Partitioning of microbial cells between clouds and precipitation

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Context

> **Microorganisms** are present in the outdoor atmosphere, clouds and precipitation.

> There are 10^3 **to** 10^5 **bacterial cells m**$^{-3}$ **of air**.

> Microorganisms originate from **various local and distant sources** and consist of very **diverse and ephemeral communities**.

> It was estimated that bacteria have a mean **residence time in the atmosphere of 5 to 6 days** and can be transported along long-distance with air mass and dust (Burrows et al., 2009)

> Most abundant bacterial taxa : *Alpha-*, *Beta-* and *Gamma-Proteobacteria*

> Notably *Pseudomonas* and *Sphingomonas* among the dominant genera observed.

Main goal

> Bacteria are not equal regarding atmospheric transport.

> For example, it was proposed in the past that bacteria with high ice nuclei activity (INA) are likely more efficiently precipitated than others (Joly et al., 2014).

> We extend this hypothesis and suggest more generally that **different bacteria taxa could exhibit different phase partitioning between aerosol particles, cloud and rainwater**, which may affect their atmospheric residence times.

> To investigate this, cloud and rain samples were collected simultaneously from single precipitation events from two meteorological stations located at different altitudes; and bacterial biodiversity was examined by 16s rRNA gene amplicon sequencing.

Sampling Sites

> Cloud and snow sampling was done at the summit of puy de Dôme Mountain, France (1465 m a.s.l.)
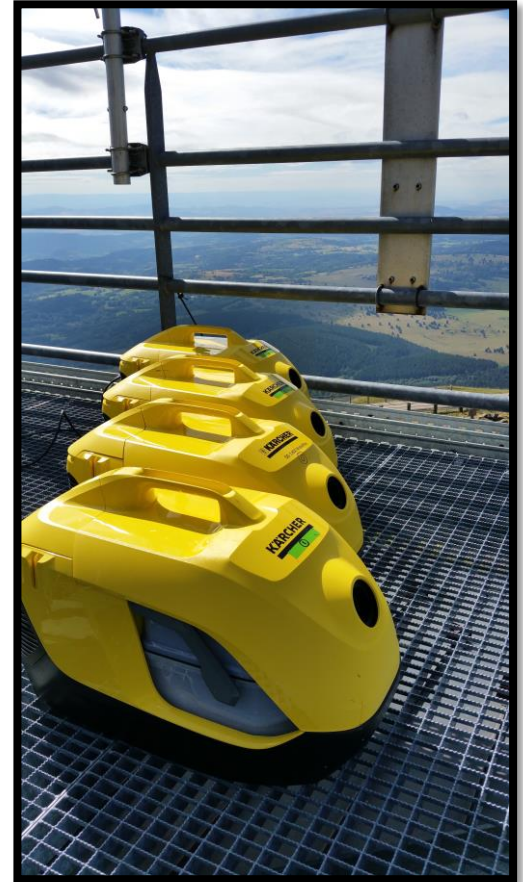> Rain sampling was done at Opme meteorological station, France (680 m a.s.l.)

