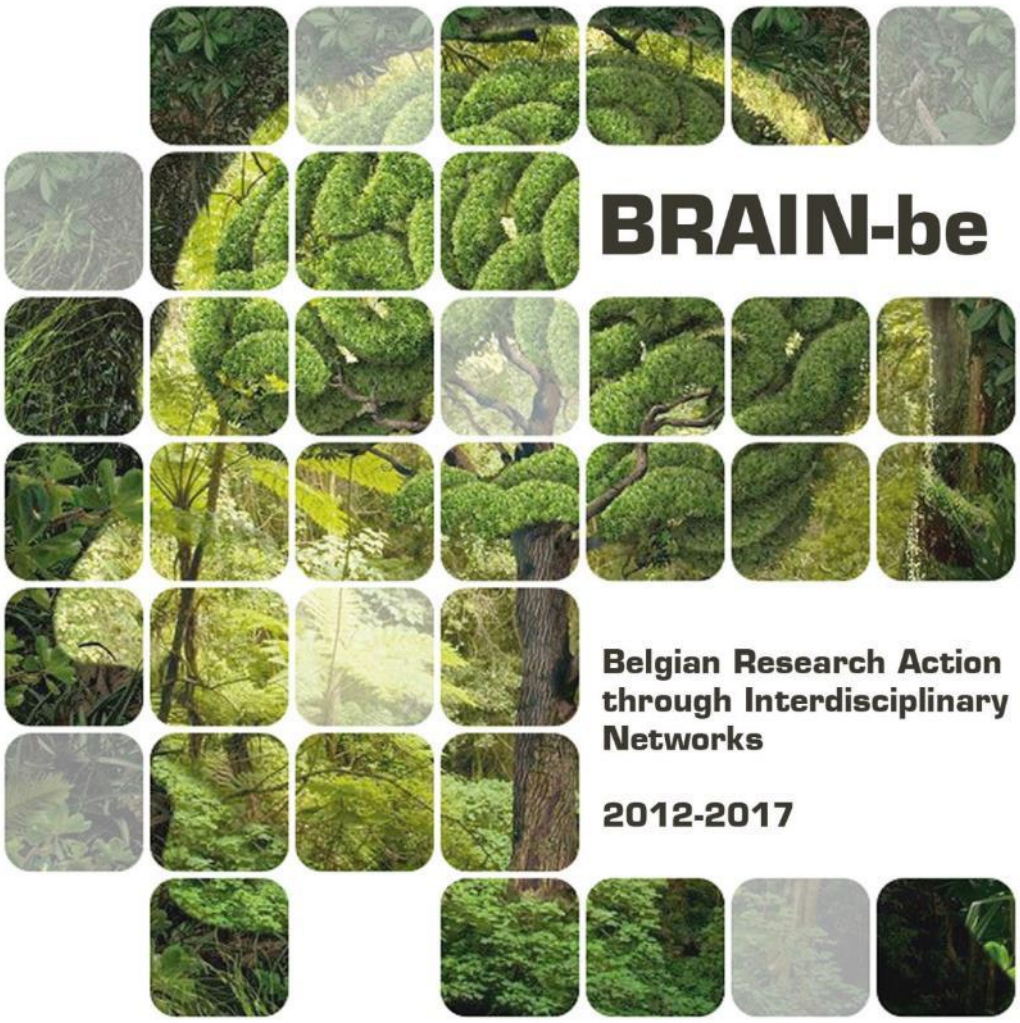
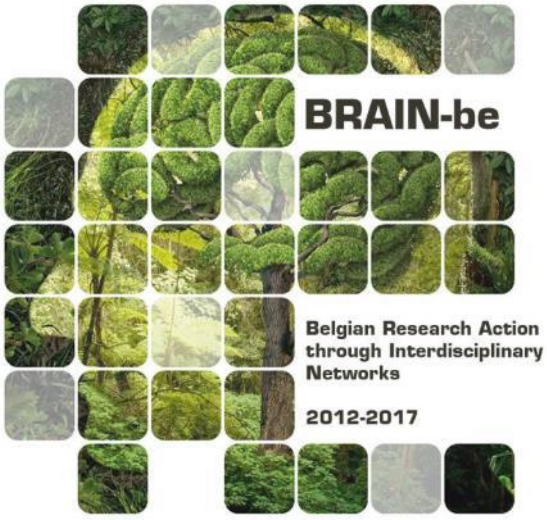


MICROBIAN

Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica

1. Wim Vyverman, Protistology & Aquatic Ecology, UGent
2. Bjorn Tytgat, Protistology & Aquatic Ecology, UGent
3. Valentina Savaglia, In-Bios Centre for Protein Engineering, University of Liège and Laboratory of Protistology & Aquatic Ecology, UGent
4. Sam Lambrechts , Laboratory of Microbiology and Laboratory of Protistology & Aquatic Ecology, UGent
5. Quinten Vanhellemont, Operational Directorate Natural Environment, Remote sensing and ecosystem modelling team
6. Bart Van de Vijver, Plantentuin Meise
7. Elie Verleyen, Protistology & Aquatic Ecology, UGent
8. Anne Willems, Laboratory of Microbiology, UGent
9. Annick Wilmotte. In-Bios Centre for Protein Engineering, University of Liège

Axis 1: Ecosystems, biodiversity and evolution



NETWORK PROJECT

MICROBIAN

Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica

Contract - BR/165/A1

FINAL REPORT

PROMOTORS:

1. Wim Vyverman, Laboratory of Protistology & Aquatic Ecology, UGent
2. Annick Wilmotte, In-Bios Centre for Protein Engineering, ULiège
3. Anne Willems, Laboratory of Microbiology, UGent
4. Quinten Vanhellemont and Anton Van de Putte, Operational Directorate Natural Environment, Remote sensing and ecosystem modelling team & Biodiversity and Ecosystems Data and Information Centre, RBINS
5. Bart Van de Vijver, Plantentuin Meise

AUTHORS:

1. Wim Vyverman, Protistology & Aquatic Ecology, UGent
2. Bjorn Tytgat, Protistology & Aquatic Ecology, UGent
3. Valentina Savaglia, In-Bios Centre for Protein Engineering, University of Liège and Laboratory of Protistology & Aquatic Ecology, UGent
4. Sam Lambrechts , Laboratory of Microbiology and Laboratory of Protistology & Aquatic Ecology, UGent
5. Quinten Vanhellemont, Operational Directorate Natural Environment, Remote sensing and ecosystem modelling team
6. Bart Van de Vijver, Plantentuin Meise
7. Elie Verleyen, Protistology & Aquatic Ecology, UGent
8. Anne Willems, Laboratory of Microbiology, UGent
9. Annick Wilmotte. In-Bios Centre for Protein Engineering, University of Liège

GHENT UNIVERSITY LIÈGE université museum Plantentuin Meise belspo .be

Published in 2022 by the Belgian Science Policy Office
WTCIII
Simon Bolivarlaan 30 Boulevard Simon Bolivar
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11
http://www.belspo.be
http://www.belspo.be/brain-be

Contact person: Maaike Vancauwenberghe
Tel: +32 (0)2 238 36 78

Vyverman W, Tytgat B., Savaglia V, Lambrechts S, Vanhellemont Q., Van de Vijver B, Verleyen E, Willems A, Wilmotte A. ***Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica.*** Final Report. Brussels : Belgian Science Policy Office 2022 – 61 p. (BRAIN-be - (Belgian Research Action through Interdisciplinary Networks))

TABLE OF CONTENTS

ABSTRACT

Context

The scarce ice-free areas in Antarctica are among the most extreme terrestrial environments on Earth. Life in these places is dominated by microbes. As a consequence, food webs are strongly truncated, with few metazoans consuming organic matter and microbial biomass. Elucidating the factors that shape the biodiversity of these microbiomes and control their contribution to biogeochemical processes, provides the scientific basis for habitat mapping and classification, for developing conservation strategies, for guiding long-term monitoring efforts and for predicting their possible response to future environmental changes. In this respect, inland nunataks in East Antarctica, like the Sør Rondane Mountains (SRM), are far less well-studied than those in more coastal locations and in the McMurdo Dry Valleys. This is surprising given their long-term exposure and their potential role as ice-free refugia during Neogene and Pleistocene glacial maxima and that the environmental conditions in these inland nunataks are different from those in coastal regions. The Sør Rondane Mountains (SRM) represent a c. 900 km² large nunatak, encompassing a large range of terrestrial habitats differing in geology and soil characteristics, exposure time and microclimatic conditions.

Objectives

The objectives of the MICROBIAN project were to (i) use a combination of remote sensing and close-range field observation techniques to map physical habitat characteristics and the presence and extent of microbial mat and biological crust communities in a radius of 200 km around the Princess Elisabeth Station Antarctica (PEA), (ii) generate a comprehensive inventory of the taxonomic and functional diversity of microbial communities in these habitats, and cultivate and characterize bacterial and cyanobacterial indicator taxa and deposit them as reference material in the BCCM collections, (iii) measure key ecosystem functions, including photosynthesis, in microbial communities representative of the major habitats in the region, (iv) use mesocosm field experiments to mimic the possible effects of future climate change on the functional and taxonomic diversity of these microbial ecosystems, and (v) conduct field experiments to inform policy-makers in view of decision making regarding environmental protection and prevention measures to reduce the introduction and spread of non-native species and to avoid cross-contamination between sites. The project will provide a proof of concept to use high resolution satellite images for identifying regions of particular biological interest in East Antarctica and more broadly make a significant contribution to understanding Antarctic terrestrial microbial ecology.

Conclusions

The use of high resolution multi-look optical satellite imagery allowed for an accurate estimation of the elevation, slope and aspect of Antarctic nunataks. These factors relate to water retention and light availability, which are important in determining the presence, composition and functioning of microbial communities in these environments. Additional drone acquisitions proved to be high performance, allowing for the estimation of centimetre scale

slope and aspects of individual sampling sites. The drone data also revealed inaccuracies in the GPS locations of the individual sampling sites, but will hence allow for refining those coordinates based on field photographs. Ground truthing for future research will also require setting up a denser regular network of temperature loggers to better analyse the effect of temperature and improve results accuracy. The Digital Elevation Model and temperature (both in situ and satellite) data collected in MICROBIAN could be used for setting up and evaluating a diurnal solar irradiance and surface heating model. These models could then be used to determine light and liquid water availability at the different mountain sides. This is important given the effect of these factors on the microbial communities and since large differences in temperature and relative humidity were observed between sampling plots along the moisture gradient in the different regions. The installed snowfences, however, mitigated temperature fluctuations compared to exposed gravels in the control zones. Sampling locations within the same bedrock type tended to have similar soil chemical characteristics. It is worthwhile to note, however, that the 'Dry Valley' region appeared to be unique in terms of the soil characteristics measured in this study, and did not resemble moraine samples from other regions (Austkampane and Yûboku Valley), probably because of the very peculiar soil and microclimatic conditions.

Analysis of the amplicon sequencing data showed that pH and bedrock type were the main abiotic factors structuring microbial communities in the ice-free regions. Bacterial communities mainly consisted of Actinobacteria, Acidobacteria and Cyanobacteria. Bacteroidetes and Chloroflexi were also particularly abundant while the remaining abundant phyla consisted of Proteobacteria, Abditibacteriota, Deinococcus-Thermus and Patescibacteria. Actinobacteria and Cyanobacteria often seemed to be inversely correlated, with Cyanobacteria being less abundant in moraine samples, while Actinobacteria being the most abundant. In contrast, Cyanobacteria represented an important portion of the Yûboku Valley diversity, where the only known lake systems within the western SRM are situated. Acidobacteria were found to be well represented in all kind of soils. In the eukaryotes, Chlorophyta was the most important phylum followed by Metazoa, Cercozoa and Ciliophora. Metazoa seemed to be well represented in all kind of soils but less in moraine soils, with Cercozoa being more abundant there and in marble soils.

Using shotgun metagenomics, we were able to reconstruct 373 draft genomes from 14 phyla with an average completeness of 86.29%. Genes for aerobic organotrophic respiration were among the most abundant, while genes for anaerobic respiration and fermentation were low across all samples. Among the genes not involved in aerobic respiration, the two most abundant genes are those encoding RuBisCO (*rbc*L; 47.6%) and form I carbon monoxide dehydrogenase (*cox*L; 23.3%) respectively. Genes involved in phototrophy were relatively rare (*psa*A; 8.21%, *psb*A; 12.4%, RHO; 6.7%). Our results suggest that atmospheric trace gas oxidation and chemosynthesis might be an important process in the Sør Rondane Mountains, supporting primary production independently of photoautotrophy. The distribution of these genes was positively correlated to elevation, and inversely correlated to moisture and total organic carbon. The highest abundance of genes involved in these alternative processes was found in the more extreme oligotrophic samples from Austkampane and the 'Dry Valley' region.

Combined, our results show that geographical aspects of the nunataks influence the presence and composition of microbial communities, in addition to bedrock type and geochemical factors such as pH. In these ultraoligotrophic environments, alternative primary production pathways seem to be crucial for the functioning of the microbial communities.
Our data were also used in policy support activities as part of SCAR and CEP meetings, e.g. for the proposal of the creation of an ASPA in the Sør Rondane Mountains. They were also presented during various outreach and knowledge disseminating activities.

Keywords
Antarctica, Princess Elisabeth Station, microbial biodiversity, habitat mapping, ecosystem function, climate change, human impact

1. INTRODUCTION

Terrestrial life in Antarctica is largely restricted to the scarce ice-free areas. These are among the most extreme environments on Earth and are therefore dominated by microorganisms (Cary et al. 2010, Cowan 2014, Tytgat et al. 2016). As a consequence, their food-webs include only few metazoans (Obbels et al. 2016) and community dynamics and ecological functions are therefore primarily regulated by microbial interactions and the chemical and physical environment (Niederberger et al. 2015). Native Antarctic terrestrial communities typically have a low diversity and may include many specialist taxa which would be poor competitors in case of invasion by competitors (Hughes & Convey, 2014), but whether this also applies to microbes is as yet unknown. A growing number of studies, including those by partners in the MICROBIAN consortium, are starting to reveal that the incidence of endemism can be surprisingly high in some of these terrestrial Antarctic microbial groups (Vyverman et al. 2010; Van de Vijver et al. 2011) and that patterns in their biogeographies are congruent with those observed in, for example, springtails, nematodes and tardigrades (Chong et al. 2015; Verleyen et al. 2021). In part, this can be explained by the long-term survival of these microorganisms and their associated microinvertebrates in glacial refugia and their long history of evolution in isolation since the start of the Cenozoic glacial-interglacial cycles. For developing conservation strategies, guiding long-term monitoring efforts, and predicting the possible future response of these unique terrestrial microbiomes to environmental changes, it is critical to elucidate how chemical and physical factors control their taxonomic composition as well as their contribution to biogeochemical processes. In this respect, there is very little information on the inland nunataks in comparison to those in more coastal locations and in the McMurdo Dry Valleys. Findings obtained in one region cannot however be simply extrapolated to others, because of regional differences in environmental gradients, (micro)climatic conditions and the composition and diversity of biological communities (Tytgat et al. 2016). Given their potential role as long-term ice-free refugia during glacial maxima (Convey et al. 2008), it is important to study these inland nunataks and their microbial communities.

