Preliminary Study of the Microbiome at Whiteface Mountain (WFM)

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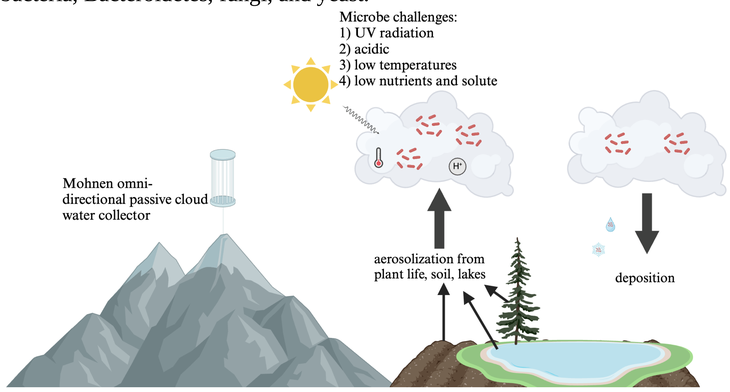
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Chemical Studies at Whiteface Mountain (WFM)

Whiteface Mountain is in the Adirondacks regions Wilmington, NY with an elevation of 1483 m. Measurements of conductivity, pH, ammonium, sulfate, and nitrate of cloud water have been taking place since 1994 with newly added measurements of water-soluble organic carbon (WSOC) being added in 2009.[1] Although these chemical measurements have been explored, microbial measurements have yet to be discussed at Whiteface Mountain. This poster offers a preliminary study of the chemicals and microbes in the clouds at WFM during the 2024 cloud water season after 0.2-micron filters were installed (July-September).

Studies of Microbes in Clouds

At WFM, microbes enter clouds through aerosolization from plants, lakes, and soil. Once here, they will experience UV radiation, acidity, low temperatures, and low nutrients. This means that microbes must either be adapted to withstand these environments or will desiccate.[2] Common bacteria previously isolated from the Puy de Dôme mountaintop observatory include Pseudomonas species that have UV-resistant phenotypes and high metabolic rates. Other microbes isolated include Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes, fungi, and yeast.[2]



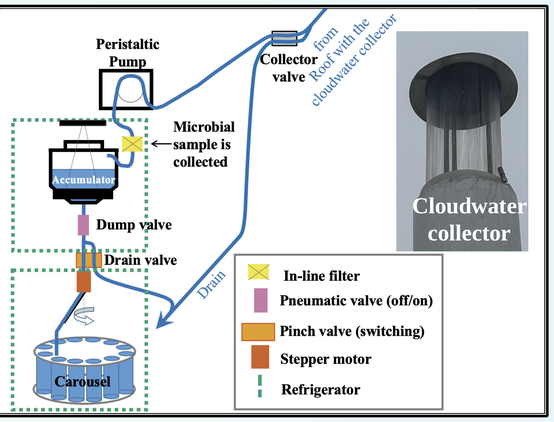
Schematic of WFM Microbes processes using BioRender

Importance

Microbes are key in understanding biogeochemical processes such as the cycling of nitrogen, carbon, sulfur, etc. There are complex reactions which occur in clouds and without the microbial piece it is difficult to understand how chemicals are being transformed. From a chemistry perspective, microbes are essential for understanding how chemicals are transformed in clouds. On the medical side, pathogens have been isolated from clouds. Understanding how these pathogens travel through clouds and into soils, vegetation, and waters could aid in our prevention of outbreaks.[2] Lastly, there has been an increase in wildfire intensity due to climate change with studies being conducted on how microbiomes are affected.[3] This preliminary study hopes to add to a longer-term study that touches on the chemistry, health effects of cloud microbiomes and wildfire effects on cloud microbiomes.

Collection Methods

- Mohnen omni-directional passive cloud water collector
- It is deployed when:
  - liquid water content (LWC) is above 0.05 g m^{-3}
  - temperature is above 2 °C
  - presence of rain is not detected
  - wind-speed is above 2 m s^{-1}
- Time resolution:
  - Cloudwater: 12 hours
  - Filters: 3-4 days



Filter Storage

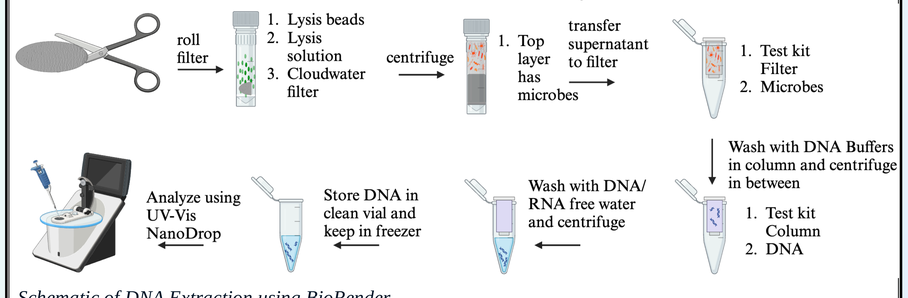
Filters are stored in 95% ethanol and fresh 15 mL tubes until DNA extraction. This method was selected as a more feasible option over freezing given the remote location of WFM and was proven a valid method by previous studies.[4] Filters are labeled "Year Julian Date Collected" Filters start white and will turn a black or brown color depending smoke levels due to black carbon and particulate matter.



Filter from August 19th, 2024 (24231)

Extraction Technique

Filters are extracted with ZymoBIOMICS DNA Microprep Kit. Lysis entails the breaking down of the cell wall to access the DNA. The DNA is stored in 100 µL of DNA/RNA free water.