Cloud sampling with HFR Impinger

Automated precipitation collector
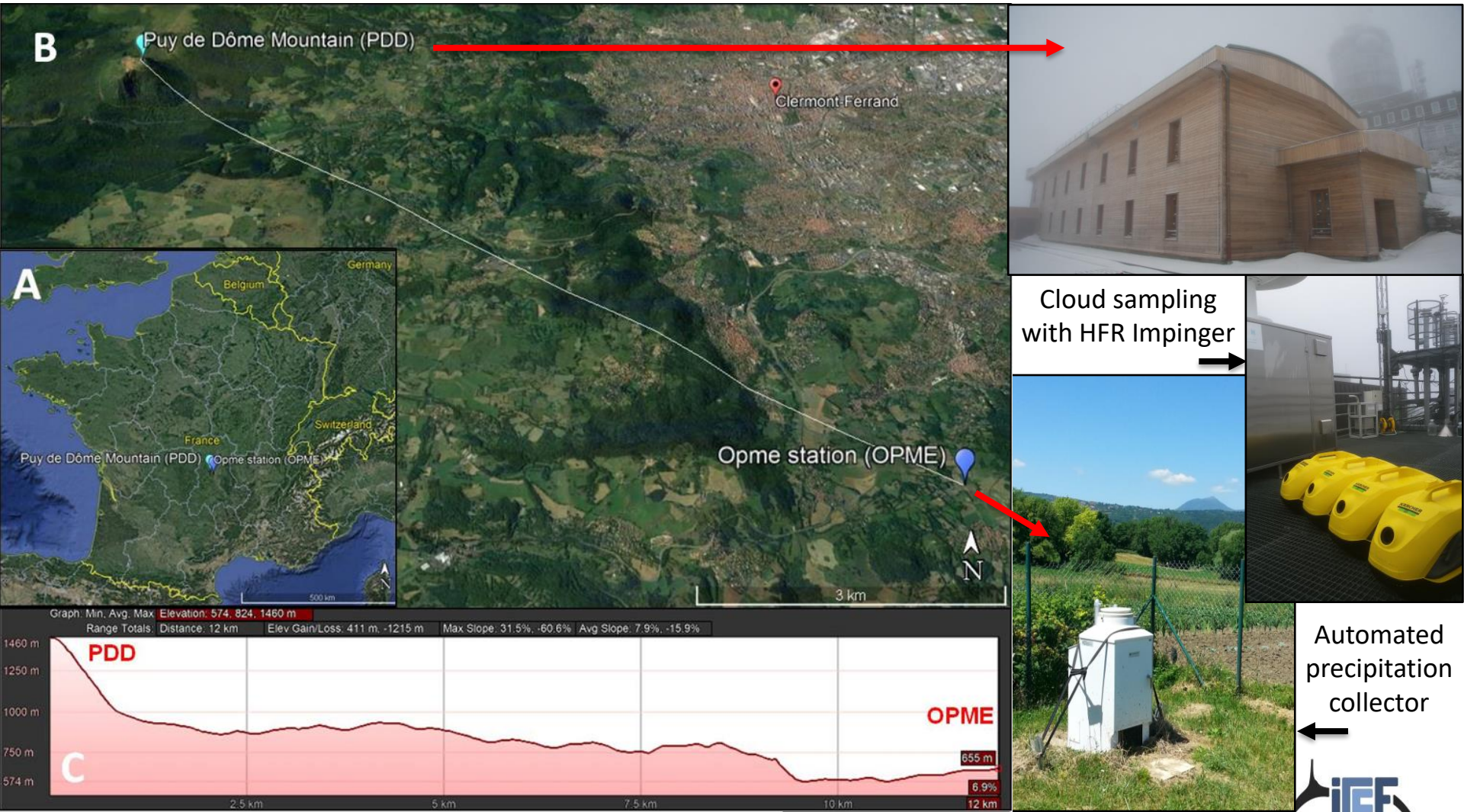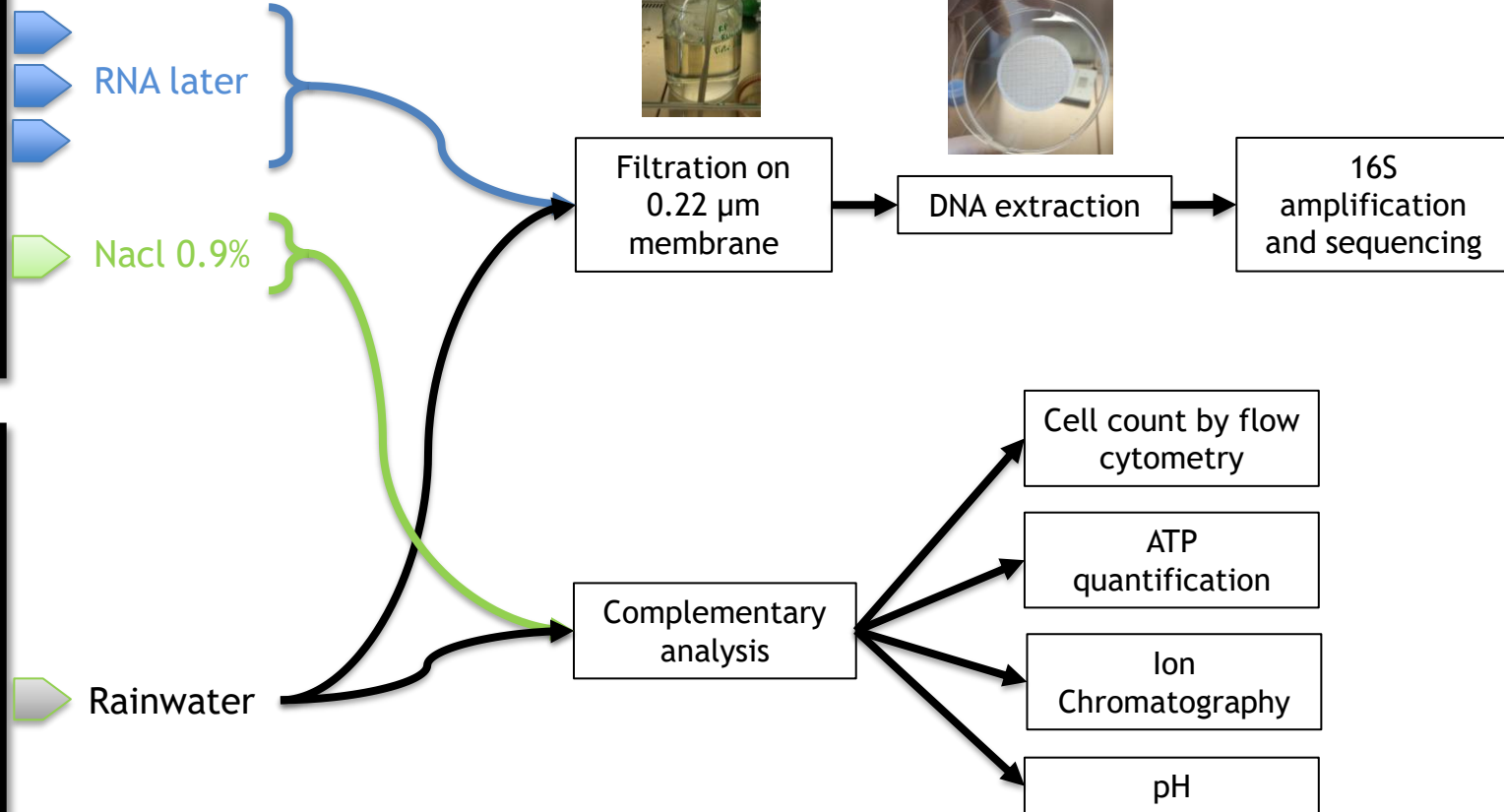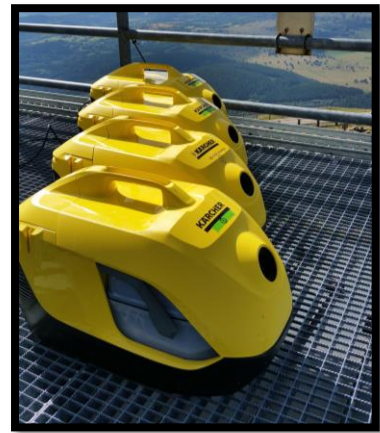
High Flow-Rate (HFR) Impinger for cloud sampling

> ## Main issues for sampling:

➔ High variability regarding bacterial communities.