2. STATE OF THE ART AND OBJECTIVES

This project focused on the Sør Rondane Mountains (SRM). Biological investigations started in the region of the Princess Elisabeth station, Antarctica (PEA) before its construction within the BelSPO ANTAR-IMPACT project (Fernandez-Carazo et al. 2011). Reconnaissance studies using high-throughput sequencing of soil and microbial mat microbiomes during the BelSPO project BELDIVA revealed that community composition varies with bedrock type and microhabitat conditions (Obbels et al. 2016), and that patterns in microbiome-environment relations are not fully congruent with those observed in the McMurdo Dry Valleys (Tytgat et al. 2016). In addition, a pilot study of pufM (light harvesting) and RuBisCO (carbon fixation) genes suggested an important role of as yet unknown groups of prokaryotes in the primary production of these ecosystems (Tahon et al. 2016).

The objectives of MICROBIAN were to:

i) use a combination of remote sensing and close-range field observation techniques to hierarchically map physical habitat characteristics and the presence/extent of microbial mat and microbial crust communities in selected ice-free regions in a radius of 200 km around the Utsteinen ridge;

ii) generate a comprehensive inventory of the taxonomic and functional diversity of microbial communities in these habitats and identify, cultivate and characterize key indicator taxa, deposit them as reference material in the BCCM collections and add their sequences to curated public databases;

iii) measure key ecosystem functions, including photosynthesis, in microbial communities representative of the major habitats as defined by differences in microclimatic conditions (temperature and moisture availability), exposure to wind and solar radiation, geochemical characteristics of the soils and the presence or absence of macroscopic life forms;

iv) use mesocosm experiments to mimic the possible effects of future climate change on the functional and taxonomic diversity and identify functions or taxa that are particularly affected;

v) conduct experiments to inform policy-makers in support of decision making regarding environmental protection and prevention measures taken to reduce the introduction and spread of non-native species and to avoid cross-contamination between sites.

3. METHODOLOGY

WP1: Remote sensing and development of Digital Elevation Models

A total of five study regions were selected in a radius of 200 km around PEA based on an existing climate and digital elevation model (DEM) developed by IP1 using satellite images, in combination with temperature and reflectance measurements obtained from Landsat 8. Tristereo imagery from the Pléiades series of satellites were acquired for the selected study sites in order to generate a high resolution DEM and to derive surface reflectance. Pléiades is a series of two identical satellites with a multispectral imager with 4 bands (blue, green, red and near-infrared) at a 2.8 m spatial resolution and a wide panchromatic band at a 70 cm spatial resolution. Multispectral an panchromatic imagery were resampled to 2 m and 50 cm by the satellite operator. The unique pointing capability of Pléiades allows acquisition of multi-stereo imagery that can be used to derive a high resolution DEM, and hence slope and aspect information. DEMs were first generated at reduced resolution (30 m) to allow for rapid development and were reprocessed in 2019 and 2020 to full resolution (approx. 1-2 m). An evaluation of the DEM data was published by Vanhellemont et al. 2021 showing good performance of the optical satellite retrieved elevation data. The DEM data are currently being further refined using images of drones equipped with high resolution and thermal cameras and a GPS (See T1.4).

Furthermore, using these multispectral observations at very high resolution could potentially resolve microhabitat characteristics and map microbial mats and soil crusts. To evaluate this, the images were atmospherically corrected and analysed for the presence of these features. The feasibility of automated detection algorithms was studied using existing and possibly novel spectral indices, but the signal of the soil crusts was found to be insufficiently strong, or inseparable from their surrounding environment. Multispectral imagery could however be used for detection of blue ice fields, and snow coverage in the mountains.

Task 1.1: Selection of ice-free regions (IP1, C, P2, P3, P4)

The selected ice-free regions included previously unexplored sites as well as regions for which samples are already available (i.e. Utsteinen ridge, Pingvinane and Perlebandet nunataks; Fig.3.1.1). However, for the latter regions, only a limited number of samples were available and there was a need to include additional samples from microbial crusts situated on a more diverse set of substrates. This was especially the case for Perlebandet, which was known to harbour a high density of microbial mats. Here, the southernmost and northernmost nunataks, consisting of gneiss with some outcrops of marble, were selected. The second site selected was the granitic Utsteinen ridge, on which the PEA is situated (at one extremity) and which also harbours a high density of microbial crusts. Both Perlebandet and Utsteinen are several kilometres away from the main mountain range, and are characterised by rather mild climatic conditions. The third selected region was Pingvinane, which is a series of granite nunataks situated close to the main mountain range. The fourth selected region was Yuboku Valley where the only two known lakes in the Western SRM occur. This area consists of chlorite-mica-epidote-amphidote schists. The fifth selected region is the unofficially called ‘Dry Valleys’ near Wideroefjellet which has a tholeiitic metatonalite substratum.

Analysis of satellite images revealed the presence of frost polygons on a moraine near Austkampane (71°44'08.24"S, 25°11'29.54"E). The frost polygons suggested the temporary

presence of liquid water through freeze-thaw cycles. However, during the BELARE 2017-18 expedition, it became clear that this sixth region is characterised by high katabatic winds due to the particular geomorphology of the nunatak and is a polar desert.
During the BELARE 2018-2019 campaign, two additional sites were surveyed in addition to the 7 sites visited during the first campaign. Pingvinane South is the southern nunatak of the Pingvinane mountain range and Petrellnuten is the nunatak situated at the south-west of this mountain range. (see Fig. 3.1.1, red points).

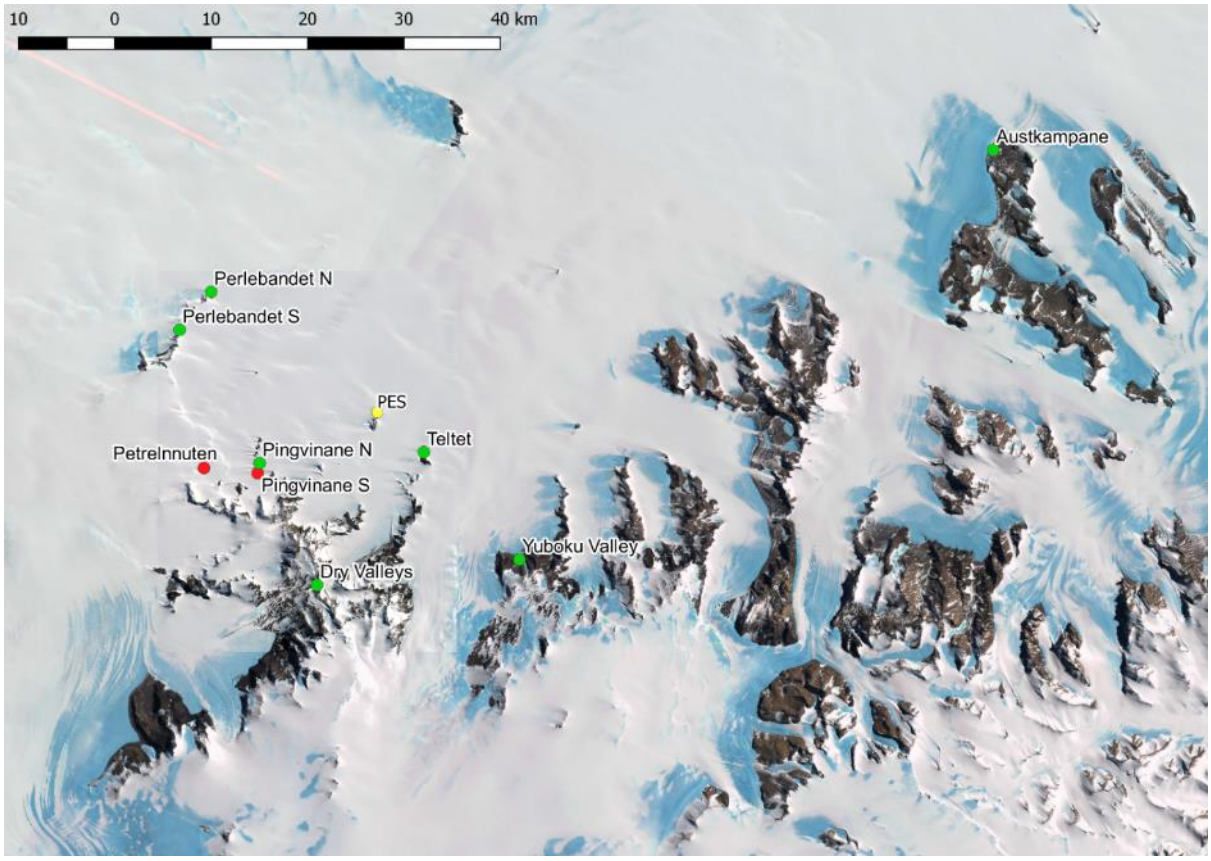


Fig. 3.1.1. Selected ice-free regions sampled during the BELARE 2017-18 and 2018-19 (green symbol) and only during 2018-2019 (red symbol) campaigns. In yellow is the Princess Elisabeth Station, Antarctica (PEA).

Task 1.2: High-resolution image processing and DEM generation (P4, C, P2, P3, IP1)
Pléiades tristereo imagery was acquired for approximately 100 square km around four study sites in 2018: Pingvinane and Utsteinen on 2018-01-28, and Utsteinen, Perlebandet, and Austkampane on 2018-02-02. Source data was produced by AIRBUS and archived, and the atmospheric correction developed by Vanhellemont & Ruddick (2018) was successfully tested, giving realistic aerosol optical thicknesses. The multi look imagery was used to generate a digital elevation model for these sites. Additional acquisitions were made in 2019: Perlebandet and Pingvinane on 2019-01-31, Utsteinen & Teltet on 2019-02-02. The new region 'Dry Valleys' was also imaged on 2019-01-31.
Tri-stereo Pléiades acquisitions were also made in 2020 for Utsteinen, Perlebandet, Dry Valleys, Pingvinane & Petrellnuten, Yuboku Valley, and for the Vesthaugen nunatak, north of

PEA. The Vesthaugen nunatak was imaged for testing the processing in a new region, and no ground reference data was collected there. Full archives of DEM datasets are processed for all 2018, 2019, 2020 imagery. Transects of elevation in the study sites were recorded using GPS loggers during the 2020 field campaign, to provide additional ground reference for the satellite derived products.

Options were evaluated for the generation of digital elevation models (DEM) from the tristereo Pléiades acquisitions. The use of epipolar geometry was abandoned in favour of the triangular mesh based SETSM (The Surface Extraction with TIN-based Search-space Minimization) model by Noh & Howat (2015, 2017), which is more robust for low contrast areas with repetitive elements. SETSM has been set up to generate a DEM for each image pair in the tristereo acquisition, with the average of the three pairs being used as final DEM. A neural network based image classification for the Pléiades imagery was developed for pixel identification. Pixels were manually identified for 5 classes (ice, blue ice, snow, mountain, shadow) with about 50 spectra in each class. A three layer neural network is used with 4 input neurons (relu, the reflectance in 4 bands), 5 hidden neurons (relu) and 5 output neurons (softmax, 5 classes).

Task 1.3: Validation of Landsat-8 surface temperature data (P4, C)

The at-sensor brightness temperature (BT) was retrieved from Landsat 8 imagery of the Sør Rondane mountains in the austral spring and summer of 2017-2018 to evaluate relative temperature patterns within a single scene, and will have to be further refined to land surface temperature (LST). The BT revealed that the surface temperature at the Tvetaggen/Austkampane site was significantly higher compared to the other sites, which could potentially be an artefact of site orientation and imaging time (Vanhellemont et al. 2021). The potential for the optical imagery was also evaluated and could include determination of e.g. the timing of snow pack melting. Full archive (2013-...) Landsat 8 data was collected and processed for the study sites. Preliminary matchups between the measurements from retrieved iButton and Landsat temperature (at sensor BT) were analysed, and showed promising performance.

A processor to generate surface temperatures (ST) from BT in the two bands on Landsat 8/TIRS was developed within the project, and was successfully used for several studies, including in the Belgian Coastal Zone (Vanhellemont 2020a), at Belgian meteorological sites (Vanhellemont 2020b), in the Sør Rondane mountains (Vanhellemont et al. 2021) and for near shore waters around the world (Vanhellemont et al. 2022). The ‘libradtran’ (Emde et al. 2016, Mayer & Kylling 2005) radiative transfer model was set up for modelling radiative transfer in the thermal domain, with resampling to the bands on the Thermal Infrared Radiometer (TIRS) on board Landsat 8. Atmospheric profiles from the balloon launches at PEA were acquired from RMI to parameterize the model, as well as profiles from the ERA5 reanalysis data, and inversion of the TIRS BT to ST was developed (details in Vanhellemont 2020a). The model was later also adapted to the thermal sensors on board Landsat 5 and Landsat 7, and will be adapted to Landsat 9 (launched September 2021) in the coming months. The processing software was made freely available as the Thermal Atmospheric Correction Tool (TACT) and is now integrated in the open source ACOLITE processor (https://github.com/acolite/acolite).

Task 1.4: Fine mapping of microbial communities (C, P4, SC1)

High resolution (cm scale) imagery was acquired during the 2020 field work for all visited sites. Several full nunataks imaging was also acquired near the end of the campaign. For each sampling, approximately 1x1 metre target markers were put out and left in the field for several hours during the drone acquisitions. These markers serve as accurate ground control points in the imagery with a well-known GPS position, both in latitude, longitude as well as in elevation. Centimetre scale models are being generated by Orthodrone, and preliminary datasets for the Northern Perlebandet and Pingvinane sites were obtained near the end of 2021. The drone data for these site were analysed, and compared to the satellite derived DEM. Drone data were used to compute the small scale (1 cm) slope and aspect information around the sampling sites.