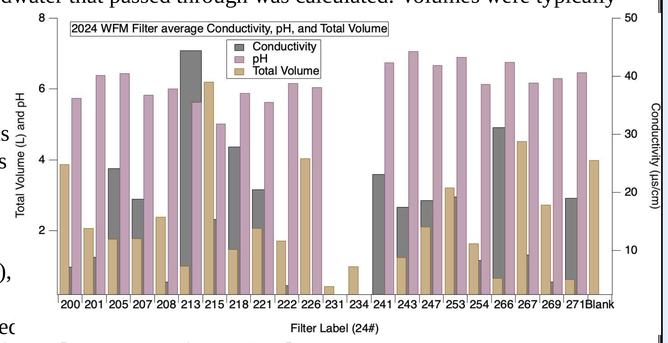


Schematic of DNA Extraction using BioRender

Chemical Background of Filters

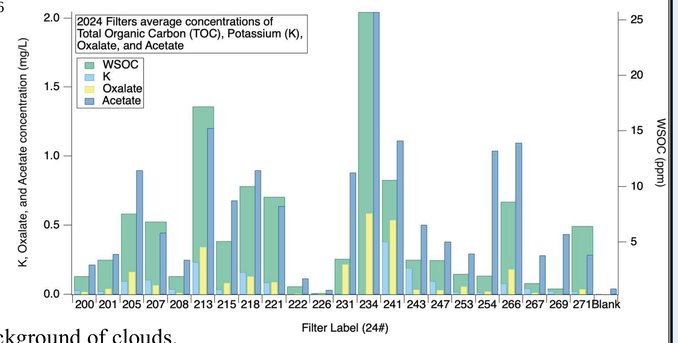
pH, Conductivity

For each filter, the amount of cloudwater along with the weighted average pH and conductivity of the cloudwater that passed through was calculated. Volumes were typically <500 mL with pH measurements being between 7-6 meaning the clouds are slightly acidic. Conductivity was more variable. Microbes can benefit from higher conductivity as this implies more ions.[5] For the blank (DI water), pH and conductivity values were not measurec



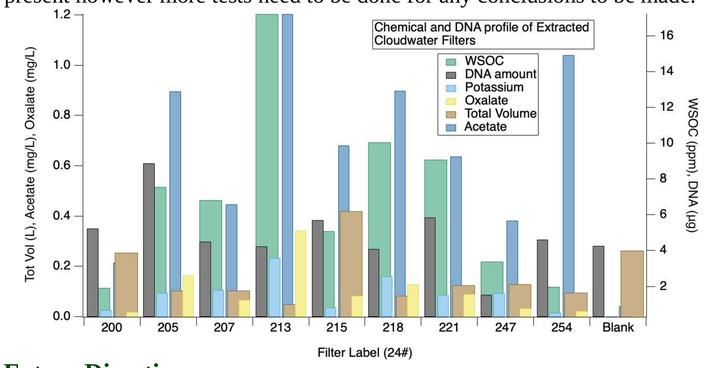
Water-soluble organic carbon, Potassium, Oxalate, Acetate

Microbes can affect water-soluble organic carbon (WSOC) level by converting organic carbon into inorganic carbon through mineralization an example of this is oxalate degrading bacteria. However, the microbes themselves can add to the WSOC through decomposition and producing acetate.[6] Lastly, potassium is a key biomarker for microbial growth as potassium is necessary for osmotic pressure, pH, and membrane potential of the cell.[7] Potassium and WSOC are also smoke indicators. In all, these weighted averages serve as a chemical background of clouds.



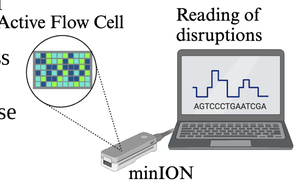
Preliminary Data: DNA concentrations

The concentration of isolated nucleic acids in the extracted samples was measured using absorbance values on a Nanodrop spectrophotometer. In all the samples measured, the amount of DNA ranged from 0.09-0.6µg. DNA was found in the blank filter (DI water passed through collector) possibly due to contamination during extraction or the collector itself. More tests need to be conducted. The results are shown below. Based on these filters, total DNA concentration does not seem correlated with the volume nor chemicals present however more tests need to be done for any conclusions to be made.



Future Directions

Future steps are to perform a 16S metagenomic sequencing of the DNA using the Oxford nanopore MinION. The extracted DNA samples will be converted to barcoded libraries using primers that amplify the entire ~1.5kb 16S rRNA gene and loaded on to a minION R10 nanopore flow cell. The changes in current caused by the strand of DNA as it passes through the pore are recorded by the MinKNOW™ software and converted into reads in real-time. Taxonomic classification of single reads from 16S rDNA amplicon-targeted sequencing will be obtained using analysis pipelines in EPI2ME software. Abundance tables with counts per taxa in all samples will be generated and enable comparative analysis of abundant taxa across the samples. Correlation of microbial composition to chemical composition in these cloud water samples will help further the understanding of the interactions existing between microbial communities and abiotic processes in clouds, as well as important insights into the aerial dispersion of microbes.



Depiction of sequencing using BioRender

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- The Kanien'kehá:ka and the Abenaki People, on whose ancestral homelands where this research took place, who have for generations stewarded the lands and waterways of this place that is now known as the Adirondacks. Furthermore, I stand in solidarity with the Land Back movement.