➔ Low biomass ($\approx 10^4$ cells/m^3 of air).

➔ Viability of cells during sampling.

> ## Avantages of the HFR Impinger:

➔ Much higher air flow rate than other impinger: 2 m^3/min.

➔ Used for aerosols and clouds.

➔ Possibility to sample in liquid solution such as preservative solution (RNA Later)

➔ Low cost sampler

Santl-Temkiv *et al.*, 2017

Methodology: sample processing
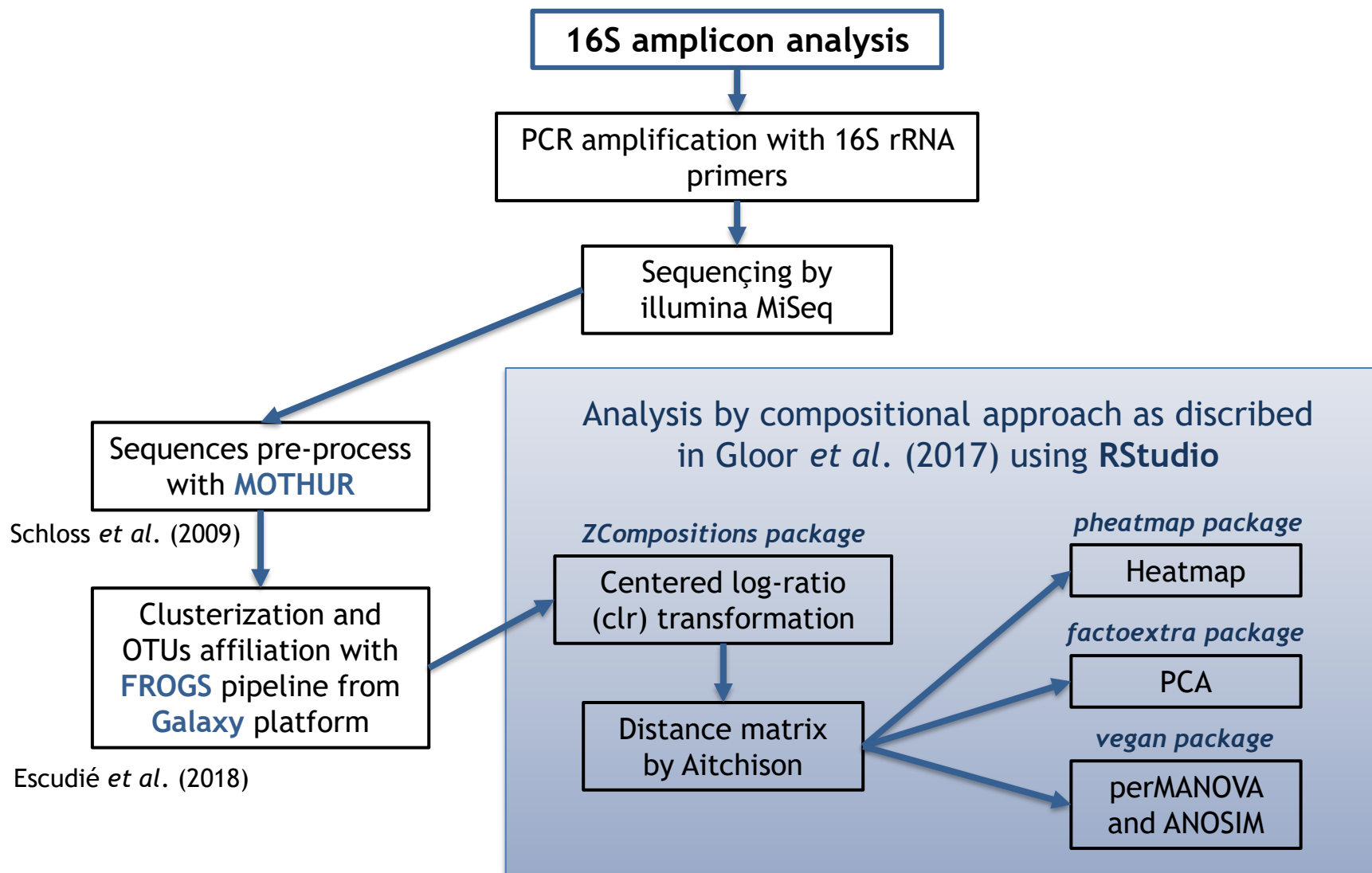
X 4 HFR Impingers

RNA later

Nacl 0.9%

Rainwater

Filtration on 0.22 μm membrane → DNA extraction → 16S amplification and sequencing

Complementary analysis → Cell count by flow cytometry

ATP quantification

Ion Chromatography

pH

Methodology: bioinformatic treatment

16S amplicon analysis

PCR amplification with 16S rRNA primers

Sequençing by illumina MiSeq

Sequences pre-process with **MOTHUR**

Schloss *et al*. (2009)

Clusterization and OTUs affiliation with **FROGS** pipeline from **Galaxy** platform

Escudié *et al*. (2018)

Analysis by compositional approach as discribed in Gloor *et al*. (2017) using **RStudio**

ZCompositions package

Centered log-ratio (clr) transformation

Distance matrix by Aitchison

pheatmap package

Heatmap

factoextra package

PCA

vegan package

perMANOVA and ANOSIM

Preliminary results

> **10 rain**, **1 snow** and **4 cloud water samples** were collected.

> **3 couples cloud-rain** were collected from a single precipitation event (same air mass).