Task 1.5: Remote sensing of other regions in Dronning Maud Land (P4, IP1, C)

Landsat processing methods are made generic for extension to other study sites, including non-Antarctic coastal and inland sites. The processing code was made publicly available under the name Thermal Atmospheric Correction Tool “TACT”: https://github.com/acolite/tact and is now integrated into the generic ACOLITE processor for L5, L7 and L8 processing of surface temperature data (https://github.com/acolite/acolite). Tri-stereo Pléiades imagery was also acquired for the Vesthaugen nunatak in 2020, and an interface to the Reference Elevation Model of Antarctica (REMA) was developed to extract DEM data for any other Antarctic site.

WP2: Ground-truthing, monitoring and field experiments

Task 2.1: Sampling for the analysis of the diversity and environmental conditions

For each region identified using the DEM, the intention was to sample all main habitats as determined by differences in pH, salinity, organic matter content, moisture availability and bedrock type (see Tytgat et al. 2016). During the first campaign, a total of 106 samples were taken along gradients in bedrock type, moisture availability, exposure and microclimatic conditions to meet these criteria at the following locations:

- Most northern nunatak of Perlebandet (21 samples)
- Most southern nunatak of Perlebandet (3 samples)
- Most northern nunatak of Pingvinane (7 samples)
- Utsteinen ridge (14 samples)
- Dry Valley (43 samples)
- Yuboku Valley (7 samples)
- Austkampane/Tvetaggen (11 samples)

During the second campaign a total of 20 samples were taken along gradients in bedrock type, moisture availability, exposure and microclimatic conditions at the following locations:

- nunatak of Petrellnuten (6 samples)
- Most southern nunatak of Pingvinane (6 samples)
- Yuboku Valley (8 samples)

The samples from both campaigns ranged from barren bedrock to substrates covered by biofilms and well-developed biological soil crusts consisting of lichens, mosses and microalgal

mats. All samples were collected aseptically using a spoon, immediately stored and transported in a cool box with ice-packs upon arrival to the station. All the samples were stored at -80 °C at the station and brought back to Belgium at -20 °C where they were stored until further processing at UGent. Sufficient material was collected for the analysis of soil characteristics including pH, specific conductance, the moisture content and the concentrations of nutrients, organic matter. All samples are georeferenced in a GIS environment and added to an existing database.

Task 2.2: Temperature and humidity loggers to measure microclimatic conditions (C, P2, P3, P4, IP1)

During the first campaign, 43 i-button Hygrochron (DS1923-F5#, Maxim Integrated Inc., USA) were installed, recording temperature and humidity every 3 hours during the entire duration of the project. These data will be compared to the climate model and Landsat 8 derived temperatures, and will provide us with a range of microclimatic conditions in different habitats potentially present for a given regional temperature range. The loggers were placed in strategic sites within the selected ice-free regions. Loggers were also placed in all newly installed OTC and snow fence plots, including their controls (16 in total). Three loggers were replaced in established OTCs. In addition, Labjack inc. (Denver, USA) Digit-TLH temperature-light-humidity sensors were placed in OTC2 and OTC11 on Utsteinen Ridge and their control plots, while the 6 remaining sensors were placed in the snow fence plots and their controls in the Dry Valley.

During the second campaign, 41 i-button temperature and humidity loggers were retrieved. The data were downloaded and the devices were installed in new sites. It was impossible to retrieve two i-buttons because of the extensive snow cover. Additional i-buttons were placed in two out of the three newly installed Open Top Chambers (OTCs, T.2.4), and 2 out of their 3 control plots (4 i-buttons in total). The remaining loggers were placed in strategic sites within the selected ice-free regions, namely in 3 out of the 6 sites on the Petrellnuten nunatak, in 4 out of the 7 sites on the southern Pingvinane nunatak, and in 4 out of the 8 sites on Yuboku Valley. Six loggers were replaced in sites along the moisture gradient established during the previous campaign. We selected sites characterized by the most extreme annual variability in temperature and relative humidity and those with the lowest amount of yearly variation. Nine i-buttons were re-installed in the already existing OTCs.

In the last sampling season, 43 i-button temperature and humidity loggers that were installed during the previous years were retrieved and collected. The data were downloaded and 41 devices were placed in all the OTCs and snow fence plots (see below), as well as in their control plots to further monitor the long term experiments.

Task 2.3: Measurements of carbon and nitrogen cycling

During the first field campaign it became clear that the amount of biomass was too low to conduct accurate field experiments for analysing processes active in the nitrogen cycle. It was therefore decided to focus on the photosynthesis in biological soil crusts and mat forming algae. In order to assess the photosynthetic efficiency in the field, measurements of photosynthetic activities were carried out in a selection of different habitats in the ice-free regions using a portable Double-Modulation Fluorimeter AquaPen-P AP 110-P (Photon

System Instruments, Czech Republic) equipped with red-orange light (630 nm) modified to work as a FluorPen. Four protocols were applied:

1) the chlorophyll fluorescence transient, recorded on dark-adapted communities during the first few seconds after the sample was exposed to the very short (1-2 s) actinic radiation (OJIP), was used to identify the photosynthetic response to stressful conditions (such as drought, low temperature or high irradiance);
2) the quantum yield (QY) used to measure the effective quantum yield (ΦPSII) on light-exposed communities was useful to estimate the photo-inhibition or any other kind of injury caused to the PSII complexes;
3) the non-photochemical quenching (NPQ) on dark-adapted communities was used as an indicator of the excess-radiant energy dissipation to heat in the PS II antenna complexes;
4) the light-curve (LC) on dark-adapted communities was useful to estimate the rETRmax (relative electron transport rate), the α parameter which represents the photosynthetic efficiency and the Ik parameter representing the irradiance at which the photo-inhibition starts. These protocols (1 to 4) were run at different moments of the day (morning, noon, afternoon and evening) in three different cyanobacterial hypolithic communities in the Utsteinen ridge. When necessary (protocols 1, 2 and 3), the communities were pre-darkened during at least 10-15 minutes.

Two Minikin RTi/QTi loggers (EMSO Brno, Czech Republic) were deployed to measure the surface and the deeper incident light, as well as the temperature. Two i-buttons® were used to simultaneously record temperature and relative humidity. The OJIP, NPQ and QY protocols were also run on communities living in different locations, namely a Lake in Yuboku Valley where cyanobacteria form large biofilms and mats on the edge of the lake and the melted edge of the windscoop near Petrellnuten nunatak where cyanobacteria colonize a thin layer under the gravel by forming very compact crusts. The measurements were always run in at least three different, but similar sites within the same location.

Task 2.4: Field experiments using Open Top Chambers and snow fences (C, P2, P3, IP2)

Fields experiments using snow fences and Open Top Chambers (OTCs) were installed in order to monitor the effects of predicted climate change such as increased snowfall and higher temperatures. Snow fences will increase snow accumulation down-wind of the snow fence. This will provide more biologically available water, protection against the cold, dry winds and radiation, but will also shorten the growing season. OTCs (Fig. 3.2.1) act as a small greenhouse, increasing temperatures in the OTC with on average 2.1 °C compared to the environment (Marion et al. 1997).

Over the course of the first two campaigns, nine new OTCs were installed in addition to the eight OTCs already in-place in the region since 2010. The locations for installation were selected during BELARE 2017-2018 in function of their accessibility and their different bedrock type, which appears to be an important factor in structuring the bacterial communities (Tytgat et al. 2016). The exact location was further guided by the remote sensing data and regional conditions in wind exposure, snow cover and geomorphology on site. One was installed on the Utsteinen ridge situated on granite bedrock; two + three on a marble vein at different

altitude and marble percentage on the northernmost nunatak of Perlebandet and three on the 'Dry Valley' moraine.

Fig. 3.2.1 OTC installed in Dry Valleys.

Two snow fences (Fig. 3.2.2) were constructed and installed in the Dry Valleys in order to experimentally assess the effect of altered snow cover and duration on the microbial communities, particularly in these extreme environments.

T_0 samples were taken in triplicate in both the experimental plots and controls of the snow fences and OTCs. The plots were resampled during the later campaigns, i-button data were downloaded and the loggers were reinstalled in place.

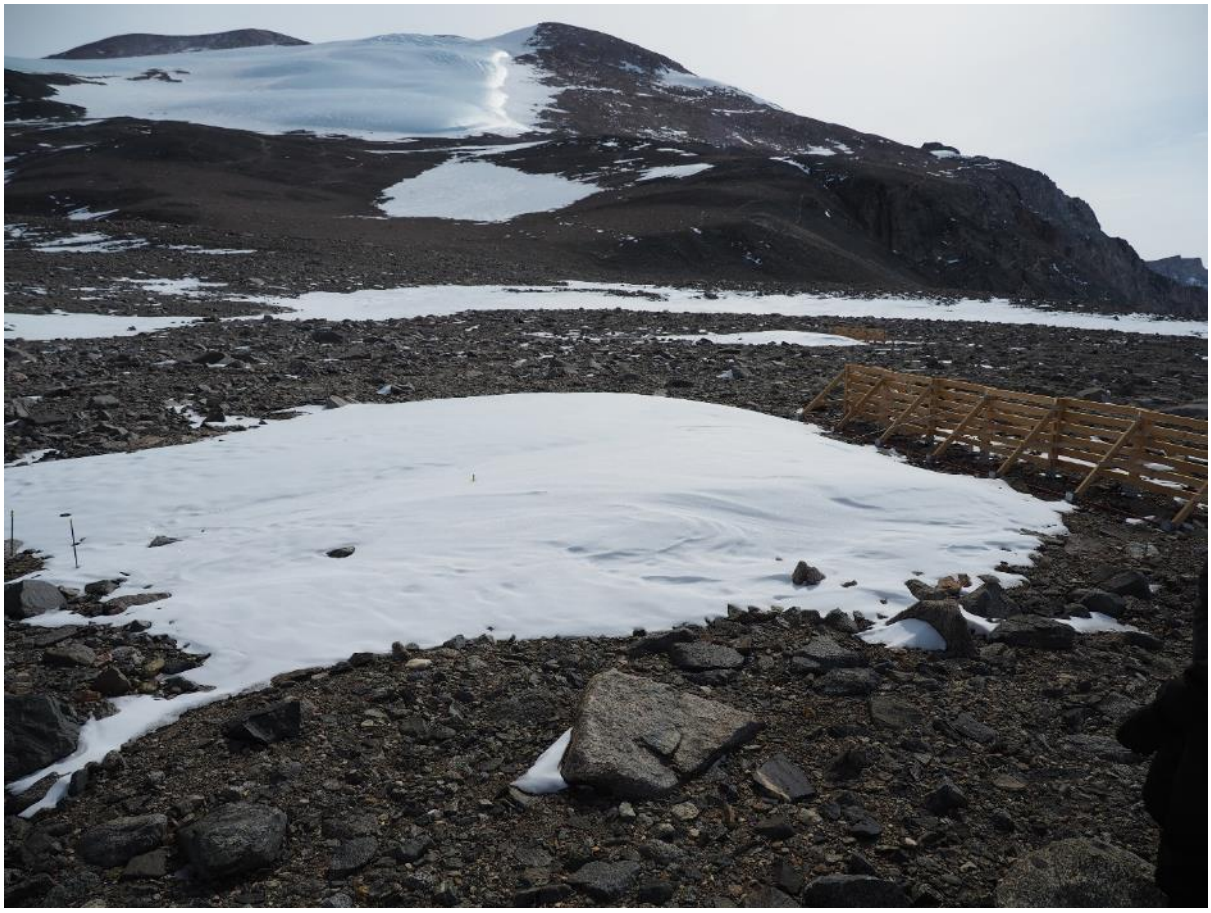


Fig. 3.2.2 Snow fences in Dry Valleys with windscoop formation behind it. The two snow fences were placed perpendicular to the wind direction, so that the snow would accumulate behind.

WP3: Biodiversity analyses and measurements of environmental factors

Task 3.1: Analysis of environmental factors

For field surveys and experiments, abiotic factors known to structure microbial communities in Antarctic soils (i.e., nutrient and ion concentrations, water availability, pH, microclimatic conditions, geochemistry; Cary et al. 2010; Tytgat et al. 2016, Verleyen et al. 2011) were measured in soil samples which were kept frozen and in the dark until analysis. Material for the analysis of the abiotic factors was subsampled from the samples taken for the microbial diversity analysis. Following defrosting, samples were homogenized prior to subsampling and sieved (2 mm mesh size). The pH (in water and in KCl buffer), electric conductivity, total nitrogen, $N\text{-}NH_4$, $N\text{-}NO_3$, $P\text{-}PO_4$, total phosphorus, and total organic carbon (TOC) in the soils were analysed following Czech and European Union standards (ISO 10390, ISO 10523, CSN EN 27888, ISO 11465, CSN EN ISO 11732, CSN EN ISO 13395 and CSN EN ISO 15681-1). To determine moisture content, soil samples were dried at 40 °C until no further mass reduction is observed (at least 24 h in similar samples from Antarctica; Tytgat et al. 2016).

Task 3.2: Measurement of gas concentrations

Insufficient biomass was present to conduct this task as mentioned above and in intermediate project reports.

