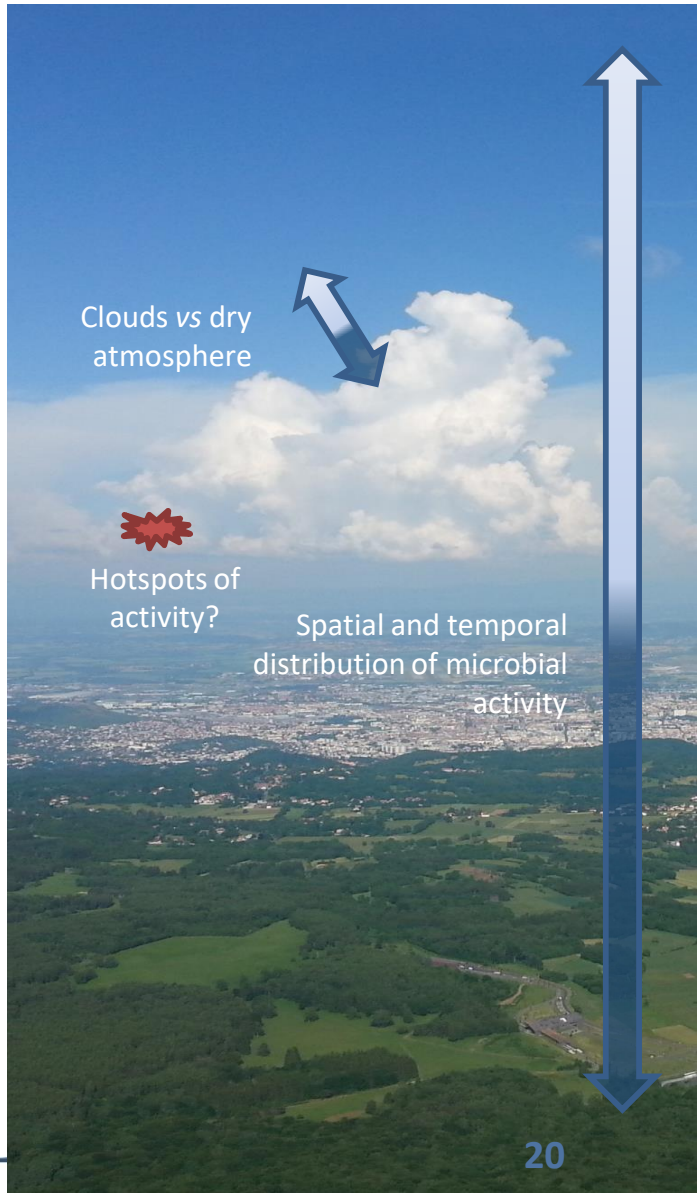
On Earth,

We accumulated knowledge over the last decades...

- **Microbial assemblages mirror to some extent the microbial diversity at the surface**
- **Certain microorganisms sustain metabolic activity in clouds**
- **Bulk cloud water supports microbial growth**
- **Microbial activity in cloud water influences the fate of some chemical compounds**
- **Compounds with medium solubility in water and volatility are best candidates for sustaining microbial metabolism/growth**
- **Certain microbial traits influence survival, maintenance and deposition**

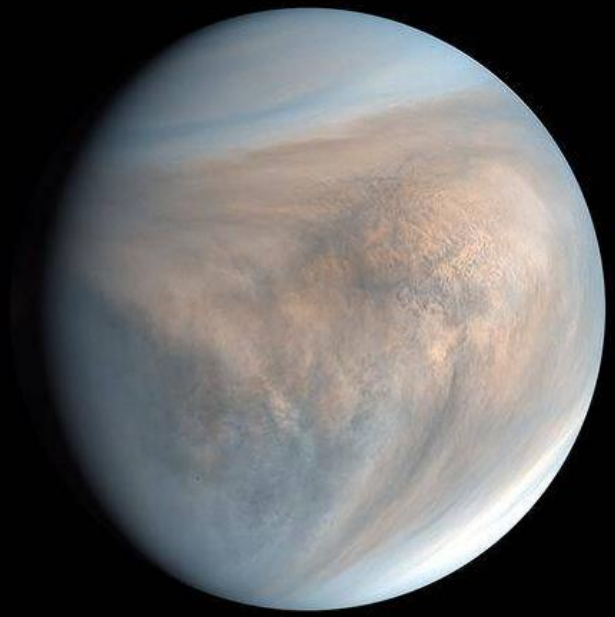
But there are still many open questions:

- **Do and how microbial activity distributes in the atmosphere?**
  - Random *vs* determined distribution of biological functions ?
  - Is microbial activity restricted to clouds?
  - Are there hotspots of microbial activity?
- **What impacts on environmental cycles?**
  - Geochemical cycles
  - Hydrological cycles
  - Ecosystem regulations
- **Do and how the atmosphere contributes driving microbial evolution ?**
  - Environmental filtering and links with biological traits?
  - Influence on the spread of genetic innovations?
  - Influence on ecosystem resilience and adaptation

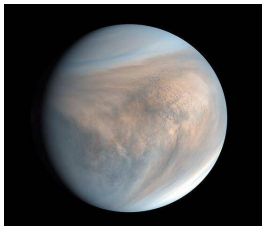


EARTH **VENUS**

General considerations for investigating microbial life in Venus' clouds



(Encouraging) considerations about the eventual presence of life in Venus' clouds



- **The theory that life in restricted to clouds on Venus implies:**

ÅLikely a lower diversity than in Earth's clouds due to:

- ➔ **The unicity of habitats** on the planet (= restricted to clouds!)
- ➔ very **constraining conditions** from a terrestrial point of view

ÅProbably higher biomass due to a permanent race against gravity, which implies:

- ➔ **Generation times shorter than cloud lifetime** or cell's residence time
- ➔ **Mixing of droplets** to avoid sedimentation of sister cells along with their mother cell

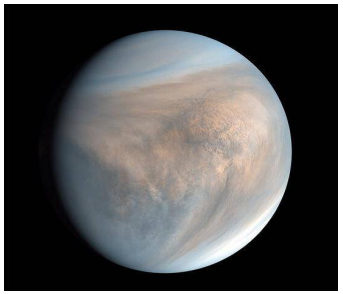
ÅSpecific trophic modes, as we know them: what are we seeking for?

- ➔ **Acidophiles**: Euryarchaea *Picrophilus* species thriving at pH 0 and 65°C (Fütterer et al 2004, PNAS)
  Microalga *Galdieria sulphuraria* able to grow at pH 0-4 and T° 45-56°C (Sidney et al., Process Biochemistry 2019)
- ➔ **Autotrophs:** photosynthetic or chemosynthesic

= Is it conceivable to **collect/accumulate/incubate samples from Venus clouds**, and if so, are **biological assays** based on life as we know it feasible and conceivable given the conditions? (ATP by bioluminescence etc; problems of acidic and anoxic conditions)

= if Venus' clouds indeed harbour life that persists, can't we simply attempt direct bulk culturing of Venus' microbes in cloud samples (including liquid + gases)?

EARTH

 VENUS

The atmosphere is considered a vector between ecosystems, not a reservoir

- **Microbial diversity is high, but biomass is low** so very sensitive methods are needed for detecting/investigating it
- **Activity yet was detected** and involved in atmospheric processes
- No or low growth under natural conditions (**time scale**, temperature)
- Biological traits possibly involved in atmospheric cycling

The atmosphere is considered the unique biome

- Due to ecological selection, **microbial diversity might be lower than on Earth**, but biomass might behigher due to physical constraints (gravity)
- **Microbial activity must have an influence** on chemistry
- Active growth or very specific traits are required (**race against gravity**)
- Biological traits might be more obvious

⇒ **On Earth, the cloudborne biomass is very low, diverse and spread, but this might not be the case on Venus if life has developped ☺**

= How fast elements and matter cycles through Venus' atmosphere? What is the time scale of physical cycles?
= Is there sufficient turbulence/ dynamics to spread biomass among droplet microenvironments?
= What are the minimal necessary population and growth rate for persistence vs physical turnover?
= Is there any expectable spatial structuration of physico-chemical conditions? (hotspots of habitability?)
= Are specific biological traits that would lengthen cloud droplet residence time and/or favor rapid spread among droplets conceivable?
= On Earth, the presence of living microbes affects cloud's chemical content: are there **any known chemical characteristic unexplained** by pure abiotic processes and that could attest of biological processes?

Thank you!

Questions? Comments?