> After OTUs affiliation (Operational Taxonomic Unit) , sub-sampling was done to keep **11,300 sequences for each samples**.

> **In total, 4553 OTUs** were integrated in the analysis.

> Cell count by flow cytometry reach **2.7x10^3 to 3.6x10^4 cell/ml** in clouds and **1.4x10^3 to 1.5x10^5 cell/ml** in rain.
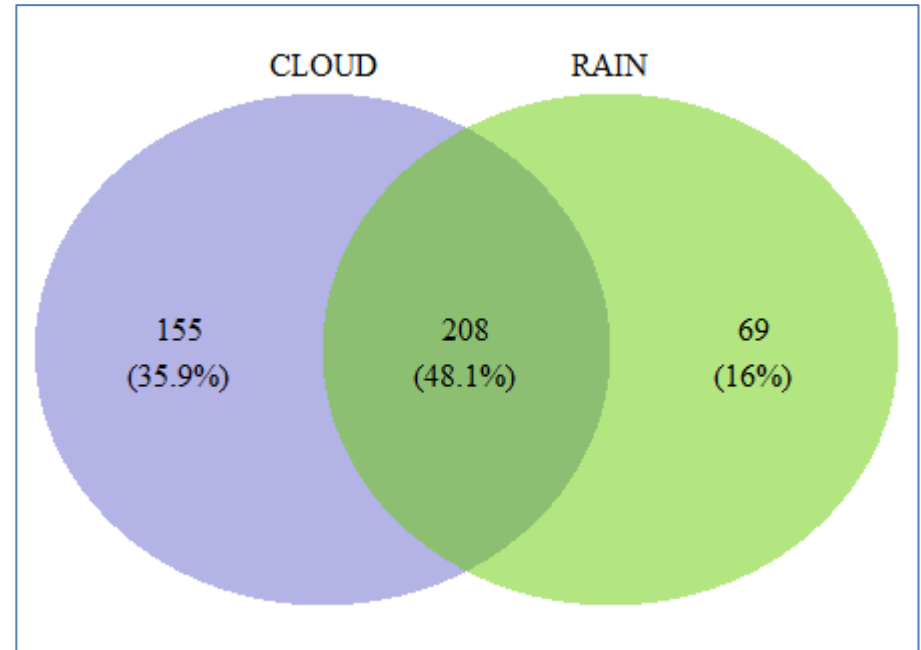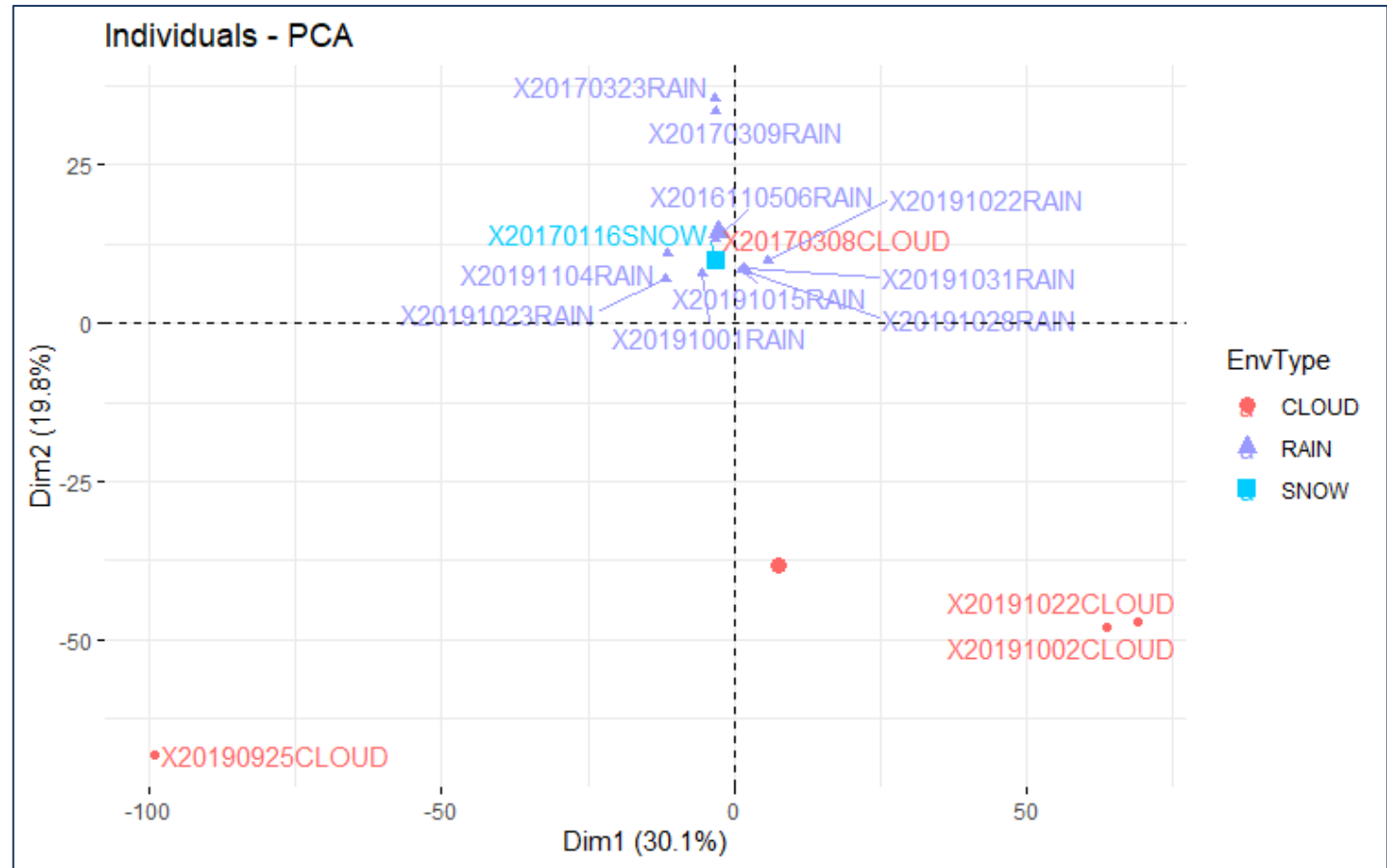

Venn diagram of the specific and shared bacterial genera for cloud and rain.