Task 3.3: Biodiversity assessments using amplicon sequencing (P2, P3, C)

The samples collected during the BELARE 2017-18 campaign were analysed using amplicon sequencing for microbiome biodiversity assessment. For each sample, DNA was extracted using the Qiagen DNeasy Powersoil kit, and different amplicon libraries were constructed by targeting the 16S and 18S rRNA genes for bacteria and eukaryotes, respectively. For the bacteria, PCRs targeting the V1-V3 hypervariable regions of the 16S rRNA gene were performed using the pA and BKL1 primers. For the eukaryotes, the V4 region of the 18S rRNA gene was amplified using the general forward eukaryote primer TAReuk454FWD1, and TAReukREV3 as the general eukaryotic reverse primer (Stoeck et al. 2010). PCRs were performed in duplicate and subsequently pooled to minimize potential biases, after which they were purified with Agencourt AMPure XP beads (Beckman Coulter Inc.). The amplicon libraries were barcoded using the Nextera XT index kit (Illumina Inc.) according to an adjusted protocol (Tytgat et al. 2016). Quality was checked using a BioAnalyzer (Agilent Technologies) and DNA concentration was measured using a Qubit (Thermo Fisher Scientific), after which the samples were pooled equimolarly and sent for sequencing on a 300 bp paired-end Illumina MiSeq platform. Sequences were analysed by a well-established pipeline within the labs of C and P3. Paired-end merging was performed using PEAR (Zhang et al. 2014), pre-processing and quality control of the paired sequences, chimera detection (Edgar et al. 2011), and OTU binning was done using UPARSE (Edgar 2013) with default OTU clustering at 97%, and taxonomic identification was done using the naïve Bayesian classifier (Wang et al. 2007) implemented in MOTHUR (Schloss et al. 2009) using curated reference taxonomic databases (PR2, Silva v132, NCBI). To validate these results, sequences were analysed using a different methodology based on amplicon sequence variants (ASV). Raw reads were processed using the DADA2 package (Callahan et al. 2016) within R (R Team 2018). For 16S rRNA, primers were removed from the raw reads by trimming the first 20 and 21 nucleotides from the forward and reverse reads respectively using 'trimLeft = c(20,21)'. Reads were truncated (at base pair 297 and 257 for forward and reverse reads, respectively, using 'truncLen=c(297,257)'), and filtered with a maximum number of 'expected errors' (maxEE) threshold of two for both forward and reverse reads. For 18S rRNA, primers were removed from the raw reads by trimming the first 20 and 18 nucleotides from the forward and reverse reads respectively using trimLeft = c(20,18). Reads were truncated (at base pair 297 and 267 for forward and reverse reads, respectively, using truncLen=c(297,267)), and filtered with a maximum number of 'expected errors' (maxEE) threshold of two for both forward and reverse reads. Reads not matching these criteria were discarded. For each sequencing run, following sequence dereplication, sequence variants for the forward and reverse reads were inferred based on an error matrix constructed from the first 1e8 bp of the sequences. Singletons were discarded, and paired-end reads were merged with no mismatch allowed and a required minimum overlap of 20 bp. Sequence tables for each sequencing run were constructed using 'makeSequenceTable(mergers)', and subsequently merged with 'mergeSequenceTables'. Finally, chimeric sequences were removed using 'removeBimeraDenovo'. Taxonomic

classification of the resulting sets of Amplicon Sequence Variants (ASVs) was done using classify.seqs in mothur with the SILVA v138.1 (Pruesse et al. 2007) and PR2 version 4.14.0 (Guillou et al. 2012) databases for the 16S and 18S ASV sets respectively. The ASVs were classified based on the rdp classifier (Wang et al. 2007) with a bootstrap of 80.
To have an estimation of the abundance of uncultured bacteria in Antarctic soil samples, we repeated the analysis of Lloyd et al. (2018) using sequence data for 508 Antarctic soil samples kindly provided by Jeff Bowman (Bowman, 2018). These results were published in Lambrechts et al. (2019). Additional downstream data analysis is being performed using several software packages and pipelines such as *paprica* (Bowman et al. 2015).
For Cyanobacteria, DNA was extracted using the Qiagen Biofilm kit and the Qiagen DNeasy Powersoil Pro kit depending on the type of sample. Different amplicon libraries were constructed and the partial V3-V4 region of the 16S rRNA gene was amplified using the cyanobacterial-specific CYA359F forward primer and either CYA781Ra or CYA781Rb as reverse primer (Boutte et al. 2006). Primers were modified to include a 10-bp sample-specific barcode tag at the end, to allow samples to be multiplexed for sequencing. PCRs were performed in duplicate and pooled for each sample, purified using the NucleoSpin Gel and PCR Clean-Up kit (Macherey-Nagel). Quality was checked with agarose gel electrophoresis (0,8% agarose, 100 V, 60 mins), and good quality PCR products were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific). Samples were finally multiplexed at equimolar concentration. Afterwards the samples were further purified with Agencourt AMPure XP beads (Beckman Coulter Inc.). Since the cyanobacterial amplicon libraries were made with adapter ligation to full-length PCR products, they were sequenced with a 300 bp paired-end Illumina MiSeq platform.
Sequences were analysed by a well-established pipeline within the lab of P2. Quality control of reads and filtering of the chimeric sequences based on UPARSE (Edgar et al. 2013) was adapted from Pessi et al. (2016). After performing paired-end merging using PEAR (Zhang et al. 2014), reads presenting in the 3' and 5' ends two and zero mismatches with primer and barcode sequences, respectively, were removed from the dataset. The maximum expected error was set to 0.5 and the reads length to a minimum of 370 bp. Singletons were subsequently removed and denoising was applied using unoise3 (Edgar, 2016) in order to remove chimeras. The resulting zero-radius OTUs (zOTUs) were eventually clustered into OTUs with a 99% similarity threshold using UPARSE (Edgar, 2013). Taxonomic identification was done using the naïve Bayesian classifier (Wang et al. 2007) implemented in MOTHUR (Schloss et al. 2009) using curated reference taxonomic databases (Greengenes, NCBI).

Task 3.4: Assessment of the diatom diversity using microscopy (P5, C)

Subsamples were also taken for the microscopic identification of diatoms. Meanwhile, other samples from related areas on the Antarctic Continent have been analysed. More than 100 historic samples from more than 10 localities on the Antarctic Continent, the Maritime Antarctic Region and the sub-Antarctic Islands (Campbell Island) have been collected at the British Antarctic Survey moss herbarium and have been analysed. In addition, samples collected during previous field campaigns on the Antarctic Continent (Vestfold Hills), sub-Antarctic (Crozet archipelago) and Maritime Antarctic Islands (Livingston Island & James Ross Island) have been reanalysed to identify taxa related to the continental taxa. All material was

chemically processed to obtain permanent slides and stubs for light (LM) and detailed scanning electron microscopy (SEM).

Task 3.5: Integration of biotic and abiotic data and species distribution modelling

Downstream data analysis and statistics were performed using several R packages used for multivariate ecological analyses such as vegan (Oksanen et al. 2015) and phyloseq (McMurdie and Holmes 2013). Ordinations and cluster analyses were used to explore the relation between amplicon data and environmental variables such as those obtained from the DEM (Task 1.2), the temperature and humidity loggers (Task 2.2) and the other environmental variables (Task 3.1). Using these results, an additional set of samples was selected for shotgun metagenomics.

WP4: Functional diversity

Task 4.1: Metagenomics (P3, C, P2)

A total of 63 samples covering all different habitats that were sampled were chosen for an in-depth shotgun sequencing metagenomics study based on potentially interesting *in situ* observations and amplicon sequencing results. Extraction of the DNA was done as described above in T3.3. No RNase treatment was added to remove any traces of RNA. Integrity and concentration of the extracted DNA was checked using a BioAnalyzer instrument (Agilent Technologies). Library preparation was done using the Illumina Nextera XT DNA Library Preparation Kit. The sequencing on an Illumina NovaSeq 6000 machine was outsourced to Genewiz (Leipzig, Germany) for 57 samples, and Baseclear (Leiden, The Netherlands) for 6 samples. Adapter removal and quality trimming of the reads were performed using the default settings of Trim-Galore (https://github.com/FelixKrueger/TrimGalore), and quality reports of the raw and final read sets were produced using FastQC (Andrews, 2010). The reads from each sample were assembled individually and collectively with MEGAHIT (min k: 27, max k: 127, k step: 10; Li et al., 2016), and assembly reports were generated using Quast (Gurevich et al., 2013). Gene-based analysis was performed using Squeezemeta (Tamames and Puente-Sanchez, 2019) and genome-resolved based analysis was done using Metagenome-Atlas (Kieser et al., 2020). GTDB-Tk (Chaumeil et al., 2020) was used to assign taxonomic classifications to the metagenome-assembled genomes (MAGs) based on the Genome Database Taxonomy (GTDB). GUNC (Orakov et al., 2021) was used to remove potentially chimeric MAGs with incongruent genomic and taxonomic properties. Using a threshold average nucleotide identity of 99%, MAGs from different assemblies were consolidated to a non-redundant set of 373 medium- and high quality draft genomes using dRep (Olm et al., 2017). Completeness and contamination of MAGs was assessed using CheckM (Parks et al., 2015). Open reading frames (ORFs) in MAGs were predicted using Prodigal (Hyatt et al., 2010).

To estimate the metabolic capacity of the sampled communities, metagenomes and metagenome derived genomes were searched against custom protein databases of representative metabolic markers using DIAMOND (query cover > 80%; Buchfink et al., 2021). Searches were carried out using all quality-filtered unassembled reads with lengths over 140 bp and the ORFs of the 373 MAGs. The metabolic markers searched are involved in sulfur cycling (AsrA, FCC, Sqr, DsrA, Sor, SoxB), nitrogen cycling (AmoA, HzsA, NifH, NarG, NapA,

NirS, NirK, NrfA, NosZ, NxrA, NorB), iron cycling (Cyc2, MtrB, OmcB), reductive dehalogenation (RdhA), phototrophy (PsaA, PsbA, microbial rhodopsin), methane cycling (McrA, MmoA, PmoA), hydrogen cycling (large subunit of NiFe-, FeFe-, and Fe-hydrogenases), isoprene oxidation (IsoA), carbon monoxide oxidation (CoxL, CooS), succinate oxidation (SdhA), fumarate reduction (FrdA), and carbon fixation (RbcL, AcsB, AclB, Mcr, HbsT, HbsC). Results were filtered based on an identity threshold of 50%, except for group 4 NiFe-hydrogenases, FeFe-hydrogenases, CoxL, AmoA, and NxrA (all 60%), PsaA (80%), PsbA and IsoA (70%), and HbsT (75%). Subgroup classification of reads was based on the closest matching database representative sequence. To search for the presence of an additional set of genes involved in oxidative phosphorylation (AtpA), NADH oxidation (NuoF), aerobic respiration (CoxA, CcoN, CyoA, CydA), formate oxidation (FdhA), arsenic cycling (ARO, ArsC), and selenium cycling (YgfK), a second set of custom databases were used. The search of these genes in unassembled reads and ORFs of genomes was carried out as described above, using the DIAMOND blastp algorithm with a minimum percentage identity of 60% (NuoF), 70% (AtpA, ARO, YgfK) or 50% (all other databases). Counts for each gene were normalized to the length of that gene and to the sequencing depth of the corresponding samples. To analyze the proportion of community members that encode each of these genes, the resulting coverage of each gene per sample was divided by the coverage of the RecA gene. This resulted in an estimation of the average number of copies of each of these genes per genome in our samples (average copy number). These results were compared to the results of the occurrence of these genes in the reconstructed genomes per phyla per sample.

A subset excluding Cyanobacteria dominated samples is being processed by P3, and focuses on the creation of Metagenome-Assembled Genomes (MAGs) of non-Cyanobacterial microorganisms. P2 focuses on the reconstruction of cyanobacterial genomes from 56 samples containing Cyanobacteria. The raw reads were quality filtered using the illumina-utils library (Eren et al. 2013) v2.6. Briefly, low quality sequences were removed using the program ‘iu-filter-quality-minoch’ with default parameters, which computes a noise filtering, as described in Minoche et al. (2011). Filtered reads were then assembled individually using MEGAHIT (Li et al. 2014) v1.2.9, with a minimum scaffold length of 1000 bp. Short reads were then mapped from the quality filtered reads to the contigs resulting from this assembly, using Bowtie2 (Langmead et al. 2012) v2.3.5.1 and the reads stored as BAM files using samtools (Li et al. 2009). Contigs were then clustered with the binning algorithm MetaBAT2 (Kang et al. 2019) using –verysensitive option. Quality assessment of the bins were then assessed using the ‘Taxonomic and Lineage-specific workflow’ implemented in CheckM (Parks et al. 2014). The information of coverage, taxonomy, completeness and size of the bins were used to select the cyanobacterial bins and assess their quality. Then, focusing on the Nostocales order, we performed a phylogenomic analysis to place these genomes in a phylogenetic context using ANVI’O. For this, we retrieved all the Nostocales reference genomes from the GenBank database. A multi-locus sequence alignment based on amino acid sequences of 23 ribosomal proteins was generated using MUSCLE v3.8 (Edgar, 2004), and a maximum likelihood tree was constructed using FastTree v2 (Price et al. 2009). Then, a pan-genome analysis was conduct in order to highlight the genes clusters that are specific to our Nostocales bins.

The overall functional metagenome analysis of all 60 samples is performed by P1 and is currently being processed using the SqueezeMeta pipeline.

Task 4.2: Cultivation, characterization and preservation of bacterial indicator taxa (P3, C)

In the BELDIVA project, Tytgat et al. (2016) showed the importance of Ellin6075-related species of the Acidobacteria phylum in dry gneiss soils and candidate division FBP in wet granite soils of the Sør Rondane Mountains. For the FBP phylum, we recently succeeded in growing an isolate which has been characterized and has now been named *Abditibacterium utsteinensis* (Tahon et al. 2018). However, polar Ellin6075-related species have not been cultivated so far. This new biodiversity assessment may allow to detect other taxa potentially sensitive to rising temperature and moisture availability, that will become new targets for cultivation.

A first isolation campaign has been set up to assess using lower pH values and a mix of amino acids as carbon source to facilitate isolation of new groups from terrestrial samples. Samples containing more than 60% Ellin6075-related species based on 16S rRNA gene amplicon sequencing were chosen. Dilution series were made and plated out for incubation at 4 and 15°C. All experiments were carried out in duplicate.

Task 4.3: Isolation and characterization of cyanobacterial strains (P2, C)

During the BELDIVA campaigns of 2009-2010, Zorigto Namsaraev sampled different soil biofilms in a zone around the Belgian Princess Elisabeth Station. Not all the samples were analysed yet and because of the highest abundance of cyanobacteria found on granite-derived bedrock (Tytgat et al., 2016; Pushkareva et al., 2018), samples from these substrates were chosen to be plated for cultivation and isolation. A total of 10 samples taken from crusts and biofilms located in Tanngarden, Petrellnuten and Pingvinane were plated and are currently incubated at 4 and 12 °C under continuous fluorescent lighting with photosynthetically active radiation (PAR) intensity of ~ 6 µmol m^{-2} s^{-1} with BG11 and BG110 solid and liquid media. Samples from cryoconites collected on Teltet and Utsteinen nunataks were cultured in liquid media with different dilutions (10^0 to 10^{-3}) and are also currently incubated at the same temperatures. Communities are often exposed to oligotrophic environments, which is the reason why it is interesting to simulate poor nutrient conditions that are normally observed in the field. We thus expect that oligotrophic solutions may lead to the growth of more recalcitrant species. So far, three different filamentous strains (*Nostoc* sp.) were successfully isolated and were deposited in the Belgian Coordinated Collection of Microorganisms (BCCM, http://bccm.belspo.be/) - Cyanobacteria Collection based at the University of Liège as ULC606, 608 and 609.

