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**AEROMICROBIOLOGY, SNOW AND
BIOGEOCHEMISTRY OVER ANTARCTICA**



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ABSTRACTS SUBMITTED TO THE (CANCELLED) SCAR 2020 OSC IN HOBART

Survey of Viridiplantae present in Air and precipitated with Snow on Livingston island. (South Shetlands)

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We accessed Viridiplantae diversity present in freshly deposited snow and air samples at Livingston Island using DNA metabarcoding via high throughput sequencing (HTS). Two surface snow samples (>1500 mL each) were obtained shortly after precipitating, melted at room temperature and concentrated with Sterivex filters (0.22 µm pore size). Also, two air samples were collected with a High-Flow-Rate Impinger (340-400 m³ of air) into sterile MQ water and concentrated on Sterivex filters. Total DNA present was extracted and sequenced. In the snow, we detected 31 OTUs affiliating with Magnoliophyta (18), Bryophyta (1) and Chlorophyta (6). *Trebouxia simplex* was the most abundant Chlorophyta and *Zea mays* the most abundant Magnoliophyta. Microalgae affiliating with *Trebouxia* sp. have ice-nucleation activity and may have relevance for cloud and precipitation formation. In air, we found 12 OTUs affiliating with Chlorophyta (4), Bryophyta (1) and Magnoliophyta (7). Among Chlorophyta, the most abundant was *Dictyosphaerium* sp. and among Magnoliophyta, *Monoon tirunelveliense*. Only five OTUs were shared between air and snow, which likely reflects the different air masses that were collected. Also snow forms at higher altitude and is likely dominated by taxa arriving through long-range dispersal. Air sampled at ground level is likely dominated by taxa from local sources. The presence of maize, a tropical crop, was unusual, but as one of the most abundant crops in the world is not unlike it may spread via atmosphere. It is important to stress that detecting the DNA of a species does not mean that the species is present.

Excess carbon induces carbon storage as polyhydroxyalkanoate (PHA) production in Antarctic sea-ice bacteria

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Sea-ice organisms experience fluctuating and harsh environmental conditions such as low temperature, high salinity and intermittent substrate supply. Bacteria in sea ice have various strategies to survive in the ice (e.g. compatible solutes and EPS-production), however, these mechanisms are not thoroughly understood. Polyhydroxyalkanoates (PHAs) are polyesters that serve as a pool for carbon storage and are readily available for different cellular processes. Interestingly, PHA is also used in bioplastic production, for which sea-ice bacteria may serve as potential production organisms i.e. due to their low temperature optima. PHA granules and *phaC* synthase genes have been detected in sea-ice bacteria, however, their production mechanism and ecological significance is not known. Our aim was to investigate whether or not sea-ice bacteria are capable of PHA production and to elucidate the cellular mechanism behind it.

PHA production was tested with two bacterial isolates, *Paracoccus* (Alphaproteobacteria) and *Halomonas* (Gammaproteobacteria), isolated from Antarctic sea ice. PHA production was verified with transcriptomic, microscopic and GC-MS methods. Also, the occurrence of *phaABC* genes were also detected from several Antarctic, Arctic and Baltic Sea ice metagenomes.

These results demonstrate that Antarctic sea-ice bacteria are capable of producing PHA. We hypothesize that PHA production in ice is related to the ephemeral feature of labile dissolved organic carbon availability (i.e. from the initial freezing). Thereafter carbon PHA granules are stored to enhance survival within ice.

Bacteria living on air: Atmospheric chemosynthesis supports primary production in cold desert soils