➢ Most bacterial genera are shared between clouds and rains. Clouds host more diversity than rains.

Results

> Clouds generally host distinct communities than precipitation.

Individuals - PCA

PCA plot based on bacterial assemblage composition.

4,553 OTUs (Operational Taxonomic Unit) were used to do PCA. The "EnvType" colour scale give the environmental type. Each sample name is like: Date-Environmental type.

Results: Biodiversity

<u>Heatmap of the 30 most abundant bacterial orders in samples communities.</u>

Green colour gradient represent the relative abundance of orders normalised in centered log-ratios (CLR). The "EnvType" colour scale give the environmental type of each sample. Hierarchical clustering is done by euclidean distance and "complete" method. Each sample name is like: Date-Environmental type.

➢ *Pseudomanadales*, *Sphingomonadales* and *Betaproteobacteriales* are the three most abundant orders and are more present in precipitation than in clouds.

➢ Predominance of *Pseudomonadales* order in rain is in agreement with the fact that some bacteria can be more efficiently precipitated as *Pseudomonas* with their ice nuclei activity (INA). This observation can support the hypothesis of bio-precipitation.
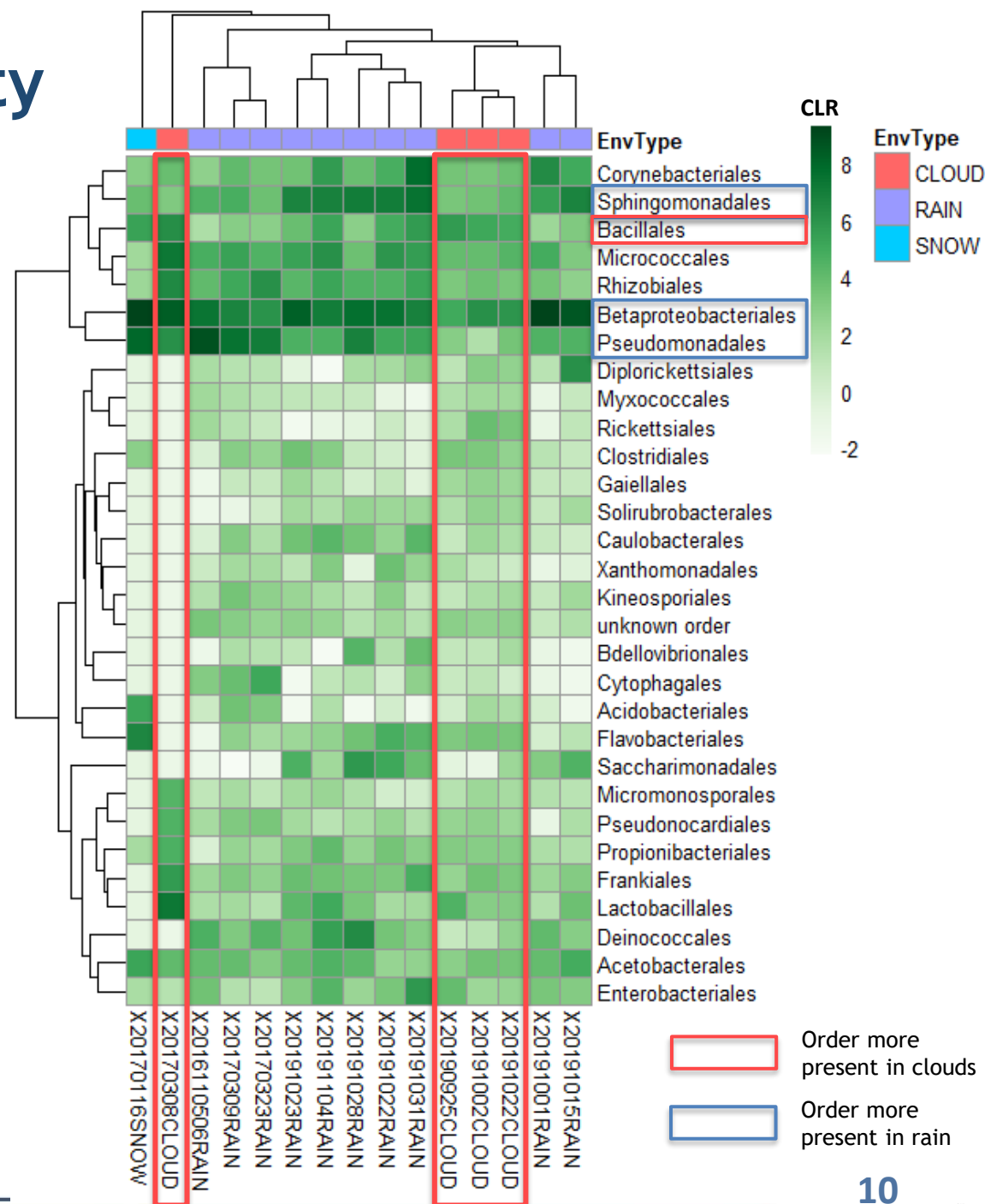
Results: Biodiversity

Heatmap of the 30 most abundant genera in *Betaproteobacteriales* order.