Work package 5: Policy support activities

In addition to the activities listed below, P1, P2 and P4 are involved in SCAR activities that are linked to policy support. P1 is member of the Steering committee of the Antarctic Near-shore and Terrestrial Observation System (ANTOS), P2 participated to the discussion about the science and implementation plan of the new SCAR SRP programme ANT-ICON (Integrated Science to Inform Antarctic and Southern Ocean Conservation) that was endorsed at SCAR2020. This SRP aims to encourage high-quality transdisciplinary research to inform

conservation and management of Antarctica. P4 is the Antarctic regional node manager of OBIS and GBIF and Deputy Chief Officer of the Expert Group on Biodiversity Informatics. P2 co-authored a paper on the strengthening of the links between science and governance for a better Antarctic environmental protection (Hughes et al., 2018).

Task 5.1: Assessment of cross contamination risks between nunataks and contamination of the environment (P2, P3)

This work is intended to produce information on the efficiency of measures currently suggested in the SCAR codes of conduct for terrestrial field research and research in geothermal areas, with the aim to avoid cross contamination of microbial populations between different nunataks, and the homogenization of their diversity.

A first cross-contamination experiment was performed during the 2018-19 field campaign. The experiment was aimed at assessing the transportation of microbes on knees while sampling terrestrial ecosystems in Continental Antarctic deserts. Chirurgical masks were put on both knees and sampled before (T_0) and after 2 minutes of kneeling (T_1). The latter was used to simulate the activity during soil sampling and consisted of two treatments, namely (i) one without prior kneeling in a snow patch and (ii) one with prior kneeling during 2 minutes in a snow patch. The latter treatment was added because we observed during previous sampling activities that snow attached to knees promotes the attachment of small rocks. The local terrestrial communities were subsequently sampled to provide a reference dataset against which the microorganisms on the knees can be compared. This experiment was repeated 3 times on the Utsteinen moraine, in three different places. All the sampled masks were stored at -80 °C at the station and brought back to Belgium at -20 °C.

A second cross-contamination experiment was performed during the 2019-2020 field campaign. The experiment was aimed at assessing the transportation of microbes on shoe soles while walking to the sampling area of terrestrial ecosystems in Continental Antarctic deserts. Soles of boots were sterilized using ethanol 70% upon arrival in the field and a first sample was immediately taken (T_0) with the help of a cotton swab, passed thoroughly around all the sole cavities. Another sample was also taken after around 30 steps in the snow (T_1). After a walk of 300 steps on bedrock, another sample was taken (T_2) followed by another sample taken after another 30 steps in the snow (T_3). The latter treatment was added because we observed during previous sampling activities that snow could almost completely “wash” the boot’s sole, by detaching all the small rocks that were stuck in the sole cavities. The local terrestrial communities were subsequently sampled to provide a reference dataset against which the microorganisms on the soles can be compared. This experiment was repeated 3 times on the Dry Valleys moraine, in three different places. All the sampled swabs were stored at -80 °C at the station and stored in Cape Town at -20 °C. Samples were shipped to Belgium at -20 °C and are currently stored in Belgium at -20 °C until further processing.

Task 5.2: Participation and preparation of CEP meetings (P2 and all partners)

A. Wilmotte (Partner P2) was member of the Belgian delegation to the Committee on Environmental Protection (CEP) of the Antarctic Treaty and participated to the discussion of the documents (co-)sponsored by Belgium during the following CEP meetings: XXth meeting in Bejing, China (22 to 26 May 2017), XXIst meeting in Buenos Aires, Argentina (13 to 16 May

2018), XXIInd meeting in Prague, Czech Republic (01 to 05 July 2019), XXIIIrd meeting in Paris, France (online, 14-18 June 2021) and XXIVth meeting in Berlin, Germany (23-27 June 2022). In 2019, she also attended a Joint SCAR / CEP Workshop on Further Developing the Antarctic Protected Area System from 27-28 June. She translated in French an Information Summary on microbial invasions for the Antarctic Environments Portal, a science-policy communication tool about environmental topics discussed at CEP (Les introductions de micro-organismes non indigènes : quel risque pour les écosystèmes antarctiques?).

A. Wilmotte (P2) is the Belgian contact point for Education and Outreach on Antarctic research, and gathers the information about Belgian activities as an input for the ATCM Forum on Education and Outreach. She also co-authored a publication on Education and Outreach by the Antarctic Treaty Parties, Observers and Experts in the frame of the Antarctic Treaty System (Xavier et al. 2019).

Task 5.3: Identification of ASPA (P2 and all partners)

This task is aimed at identifying specific regions that need protection based on biodiversity assessments and other values listed in Annex V of the Madrid Protocol. During CEPXX in 2017, in the Working Paper WP42 presented by Belgium, a number of isolated ice-free areas (Tanngarden Ridge, Petrelnutten Nunatak, range of Pingvinane Nunataks, Perlebandet range, and a part of the Teltet Nunatak) were proposed as ASPA sites, with the glacial areas in between remaining free for transportation. During the first field campaign, we discovered the presence of well-developed lacustrine microbial mats in the lakes in Yuboku Valley. Because lacustrine microbial mats are, to our knowledge, only present in this specific region of this part of the Sør Rondane Mountains, we proposed to include the lakes in Yuboku Valley to the list of protected ASPA sites. During the 2018-2019 campaign, other ice-free regions of the Pingvinane nunataks than those visited during the first campaign were surveyed. This survey revealed the presence of well-developed BSCs and a higher visible biomass compared with the most northern Pingvinane nunatak visited during the 2017-2018 campaign. This is in agreement with an initial survey within the BELDIVA project. The most problematic site was the Utsteinen ridge, that is also very rich in visible biomass, but its proximity to the station makes it difficult to designate it as an ASPA site. Thus, there were two possibilities: 1) along the 700 m long ridge, find an area that can be designated as ASPA site without causing problems for the functioning of the station, or 2) to develop 'site guidelines' (as is done for touristic sites) for the visitors to protect the biological communities. Two meetings with members of IPF to discuss IPF's critiques of the draft of the ASPA in the Sør Rondane Mountains highlighted that the inclusion of a part of the ridge was not acceptable for IPF.

Three steps were needed to follow the procedure outlined by the CEP:
1) In 2017, for CEPXX, Belgium presented WP42 on a preliminary assessment of the creation of a Antarctic Specially Protected Area (ASPA) in the region of the Sør Rondane Mountains. The CEP "*agreed that the environmental and scientific values found at the Sør Rondane Mountains site, including generally poorly studied organisms, merited further consideration for potential designation as an ASPA, enhancing the representation of ASPAs in the Antarctic Conservation Biological Region ACBR 6*". It was also noted that information provided to ATCM XL indicated a potential increase in traffic in the area in the future, which could underpin the

need to protect pristine areas in this region. The Committee welcomed Belgium's intention to further consider the development of a draft management plan for the area, and noted that several Members had expressed an interest to contribute to the work. The Committee noted a range of areas and topics for possible further consideration by Belgium. These included: consideration of further explanation of the values of the area in light of the provisions of the Annex V, including its 'outstanding values'; consideration of the merit of designating the area as an ASPA in light of existing management arrangements; consideration of the implications of a possible increase of activities in the area; consideration of historical activities which could inform the identification of possible inviolate areas that may warrant further specific protection; the possible exclusion of ice-covered areas between the ice-free areas; the possible inclusion of the Utsteinen Ridge within the proposed area; the identification of possible risks associated with interactions between the station activities and the area in question; and the provision of further information about the presence of a petrel colony and the possible presence of endemic microbes, invertebrates and lichens. It follows that the MICROBIAN project was able to provide this information useful for the draft management plan, and thus, contributed to the science-policy interface.

2) In 2018, for CEPXXI, the MICROBIAN partners participated to the redaction of IP42 on "Update on the proposed Antarctic Specially Protected Area (ASPA) in the Western Sør Rondane Mountains". This Information Paper provided updated information to the Committee on the works carried out during the inter-sessional period. As "Outstanding values of the proposed area in light of the provisions of the Annex V", it referred to the published data on microbial biodiversity, the unique representativeness of mountainous ecosystems in ACBR6, the hypothesis that the region potentially acted as a glacial refugium for living organisms during the glacial cycles, and the scientific experimental value of the region. All the points raised by the CEP in 2017 were answered in IP42. As Information Papers are not discussed during the session, Belgium proposed to discuss the text with interested parties during side-meetings.

3) In order to submit a Working Paper with a draft Management Plan at CEPXXIV in 2022, maps with a sufficient resolution of the different sub-regions were needed. This was necessary to clearly identify the access paths, calculate the exact surface area and delineate the areas that require protection. The participation of P4 during the field campaign of 2019-20 facilitated the creation of these maps based on remote sensing data. In April 2022, after redaction by the MICROBIAN partners, a final draft Management Plan was sent to the involved Ministries and, after their agreement, it was presented by Belgium during CEP XXIV as WP15. The CEP discussed the document and agreed to advise the ATCM that it had decided to forward the following draft management plan for protected areas to the Subsidiary Group on Management Plans for review during the intersession. The MICROBIAN project will continue to provide useful information for the management plan, and thus, to the science-policy interface.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

Work package 1: Remote sensing and high-resolution mapping

This work package used a combination of airborne, satellite and climate model data with a hierarchical spatial resolution, ranging from 10 kilometres to a few centimetres. The aim was to map microbial communities with a high spatial resolution and link these to satellite images in order to develop a proof of concept for using remote sensing data to identify regions supporting different types of biological communities and to upscale local process measurements to a regional context. Importantly, this information can also guide scientists to conduct ground-truthing sampling campaigns and was used to select study sites in WP2. Moreover, the data was used to map the sites selected for designation as ASPA.

DEMs were generated at different resolutions, and were compared to other data sources for evaluation. The DEMs were generated using SETSM from the panchromatic channel of Pléiades (resampled to 50 cm), and slope and aspect were computed using gdal. An example, the model of Pingvinane is provided in Fig. 4.1.1, with the aspect converted from degrees (0-360°) to general aspect direction in the main compass direction.

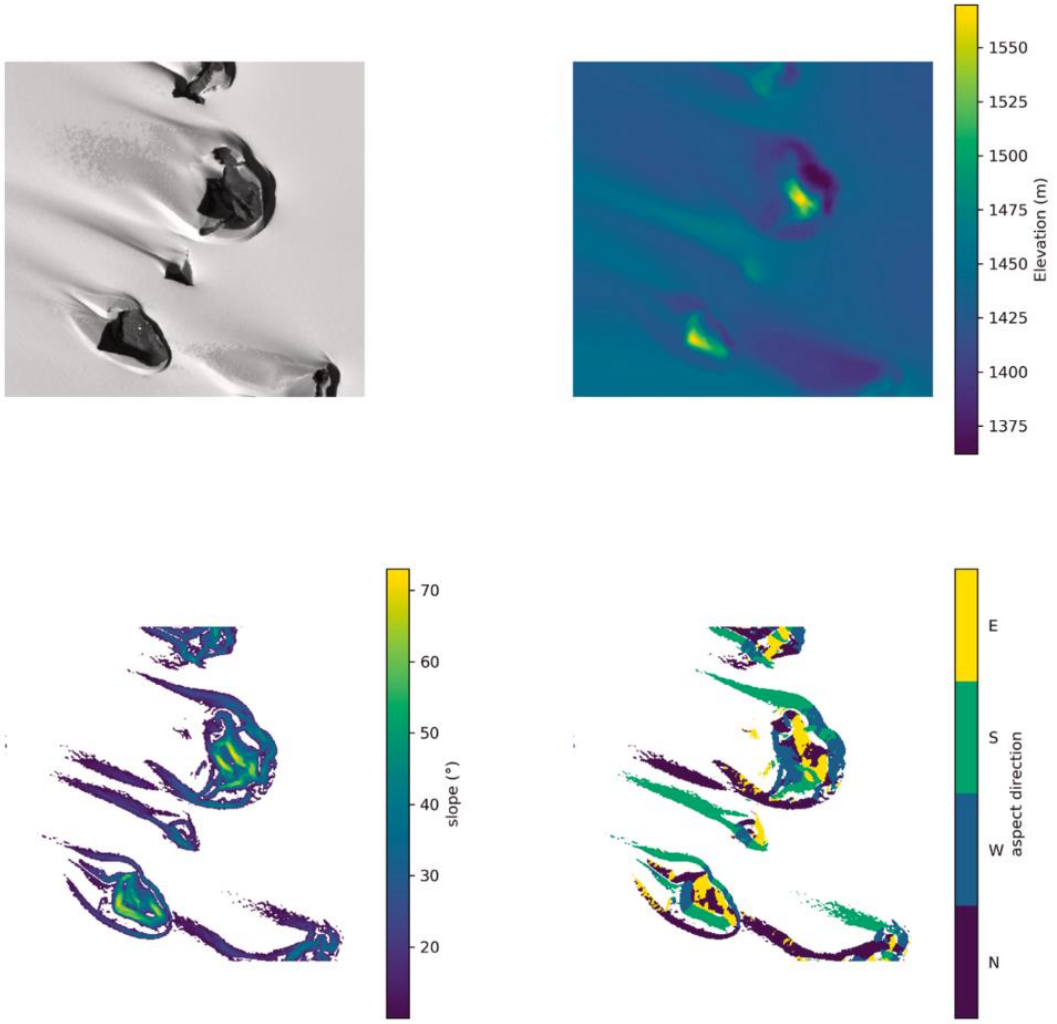


Fig. 4.1.1: Example of the Pléiades derived elevation, slope, and aspect for the Pingvinane nunataks. Taken from Vanhellemont et al. (2021).

The Pléiades derived DEM showed a low bias when compared to other datasets (around -6 m), with a scatter of <10 m (Fig. 4.1.2). The bias can be easily corrected for by using the matchups with spaceborne lidar data (left panel of Fig. 4.1.2). Slightly higher bias and scatter were found for the handheld GPS tracks due to the small scale variability of relief near the tracks (e.g. nunatak or wind scoop slopes) and low accuracy of instantaneous measurements from the moving GPS loggers. Overall, the use of high resolution multi-look optical satellite imagery allows for accurate estimation of the elevation, slope and aspect of Antarctic nunataks.