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Antarctica is the coldest, driest and windiest continent on the planet. Yet the desert soils of Antarctica host a great diversity of microbial communities that have evolved unique strategies to survive under the extremely harsh conditions. Microbes dominant terrestrial Antarctica and in doing so must endure frequent freeze-thaw cycles, complete winter darkness, limited nutrient and water availability and intense UV radiation. We have discovered that in the dry desert surface soils of the Windmill Islands and Vestfold Hills regions, Eastern Antarctica very few phototrophs such as cyanobacteria or algae exist. Instead, novel bacterial phyla with new functional capacities are thriving under these stressful conditions. By combining metagenomics with differential coverage binning and functional assays we proposed that atmospheric chemosynthesis, a new form of primary production was supporting microbial communities living in these cold, nutrient poor environments. This aerobic energy-capture process relies on the oxidation of atmospheric levels of hydrogen and carbon monoxide gas to provide the fuel required to fix CO₂ via the Calvin-Benson-Bassham cycle and provides new understanding of the nutritional limits required for life. Today I will focus on the significance of this alternative form of primary production, by providing new evidence that soil microbiomes across the three poles also perform atmospheric chemosynthesis. At the same time, I will provide new information on the physiology and environmental drivers of bacteria with the genetic capacity to live on air, particularly focusing on *Candidatus Eremiobacteraeota* (WPS-2), a yet-to-be cultured bacterial phyla.

Airborne microbial communities along a zero-emission traverse on the Antarctic Plateau

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Despite the harsh environmental conditions of the Antarctic plateau, microorganisms can be found in the air. In this communication we present the Windsled expedition, conceived as an ultra-clean scientific campaign, perfectly adapted to our purposes of investigating the microbial community in the plateau's atmosphere. Extreme cold conditions, absence of a non-renewable energy source and constant shocks caused by the movement of the WindSled when passing over the sastrugi, were a technological challenge that required development of new methods and instruments. The expedition covered a transect of 2,538 km in 52 days, in which 62 air samples were collected with three specially designed zero-emission collectors that operated with different configurations to maximize the capture of airborne microorganisms. Environmental data was also recorded using the adapted automatic weather station M-AWS. Position, temperature and relative humidity were recorded every 30 minutes, providing a dataset of 1,732 measurements for each variable at 522 different locations. Overall in this expedition we captured an average of 8.31×10^2 cells per m^3 , with a standard deviation of 1.06×10^3 cells per m^3 , indicating extremely high variability in the airborne bacterial concentrations. The relationship of this variability with the environmental conditions is analyzed. Our results indicate that the Windsled is an excellent vehicle for these remote and extreme locations, allowing scientific projects to be developed under the principle of inexpensive transits, zero emissions and ultra-clean technologies.

Active and Dormant Antarctic Snow Algae Reduce Coastal Snow Albedo

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Snow algae bloom is a common occurrence in coastal snowpacks of the northern Antarctic Peninsula and adjacent islands during austral summer. We collected surface spectral albedo and total chlorophyll concentrations from three algae bloom sites in the Antarctic Peninsula region. Here snow algae is comprised of green algae (chlorophyta) that appear green during reproduction and red during dormancy. Spectral albedo in areas of intense algae bloom was reduced in the visible wavelengths to 0.49 (red communities), 0.49 (mixed) and 0.31 (green). Relative to clean snow, the reproductive green-phase snow algae reduced snow albedo in the visible wavelengths by up to 61%, almost three times more than the dormant red-phase, 24%. Also, algal biomass in the snow was well-correlated with albedo reduction, ($r^2 = 0.68$). Given the intense warming in the region over the past 6 decades, it is likely that the snow algae bloom season has been extended, exacerbating melting and snow retreat in coastal snow areas. These data may eventually be used in algorithms aimed at mapping the spatial and temporal variability of snow algae across their reproductive phases and tracking algal bloom expansion using multi-spectral satellite remote sensing.

Mat-building ability of Antarctic cyanobacteria strains

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On the Antarctic continent, where the extreme climate places severe limitations on terrestrial life, cyanobacteria form a range of complex mats, across a variety of aquatic and semi-aquatic habitats. Mat formation is often thought to benefit survival under adverse conditions. It is a challenge to understand the interactions among mat-forming microorganisms and which organisms benefit most from, or are most active in the production of, the mat morphology. Here we examined the production of microbial mats role by individual strains and mixed cultures of five cyanobacteria belonging to the Nostocales, Oscillatoriales and Chroococcales orders, isolated from Antarctic mats. The results were focusing on the chlorophyll-a, exopolysaccharide and organic matter production. Despite, some morphotypes performing better than others, we did not find strong evidence that mixed cultures performed better than the best single isolates. To a large extent different strains were able to “substitute” for each other in forming a mat, indicating the potential for a resilient response to changing stress profiles. The morphotype *Phormidium* cf. *autumnale* showed the best performance displaying greater production of EPS, organic matter and Chl-a contents.