Green colour gradient represent the relative abundance of orders normalised in centered log-ratios (CLR). The "EnvType" colour scale give the environmental type of each sample. Hierarchical clustering is done by euclidean distance and "complete" method. Each sample name is like: Date-Environmental type.

➢ *Betaproteobacteriales* order is the most abundant order and is more present in precipitation than in clouds.

➢ But, if we look at genus level, groups like *Undibacterium* or *Comamonas* and *Limnobacter* are more present in clouds. Moreover, many sub-abundant groups are more present in clouds.

Genus more present in clouds

Genus more present in rain
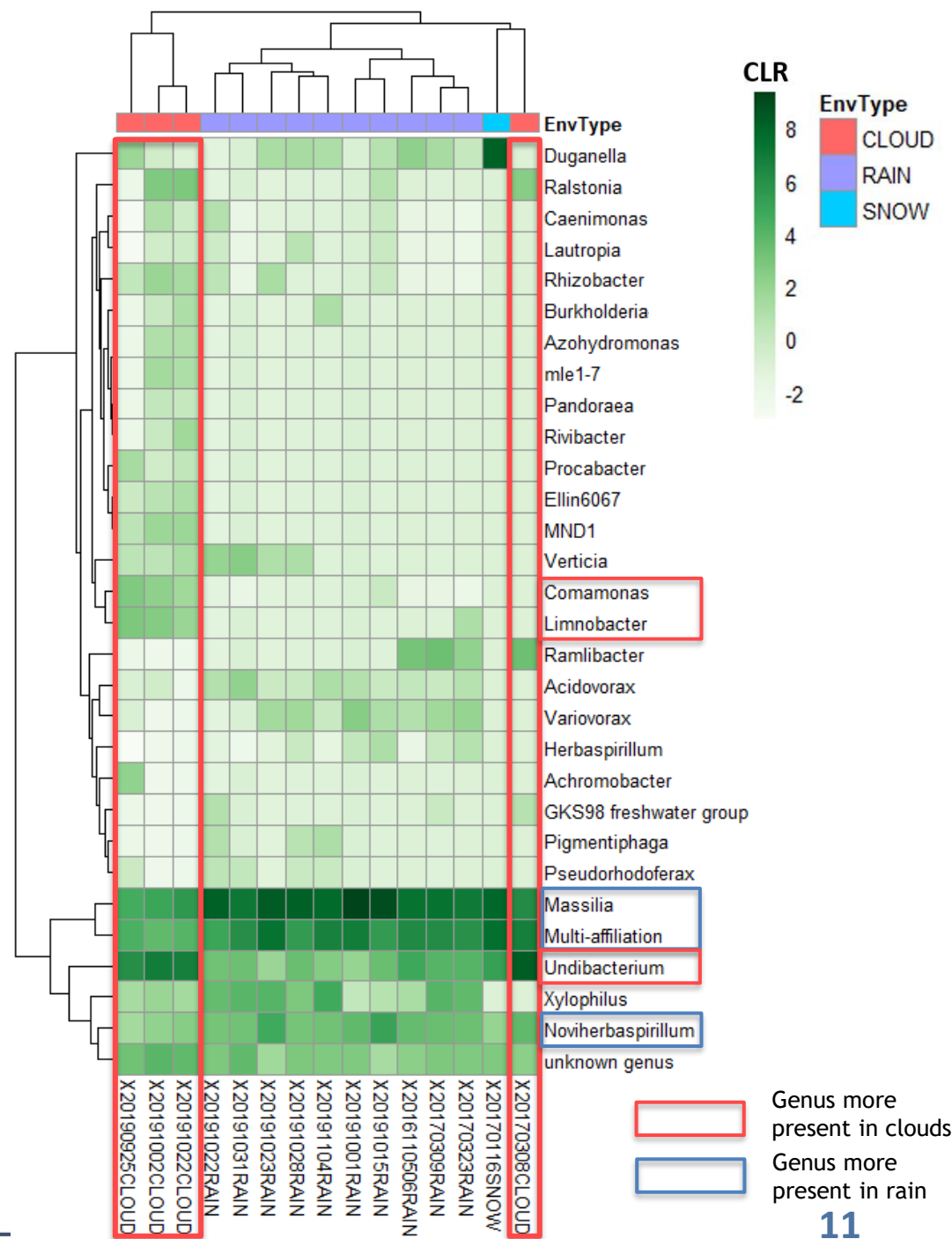
Example of associated cloud-rain samples from october 2019

<u>Venn diagram for two associated cloud and rain samples collected from the same air mass, at one day interval.</u>
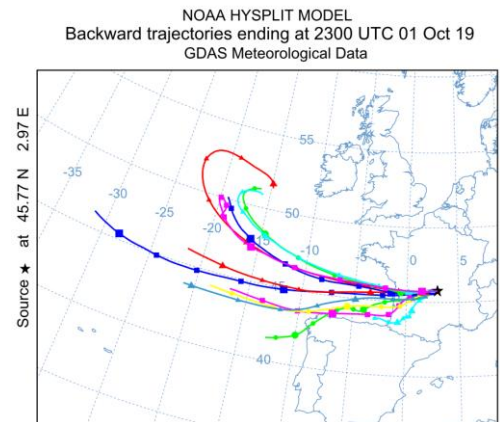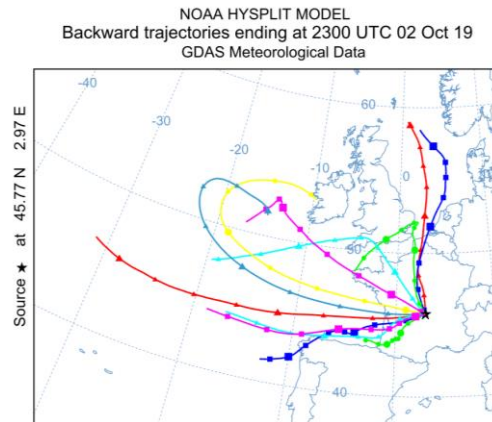Numbers and percentages of genus specific and shared by the two samples.