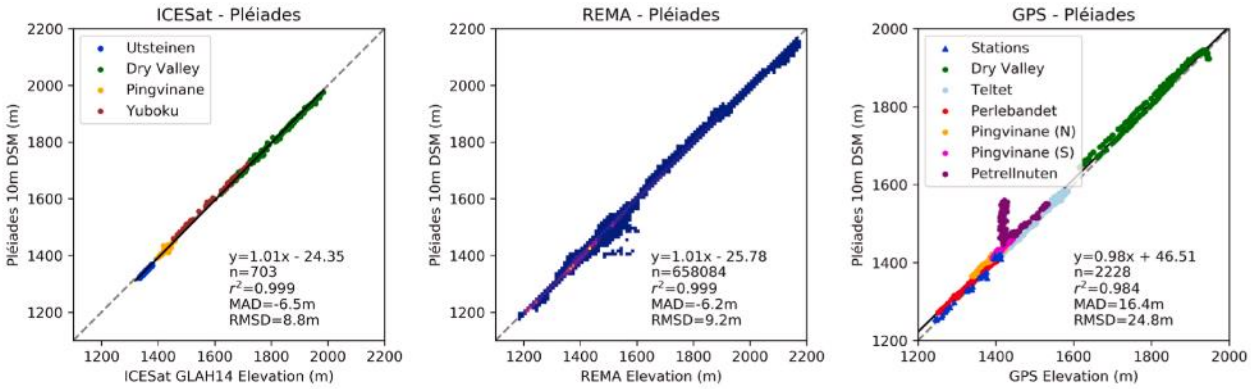


Fig. 4.1.2 Comparison of the MICROBIAN DEMs generated from Pléiades tri-stereo imagery with other datasets, (left) the ICESat LIDAR, (middle) the Reference Elevation Model of Antarctica (REMA), and (right) handheld GPS tracks collected in situ. Taken from Vanhellemont et al. (2021).

The satellite derived DEM data were used in the 2020 campaign to accurately plan drone acquisitions (i.e. to avoid hitting nunataks with the drone), and allowed for the development of the centimetre scale DEMs (Fig.4.1.3). Slope and aspect information at different scales is expected to provide useful information for explaining the differences in biodiversity at the sampling sites. First results indicate high performance of the drone acquisitions, allowing for the estimation of centimetre scale slope and aspects of individual sampling sites. The drone data revealed inaccuracies in the GPS locations of the individual sampling sites, but will also allow for refining those coordinates based on field photographs.

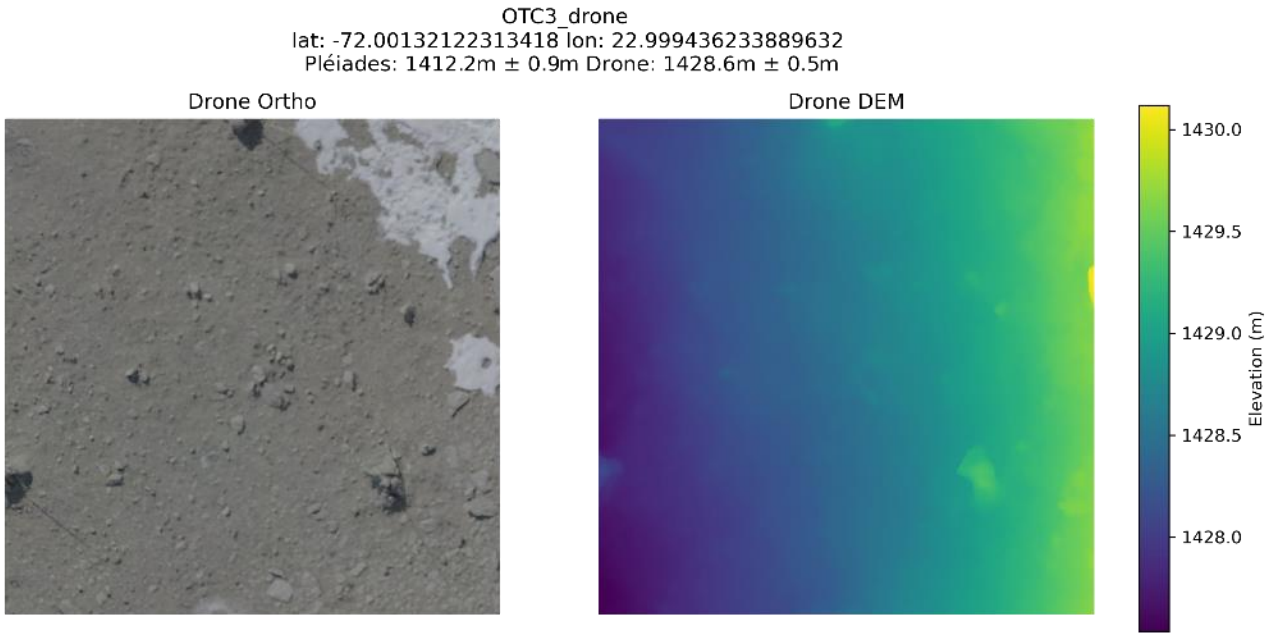


Fig. 4.1.3 Drone orthophoto and elevation for a 10x10 metre subset centred on the OTC3 site at Pingvinane North.

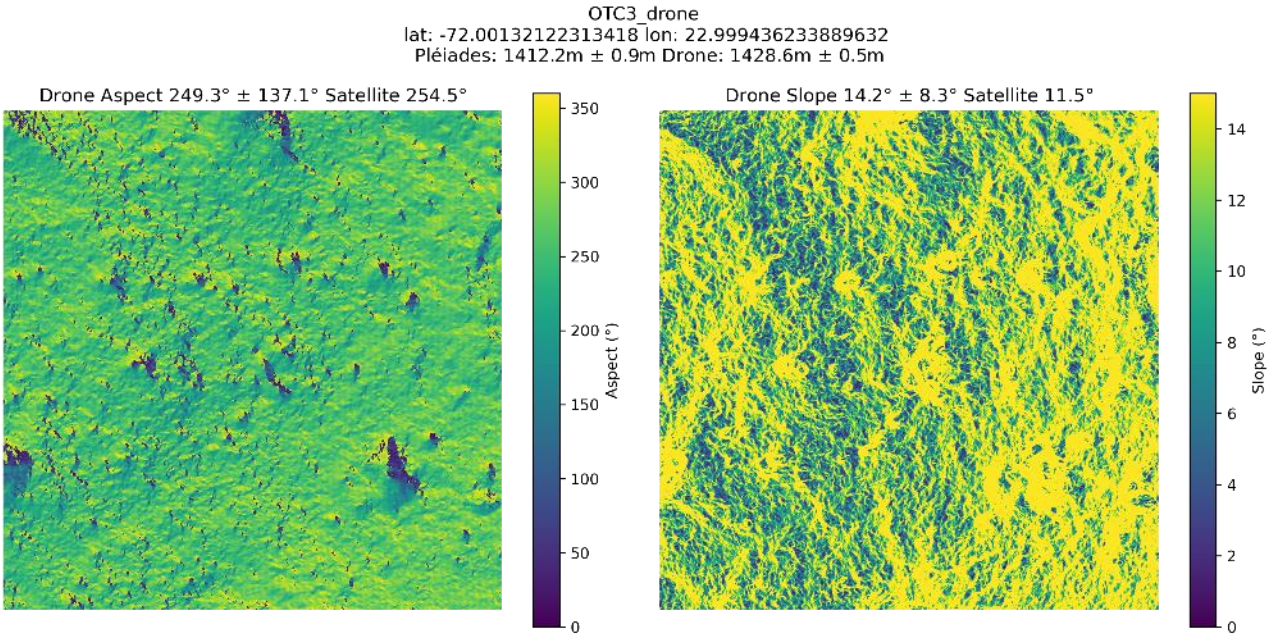


Fig. 4.1.3 (continued): the Aspect and Slope from the centimeter scale drone data at Pingvinane North. The average aspect is within 5 degrees of the Pléiades derived result, and the slope within 3 degrees, but a large variability is found in the drone data due to the capability of the drone to resolve individual rocks.

The Thermal Atmospheric Correction Tool (TACT) developed in MICROBIAN was initially validated using liquid water targets in the Belgian Coastal zone, due to their rather homogeneous temperature across several satellite pixels, and the well-known emissivity of water. For these targets, a low bias (0.05 °C) and scatter (0.69 °C) were found for atmospheric profiles from the ERA5 reanalysis model (Vanhellemont 2020a, Fig. 4.1.4).

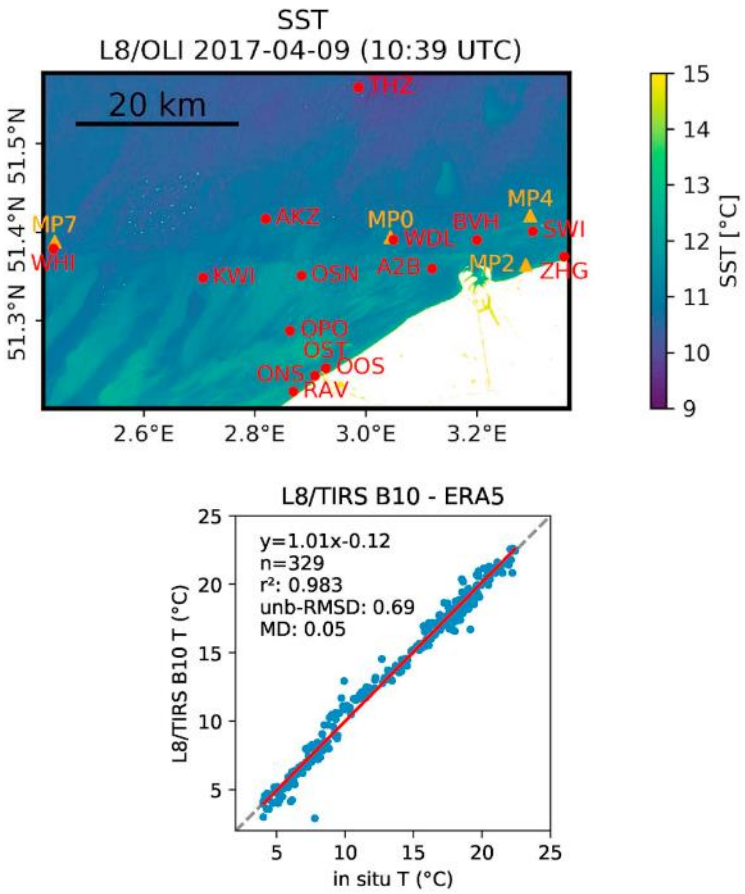


Fig. 4.1.4: A map of the L8/TIRS derived Sea Surface Temperature for 2017-04-09 and the location of the Meetnet Vlaamse Banken (MVB) stations, and the matchup results of TACT processed L8/TIRS B10 data with the MVB temperature data (taken from Vanhellemont 2020a).

Further evaluations were done using meteorological sites in Belgium, retrieving a bias of (-0.1°C) and scatter of (2.8°C) for the best performing configuration (Vanhellemont, 2020b), and of near shore data collected using surfers, showing that TACT performs better than the new standard products provided by USGS (Vanhellemont et al, 2022). Analysis of the MICROBIAN iButton data revealed higher biases and scatter (Vanhellemont et al. 2021), that could be explained by the characteristics of the study sites in the Sør Rondane mountains, and the deployment of the iButtons (typically in gravel, fixed in place with a small rock). Fig. 4.1.5 shows the results for 901 matchups collected during MICROBIAN. Site specific performance can be explained by the environments in which the iButtons were installed, e.g. in the 'Dry Valleys' a positive bias is found, since the iButtons were located in extensive rock fields, and for Utsteinen Ridge, a negative bias is found since the iButtons were installed in a narrow rock outcrop in the ice (details in Vanhellemont et al. 2021). The state of the iButton (either frozen in ice/snow, or not frozen) impacts the performance, due to the higher diurnal variability of the non-frozen loggers as a result of direct solar heating. In the future, these effects could be studied in more detail *in situ*, using thermal cameras, and by setting up a denser regular network of temperature loggers. A comparison with contact thermometers and radiometers could be performed in order to estimate the differences between these methods.

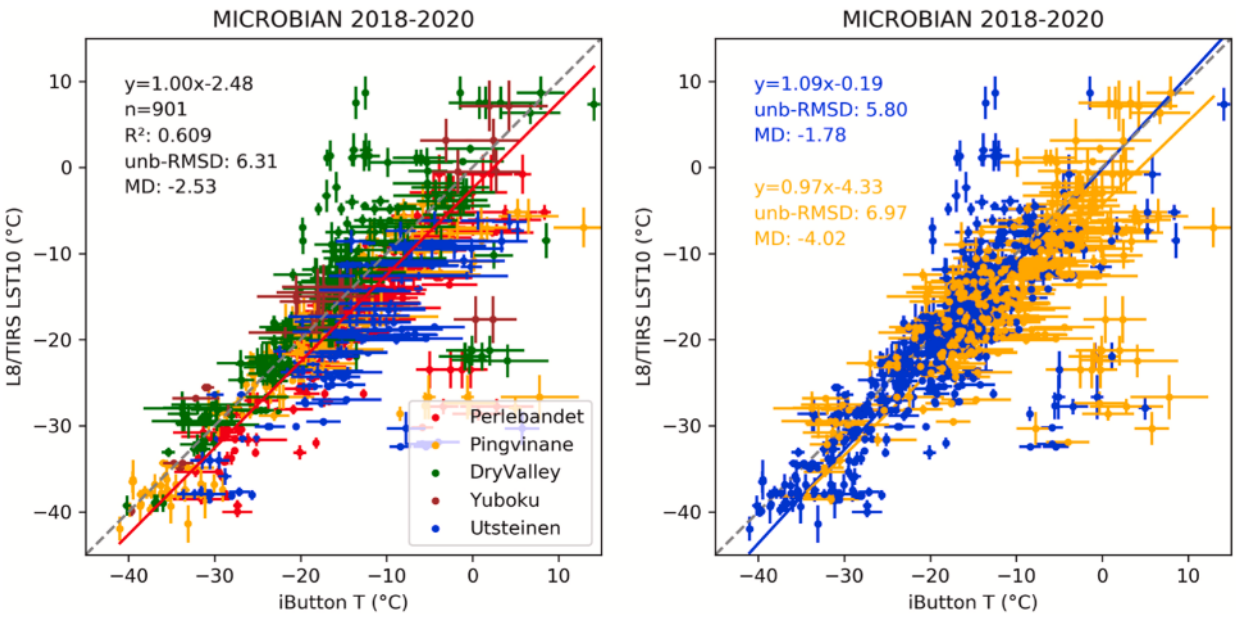


Fig. 4.1.5. Scatter plots of the L8/TIRS derived temperatures as matched up with the MICROBIAN iButton data. Both plots show the same points, but separated by site (left) and by state (right) of the iButton (either frozen, Blue, or not frozen, Orange). Statistics shown in the left plot are across all matchups, on the left they are given per state. Note the site specific performance (left) and the state specific performance (right). Figures taken from Vanhellemont et al. (2021).