Living on Air- Antarctic bacteria that use atmospheric trace gasses to survive and thrive.

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Antarctic soil is known to contain unique microbial communities as a result of the isolation and extreme environmental conditions experienced in Antarctica. This study has focused on an enigmatic bacterial phylum found in high proportions within Antarctic soils. Formerly known as Candidate Division AD3, *Ca. Dormibacteraeota* are found in soils across the globe, usually representing less than 1% of the microbial community. In arid desert soils from Robinson Ridge and Mitchell Peninsula in Eastern Antarctica, *Ca. Dormibacteraeota* represented up to 15% of the microbial community, suggesting that they thrive under these harsh conditions. We used shotgun sequencing and differential binning of soils from these two sites to obtain six metagenome assembled genomes (MAGs) belonging to this phylum. The MAGs provided insight into the lifestyle and survival strategies of these bacteria. Of primary interest was the ability of the microbes to assimilate carbon dioxide and carbon monoxide as a carbon source while using atmospheric hydrogen gas as an energy source. Further experimentation using stable isotope probing confirmed the ability of the microbes to assimilate carbon dioxide from the atmosphere using ¹³CO₂. Finally, we aimed to visualise the cells using a range of fluorescent in-situ hybridisation (FISH) techniques which revealed the cells to be small cocci approximately 250-300 nm in length. We conclude that the unique ability of *Ca. Dormibacteraeota* to assimilate trace gasses from the atmosphere and their small size and shape make them uniquely adapted to the Antarctic environment, allowing them to thrive.

Microbial diversity and prediction of ecological processes beneath the West Antarctic ice sheet

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Non-aquatic subglacial ecosystems in Antarctica may be spatially more extensive and variable in physical-chemical characteristics when compared to the subglacial aquatic habitats, however their microbial diversity is still poorly explored. In this study, we aimed to understand the microbial community structure deposited on the West Antarctic ice sheet through 16S rRNA gene sequencing, followed by a prediction of the metabolic and ecological processes. We aseptically excavated a pit structure near Criosfera1 remote Brazilian laboratory/CrioLab1 (670km from south pole), in which we collected snow/firn samples among six different depths between the surface and 200cm. The abundant phyla were classified as Proteobacteria, Firmicutes, Parcubacteria, Cyanobacteria, Bacteroides, Actinobacteria, Thaumarchaeota, Marinimicrobia, Woesearchaeota, Euryarchaeota and Chloroflexi. We found a higher proportion of marine members within Thaumarchaeota and Thermoplasmatales at the superficial strata, whereas Cyanobacteria was detected mainly at the deeper layers. Atmospheric modeling of air incursions at the study site suggest a high marine influence from the Weddell and Indian sea, as well as from the sub-antarctic environment. We also detected sequences classified as hyperthermophiles within Aquificae and Euryarchaeota. Microorganisms associated with nitrogen metabolism were more abundant among deep layers (110-180 cm), while those in superficial layers were related to functions as chemoheterotrophy, degradation of aromatic compounds and animal parasites and symbionts. The prevalence of members that occupied such distant ecosystems as marine, hydrothermal and animal bodies suggests a heterogeneity of the ice-sheet microbiome probably due to the largely aeolian dispersion over the Antarctic continent and their long-time persistence in this extreme and isolated environment.

Geomicrobiology studies in the Ellsworth Mountains, Antarctica; interaction of microorganism with rocks as a strategy to survive in this hyper-extreme environment.

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Close to the Chilean Union Glacier scientific camp, located in the Ellsworth Mountains about 1000 km from the South Pole, is the mountain called Elephant Head. This zone presents temperatures below freezing throughout the year and high UV radiation during the summer. Mineralogical and elemental composition of Elephant Head soil samples were determined through XRD and SEM-EDS, respectively. XRD analysis revealed the presence of calcite, dolomite, and quartz, and the elemental analysis revealed the presence of O, Si, Ca, Al, K, Mg, and Fe. Also, using X-ray microtomography (Micro-CT) we determined that the rocks of this site have greater porosity than those found in other areas of the Ellsworth Mountains (Mount Rossman or Charles Peak).