➢ Most of the diversity detected in the cloud is not detected in the rain originating from it (73.7%). The diversity decreases from cloud to rain. Bacteria in rain mostly originate from the cloud and very few genera are specific of rain, indicating probable low below-cloud scavenging processes.

20191002CLOUD   20191001RAIN

165
(73.7%)

46
(20.5%)

13
(5.8%)
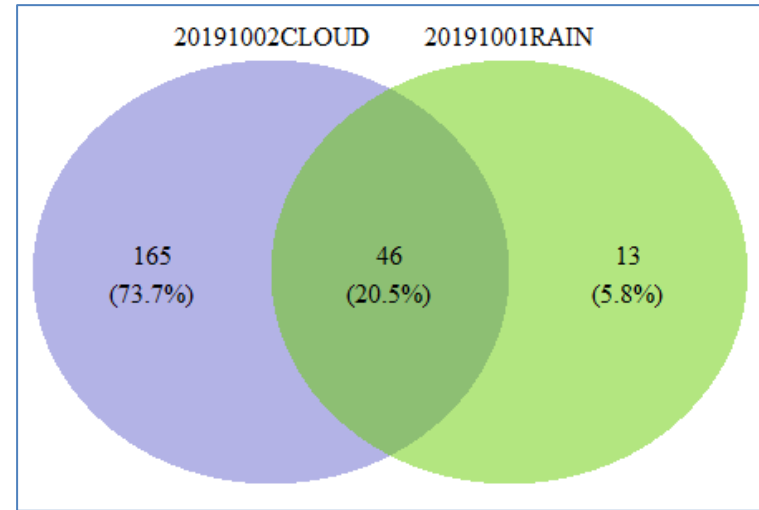
<u>48 hours backward trajectories for the 1st and 2nd of October 2019.</u>
Done with HYSPLIT model using GDAS1 meteorological data at puy de Dôme mountain localisation (45°46'20" N, 2°57'57" E) and altitude (1465 m a.s.l). A new trajectory is generated each 6 hours.

NOAA HYSPLIT MODEL
Backward trajectories ending at 2300 UTC 02 Oct 19
GDAS Meteorological Data

NOAA HYSPLIT MODEL
Backward trajectories ending at 2300 UTC 01 Oct 19
GDAS Meteorological Data