Due to the limited range of Landsat overpass times, the nunataks are imaged at similar solar positions, and hence at similar illumination and surface heating conditions for the different nunataks. This causes the north facing slopes (e.g. Fig. 4.1.6 for Yuboku) to be warmer during acquisition, while maximum temperatures can also be reached in different mountain slopes, as is evident from the *in situ* iButton data collected (e.g. Fig. 4.1.7). Interpretation of Landsat ST for Antarctic sites hence has to be done with care, taking these effects into account. The DEM and temperature (both in situ and satellite) data collected in MICROBIAN could be used for setting up and evaluating a diurnal solar irradiance and surface heating model. These models could then be used to determine light and liquid water availability at the different mountain sides.

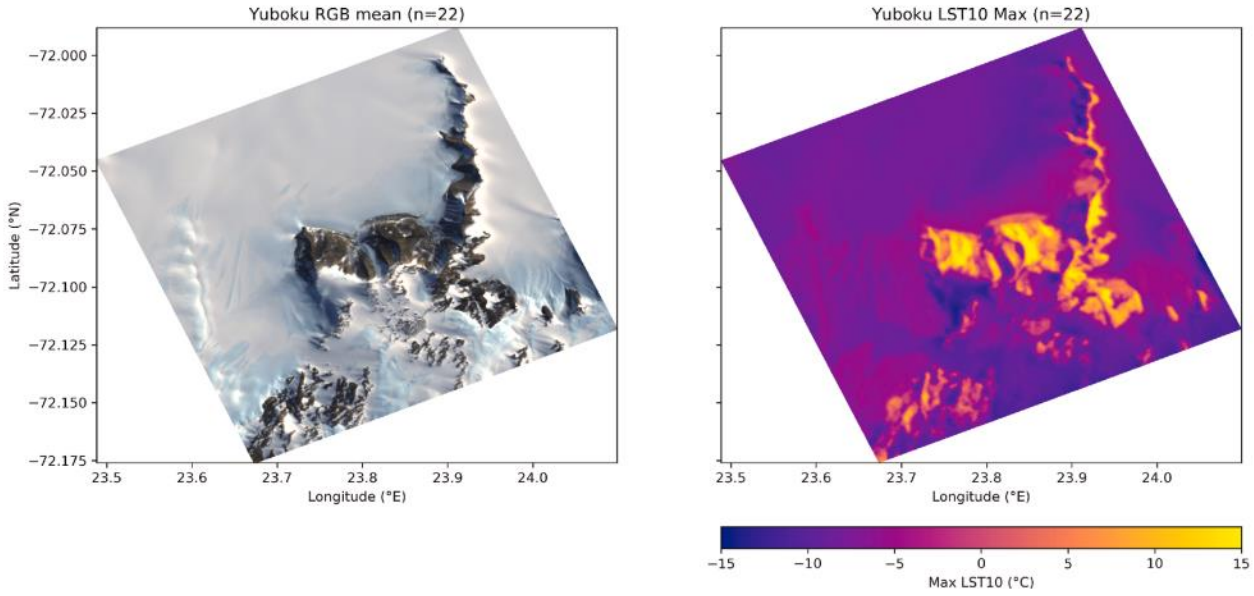


Fig. 4.1.6. L8 mission average surface reflectance RGB and maximum surface temperature for the Yuboku site. Figures taken from Vanhellemont et al. (2021).

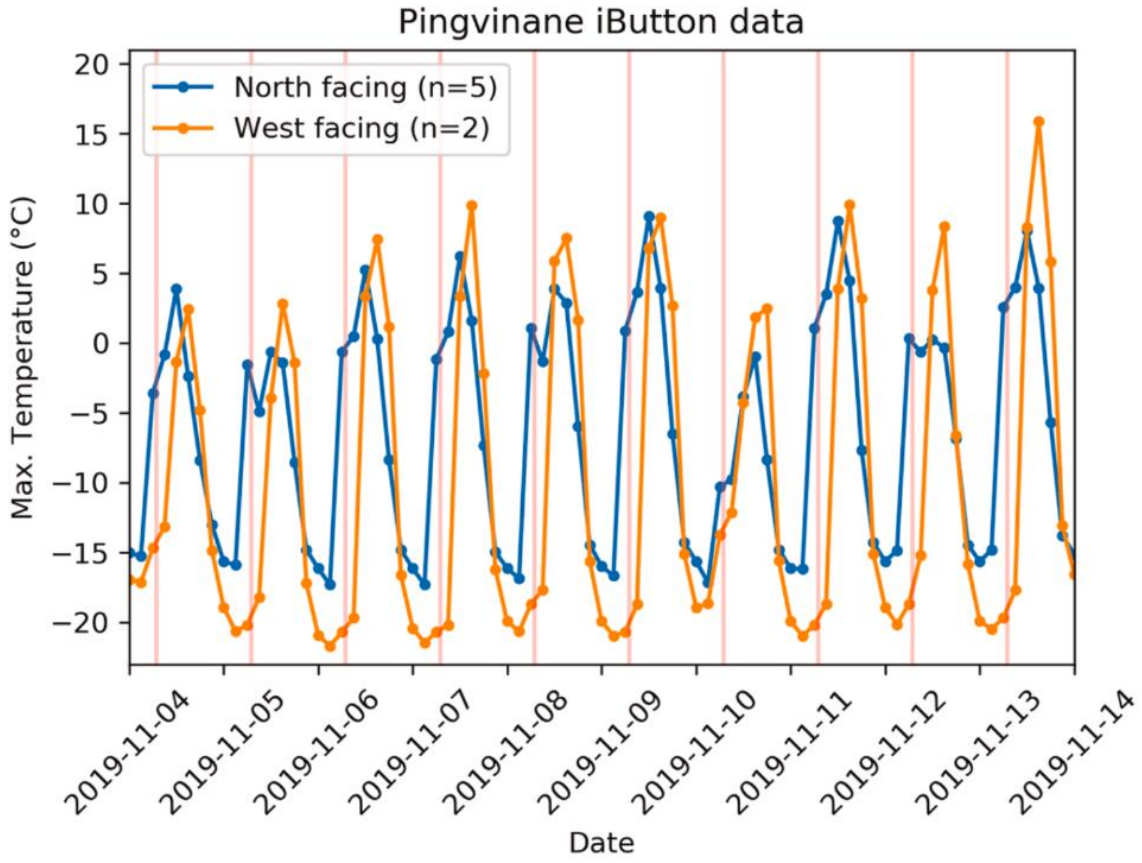


Fig. 4.1.7 Average iButton temperatures for several dates at a north and west facing slope at Pingvinane. The vertical pink lines represent typical Landsat overpass times, at which the north face is warmer. The west face reaches higher temperatures later in the day. Figure taken from Vanhellemont et al. (2021).

Work package 2: Ground-truthing, monitoring and field experiments

Except for the drone analysis, all planned sampling activities and field experiments were conducted according to the initial planning. In total, 142 samples from 10 regions (Table 1) were taken over the course of the 3 field campaigns and used for DNA extractions. In addition, 90 samples and 36 controls from the installed snowfences (see below) and 70 samples and 21 controls from the new OTC were also taken over three campaigns. Samples were stored at – 20 °C and shipped frozen to Belgium.

Auskampane	Dry Valley	Pingvinane	Pingvinane South	Perlebandet	Perlebandet South	Petrellnuten	Teltet	Utsteinen	Yobuku
6	41	7	7	31	3	6	7	20	14

Table 1: number of samples taken from the 10 different regions.

The i-button data were retrieved from the first campaign and stored in a local database shared between the partners. Large differences in temperature and relative humidity were observed between sampling plots along the moisture gradient within the different regions (Fig. 4.2.1). For example, in the northern nunatak of the Perlebandet range, an i-button placed at the base of the nunatak (PB_3) showed lower temperatures during the summer period compared to one placed at the top of the same nunatak (PB_12; Fig. 4.2.1).

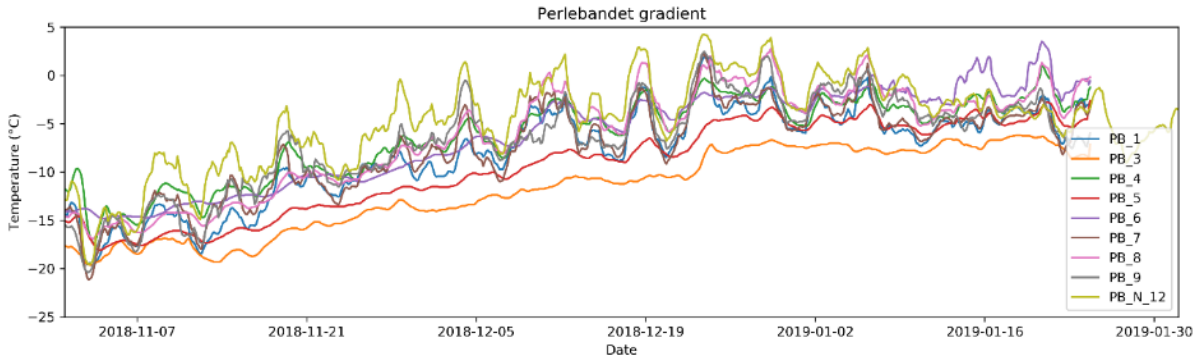


Fig. 4.2.1 Temperature gradient in the most northern nunatak of the Perlebandet range during the summer months of 2018-2019. Colours indicate the different i-buttons that were placed along an altitude gradient. PB_3 (in orange) showed the lowest temperatures whereas PB_12 (in light green) the highest.

PB_3 i-button was placed inside a N-E facing cavity of gneiss rock at the base of the nunatak (1255 m) and it was composed by fractured gneiss bedrock covered by visible black lichen community (Fig. 4.2.2). By contrast, PB_12 i-button was placed higher on the nunatak (1290 m) on relatively flat fractured gneiss/moraine bedrock without any visible established microbial communities (Fig. 4.2.3). Because of the higher exposure to winds, no snow would accumulate here compared with PB_3, and the rocks would accumulate more heat compared to the base of the nunatak (Fig. 4.2.1).

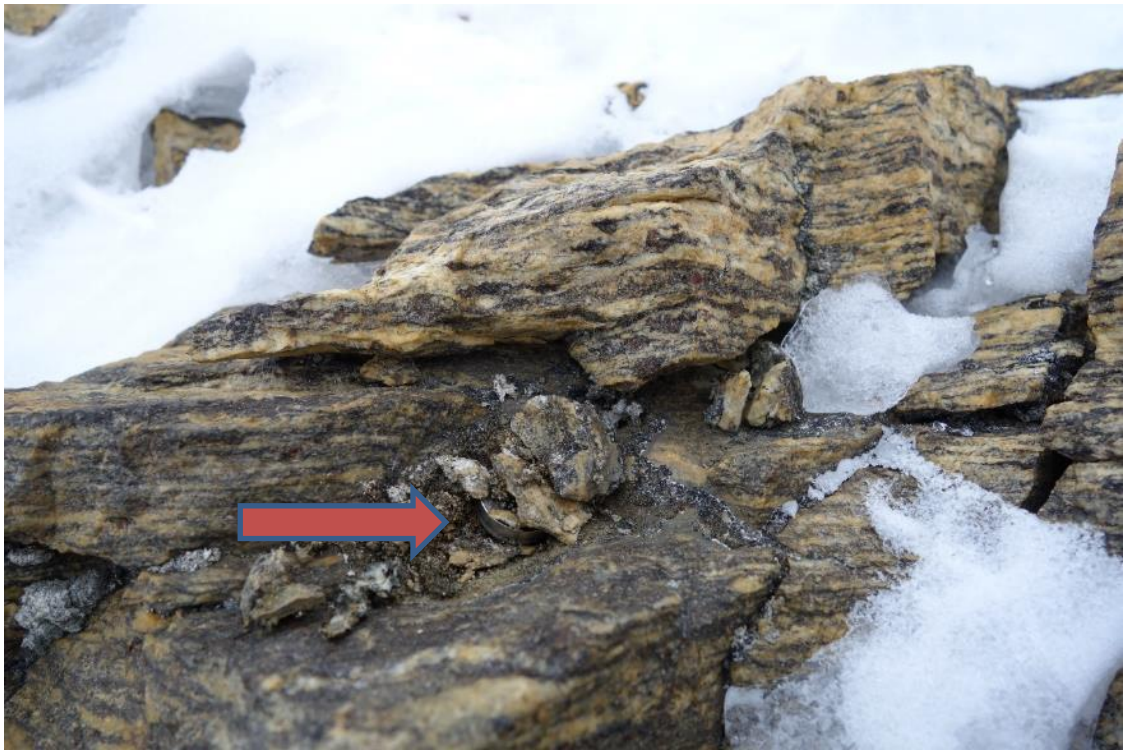


Fig. 4.2.2 i-button placed in PB_3 (S71.84355, E022.83801), inside a N-E facing cavity of gneiss rock at the base of the nunatak (1255 m) where small lichen communities were developed and some snow would accumulate.

Fig. 4.2.3 i-button placed in PB_12 (S71.84418, E022.83209) at cm 32 onto exposed fractured soil without any visible established microbial communities at a higher altitude (1290 m) than PB_3 (S71.84355, E022.83801).

These data are currently being analysed and will be used in direct ordinations of the amplicon sequencing data (Savaglia et al, *in prep*.).

Snow fences and Open Top Chambers were installed, T_0 samples were taken, and temperature and humidity loggers and light sensors were installed. OTCs were modelled after those originally installed (Fig. 4.2.4). More robust snow fences were installed compared to the initially proposed plastic nets. The effectiveness of the snow fences is shown in Fig. 4.2.5, causing the snow to remain accumulated compared to the snow in the vicinity, which is blown away by the katabatic winds. To withstand these winds, too, the snow fences were additionally strengthened, as can be noticed in the picture.