Through 16s rRNA gene sequencing we identified the microorganisms present in soil samples obtained during two campaigns (2017 and 2018) at different depths and altitudes on Elephant Head. Obtained results revealed the presence of cyanobacteria (particularly in surface samples), a result that allowed us to establish possible associations between the type of rock present in Elephant Head and the microbial communities. In this sense, quartz is a translucent mineral that permits the passage of light inside the rock and considering their porosity could act as a shelter protecting the microorganisms from hyper-extreme environmental conditions allowing a light-driven metabolism mediated by cyanobacteria.

Finally, we isolated endolithic bacteria and we are studying their potential in biomineralization processes to understand mineral-bacteria interaction. In particular we studied calcite bioprecipitation and the generation of metal nanocrystals.

New zero emissions collector for the capture of airborne microbes in the Antarctic Plateau.

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Air Sampling has been demonstrated a complex task with some crucial aspects that may create difficulties in the interpretation of results. When airborne microorganisms are to be collected from extreme remote locations with extremely low cell concentration, as the Antarctic Plateau, adapted capturing methodologies should be developed. We present the methodological development of an airborne bacterial capturing technology created specifically for an Antarctic Plateau transect (2540 km), the Antarctica Unexplored Expedition 2019, with very limited energy availability. We present the capturing technology and methodology for the analysis of bacterial community collected from the Antarctic Plateau's atmosphere. We measured airborne cellular concentration, assessed its potential viability by flow cytometry and extracted DNA for sequencing. The collector consisted of a forced air system with a precise air flow and a turbine that converted the flow in turbulent before the retaining device based on a solid petroleum jelly, which remained viscous at extremely low temperature. The energy demand of the system was very low and handy solar panels provided the requirements. Cell extraction from the viscous matrix was optimized in the lab, and allowed high recovery rates, antibody identification, staining and flow cytometry analyses and DNA sequencing of the airborne community. We collected 62 samples from which we have obtained microbial airborne concentration, its viability through membrane integrity and the molecular identification of those microbes.

Bacterial diversity in the Antarctic plateau atmosphere and accumulated snow

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The extreme environmental conditions dominating the Antarctic Plateau constrain the biological activity in this extensive continent. The WindSled expedition navigated the Antarctic Plateau in a special wind-powered vehicle for 52 days and 2538 km. Air samples were collected using a collecting device designed ad-hoc for the expedition along this transect. The airborne cells collected were enumerated by flow cytometry and identified by fluorescence microarray immunoassay with a life detector chip (LDChip) and massive 16S rRNA gene sequencing. Simultaneously, samples from snow profiles (down to 4 m depth) at three sites were obtained and analysed in situ with LDChip and DNA sequencing in the laboratory. In this communication we discuss the differences in the bacterial communities found in both matrixes (air and snow) considering that biological activity at the plateau should be extremely low (given summer mean temperature was -30 °C during the expedition) and thus the bacterial composition of the air community should be a combination between the advent of cells from snow fields in Antarctica and those coming from long range wind trajectories. In this study, we also investigate the long-range trajectories of the air masses that transport cells during the capturing period. We discuss our hypothesis about the Antarctic Plateau as an immense airborne bacterial trap that accumulates cells (i.e. DNA) from different regions on Earth.