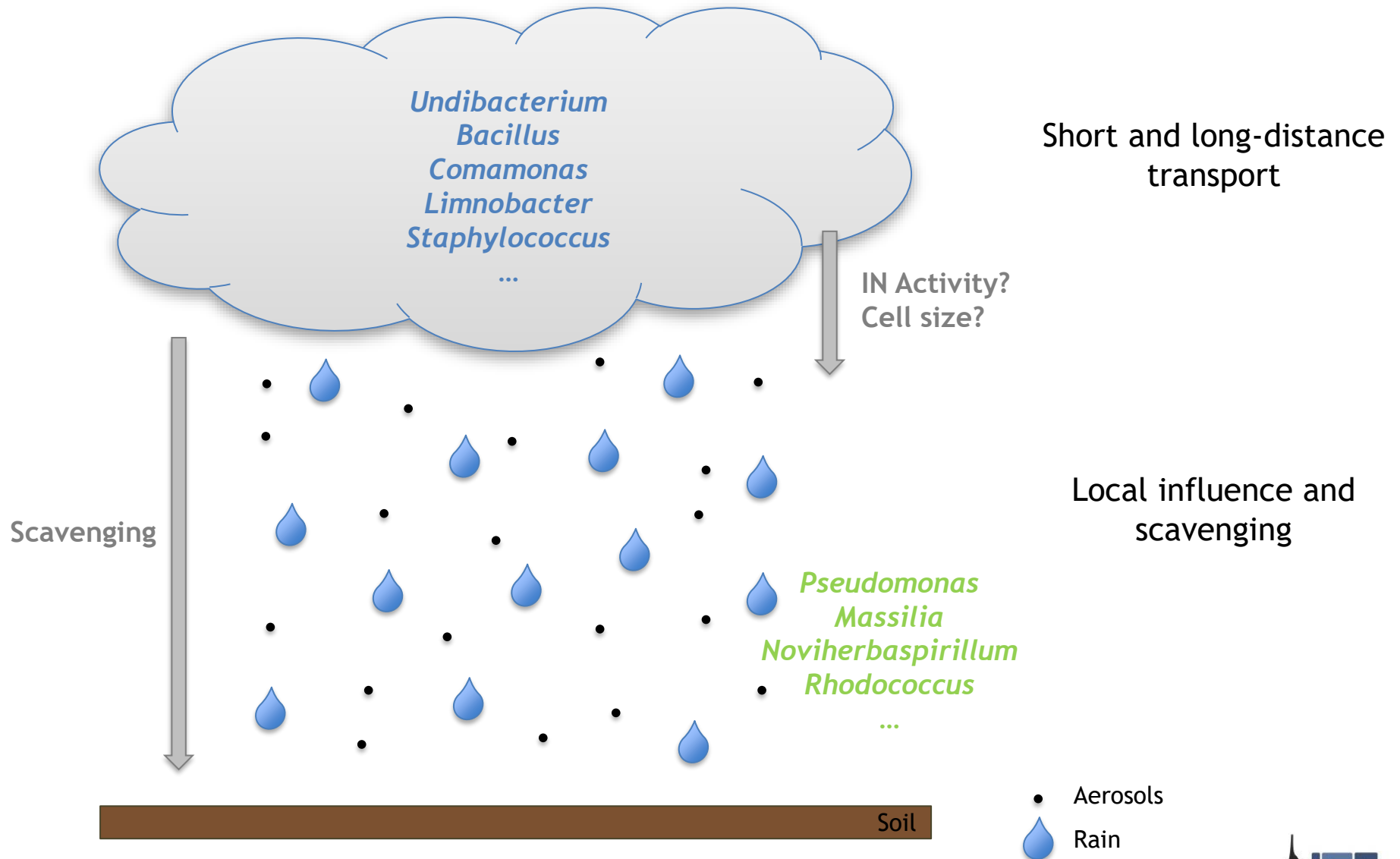# Example of associated cloud-rain samples from october 2019
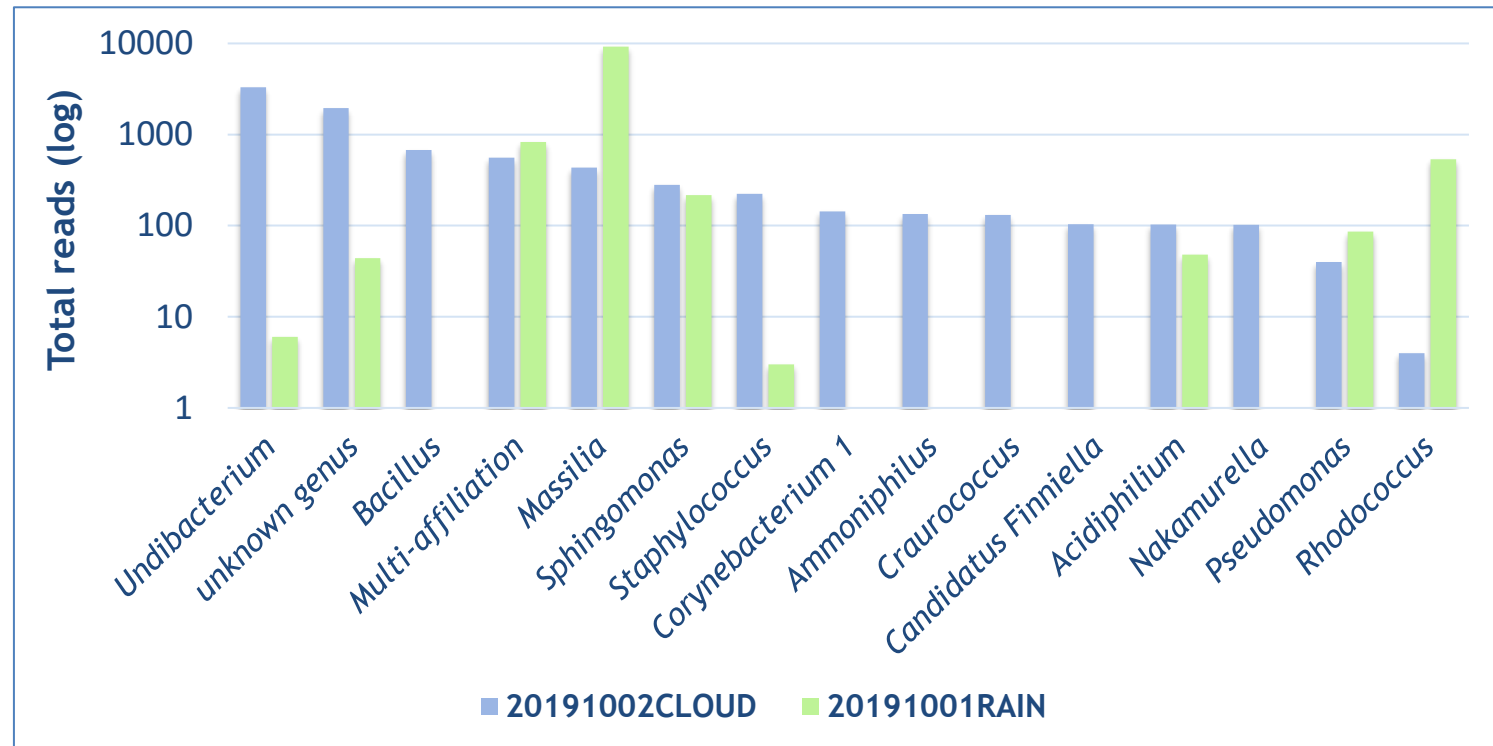
Histogram of the 15 most abundant genus in the couple cloud-rain.
Total reads for each genus for cloud sample and rain sample. Scale is in logarithm.

➤ *Undibacterium* more present in cloud than in rain, so likely of distant origin, while, *Pseudomonas* and *Massilia* are more present in rain, so likely of local origin.

Results summary

Undibacterium
Bacillus
Comamonas
Limnobacter
Staphylococcus

...

Short and long-distance transport

IN Activity?
Cell size?

Scavenging

Local influence and scavenging

Pseudomonas
Massilia
Noviherbaspirillum
Rhodococcus

...

Soil

Aerosols

Rain

Perspectives

> What are impacts of cloud/rain partitioning of bacteria on their capacity of dispersion as airborne and on their atmospheric life cycle?

> What biological properties or processes are driving the physical behaviour of cells in the atmosphere?

> Are there microbial acclimatization and/or adaptation processes linked with atmospheric partitioning?

> Results are still be processing for this study

> In further studies, aerosols will be also collected and bacterial activity in each atmospheric compartment (clouds, rain and aerosols) will be investigated.