Uncultured fungal diversity in recent superficial snow on Livingston Island, Antarctica

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We assessed uncultured fungal diversity present in freshly deposited snow and in air samples obtained from Livingston Island, Antarctica, using the DNA metabarcoding through high throughput sequencing (HTS). Superficial snow obtained shortly after deposition was kept at room temperature and yielded 3.760 L of water, which was filtered using sterivex membranes (0.22 µm). Also, 740 m³ of air at the same region were pumped through an 0.22 µm membrane. The total DNA present in the membranes was extracted and sequenced. In the snow sample, we detected 97 fungal operational taxonomic units (OTUs) dominated by the phyla Ascomycota, Basidiomycota, and Mortierellomycota. The fungal assemblages present in the air and recent snow displayed high diversity and richness, but low dominance indices. Twenty-seven OTUs occurred only in the air, 30 in snow, and 36 in both samples. Within both the air and recent snow communities, the Ascomycota taxa *Cladosporium* sp. and *Pseudogymnoascus roseus* were dominant (>20,000 reads). Use of an HTS approach revealed the presence of more diverse fungal communities than have been detected using traditional isolation methods. The communities included cold-adapted and cosmopolitan taxa. Evidence of their presence in the airspora supports the possibility of dispersal around Antarctica in the air column. Further aeromicrobiology studies are required to understand the dynamics of fungal dispersal within and beyond Antarctica.

Potential export of organic carbon and nutrients from the coastal Antarctic ice sheet

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Physico-chemical and biological processes could influence the concentration of crucial biogeochemical components on glacier surface and those discharged to aquatic environments through melt water runoff. Here, we present data on the spatial and temporal variation of the chemistry of surface glacier samples collected along a 1.2 km transect (45 day period, 11 day interval) during summer. Samples were collected beginning at the ice cap in Grovnes peninsula, Larsemann Hills (East Antarctica), where meltwater from snow slush feeds a glacial stream before traversing through a cryoconite hole zone and eventually discharging into the Thala fjord. High debris loading on snow surface resulted in reduced albedo compared to clean snow, contributing to increased melting in this region. Concentration of major inorganic ions (Na⁺, Ca²⁺, K⁺, Mg²⁺, Cl⁻, SO₄²⁻, NO₃⁻) did not change substantially along the transect, but increased towards the end of melt season. Dissolved organic carbon concentrations in snow were below detection limit at the beginning, but increased considerably towards the end (up to 839.0 µg L⁻¹) of the season, indicating high biological activity on the glacier surface during summer. Trace element (Al, Mn, Ni, Cu, Se, Sr, Ba, Fe and Si) concentration increased in the order clean snow < dirty snow < meltwater < cryoconite holes, and was higher at the end, compared to the beginning of melt season. The high concentration of chemical constituents in the melt water suggests that during summer, the coastal Antarctic sheet could be an important contributor to DOC and nutrients to the surrounding coastal waters.

Reconstruction of the Functional Ecosystem in the High Light, Low Temperature Union Glacier Region, Antarctica

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The identity and functionality of microorganisms from polar glaciers are defined. However, little is known about microbial communities from the high elevation glaciers. The Union Glacier, located in the inland of West Antarctica at 79°S, is a challenging environment for life to survive due to the high irradiance and low temperatures. Here, soil and rock samples were obtained from three high mountains (Rossman Cove, Charles Peak, and Elephant Head) adjacent to the Union Glacier. Using metagenomic analyses, the functional microbial ecosystem was analyzed through reconstruction of carbon, nitrogen and sulfur metabolic pathways. A low biomass but diverse microbial community was found. Although archaea were detected, bacteria were dominant. Taxa responsible for carbon fixation were comprised of photoautotrophs (Cyanobacteria) and chemoautotrophs (mainly Alphaproteobacterial clades: Bradyrhizobium, Sphingopyxis, and Nitrobacter). The main nitrogen fixation taxa were Halothece (Cyanobacteria), Methyloversatilis, and Leptothrix (Betaproteobacteria). Diverse sulfide-oxidizing and sulfate-reducing bacteria, fermenters, denitrifying microbes, methanogens, and methane oxidizers were also found. Putative producers provide organic carbon and nitrogen for the growth of other heterotrophic microbes. In the biogeochemical pathways, assimilation and mineralization of organic compounds were the dominant processes. Besides, a range of metabolic pathways and genes related to high irradiance, low temperature and other stress adaptations were detected, which indicate that the microbial communities had adapted to and could survive in this harsh environment. These results provide a detailed perspective of the microbial functional ecology of the Union Glacier area and improve our understanding of linkages between microbial communities and biogeochemical cycling in high Antarctic ecosystems.

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