Fig. 4.2.4 Sampling the microbial communities inside a newly installed OTC in the northern nunatak of the Perlebandet range, placed on marble bedrock.

Fig. 4.2.5: One of the two snow fences in the Dry Valleys region. Turbulence caused by the fences allows snow to collect downwind of the snow fence.

The effect of the snow cover is notable in the i-button data, with less extreme temperature fluctuations (~ - 10 °C to - 30 °C) compared to the wind exposed gravel where temperature ranges from 18 °C in summer and - 40 °C in winter (Fig. 4.2.6).

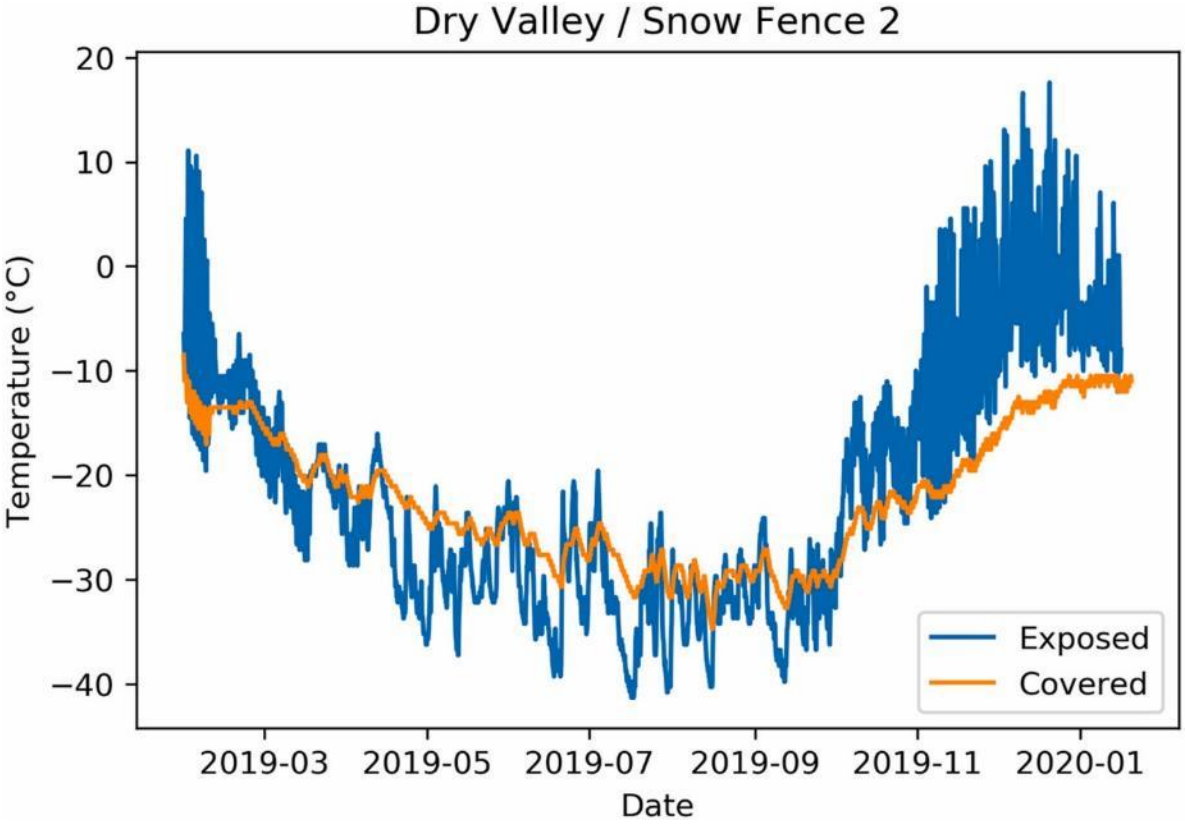


Fig. 4.2.6 i-button temperature time series for one of two snow fences installed at the Dry Valley study site. Two loggers were placed <5 m apart; one on exposed gravel (blue) and one was covered under the snow pack created by the snow fence (orange). This Figure was taken from Vanhellemont et al. (2021).

The Labjack light sensors unfortunately failed, so no information is available about the differences in light availability, and hence i.a. onset and duration in snow cover.

Photosynthetic measurements were performed on different cyanobacterial communities (biocrusts/ benthic mats) located in Utsteinen ridge, Petrellnuten nunatak and Yûboku Valley with a PSI (Photon System Instruments) Aquapen 2100 Fluorometer device. Additionally, 2 light and temperature loggers (Minikin RTi/QTi, EMSO Brno) were deployed in proximity of two monitored sites in the Utsteinen ridge. Data were retrieved before the end of the campaign (Fig. 4.2.7) and the loggers were left *in situ*. Not surprisingly, the photosynthetic activity of the three monitored communities followed the daily irradiance intensity (Fig. 4.2.8). Also temperature importantly contributed in enhancing the photosynthetic activity of the studied communities (Fig. 4.2.9).

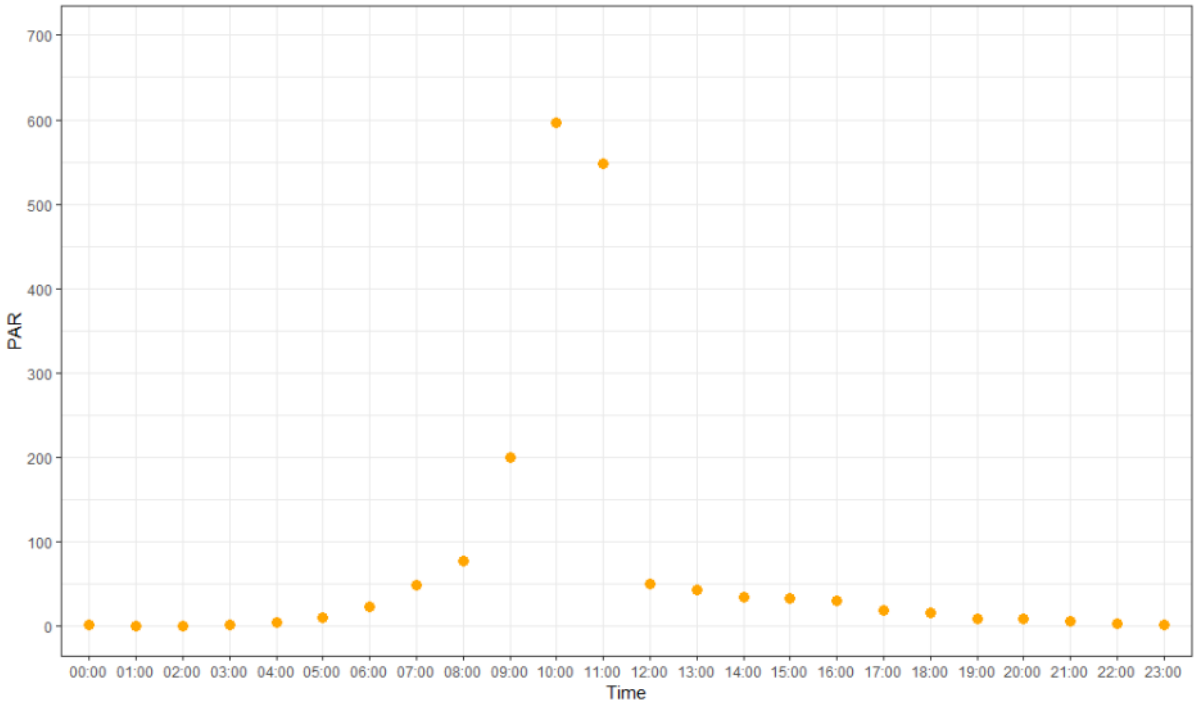


Fig. 4.2.7 Photosynthetically active radiance over a sunny day in the Utsteinen ridge.

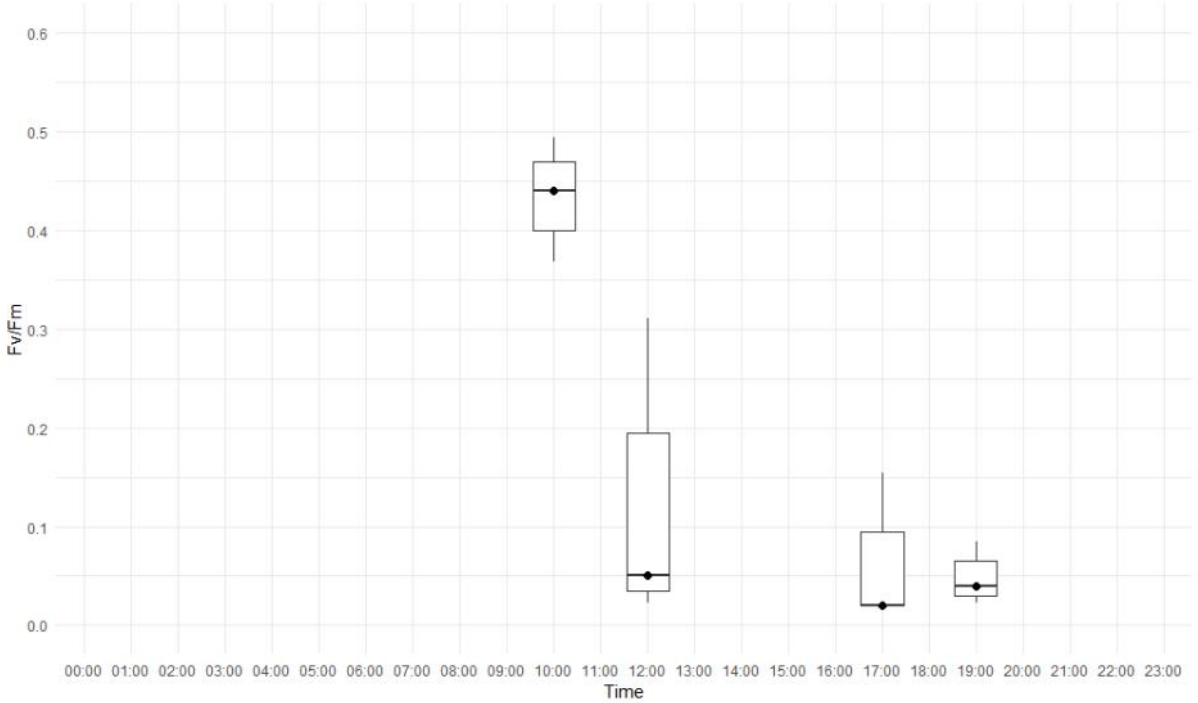


Fig. 4.2.8 Boxplots of the measured F_v/F_m parameter after 15 min of darkness on a cyanobacteria community during a sunny day in the Utsteinen ridge.

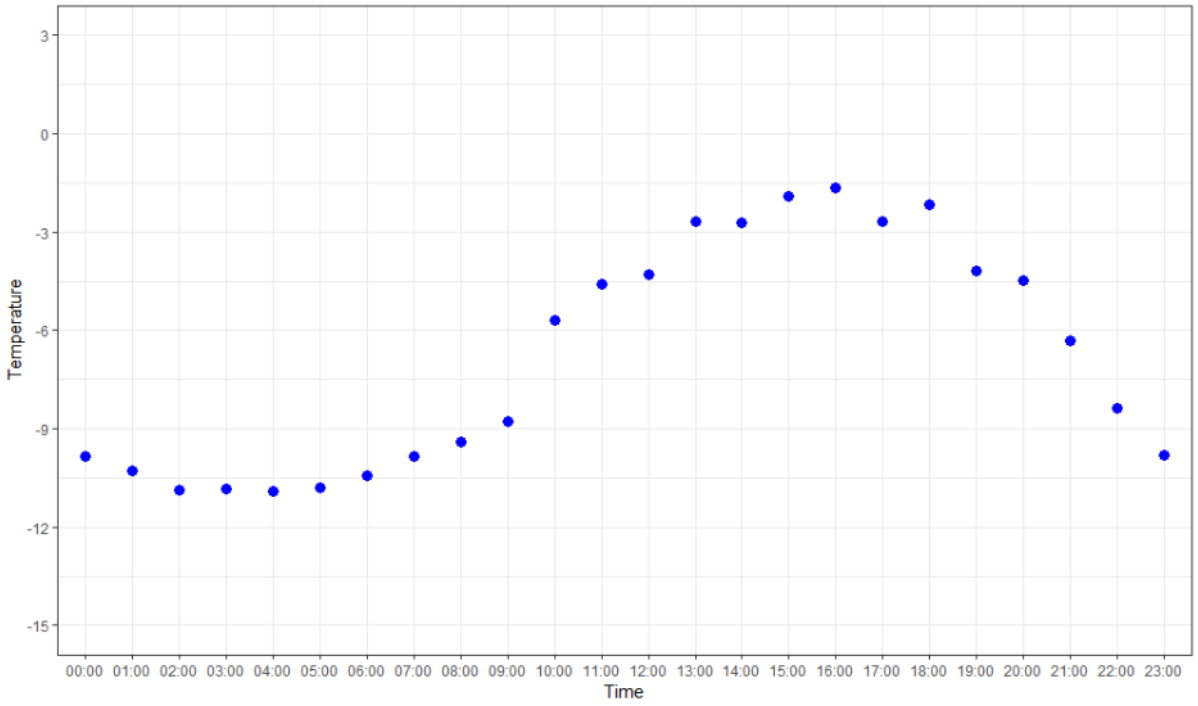


Fig. 4.2.9 Temperature over a sunny day in the Utsteinen ridge.

Work package 3: Biodiversity analyses and measurements of environmental factors

Because of the restricted amount of sample material we could collect, only the major parameters were measured, these were N-NH4, N-NO3, total nitrogen, total phosphorus, P-PO_4 and orthophosphate, total organic carbon, pH, dry weight, and electric conductivity (Fig. 4.3.1). These abiotic factors measured, including pH, moisture content (based on dry weight) and electric conductivity, showed that the most alkaline soils were composed of marble and that the highest TOC concentrations were found in granite soils. These latter soils also had the highest total phosphorus concentrations. Very low amounts of ammonia were found in moraine and marble samples, but only in moraine soils, total nitrogen was found to be close to zero. Interestingly, orthophosphates and NH_4 were found to be very low in marble soils compared to the other types. Total nitrogen and TOC values were the lowest in moraine soils, which also showed very low NH_4 concentrations, similar to marble soils.

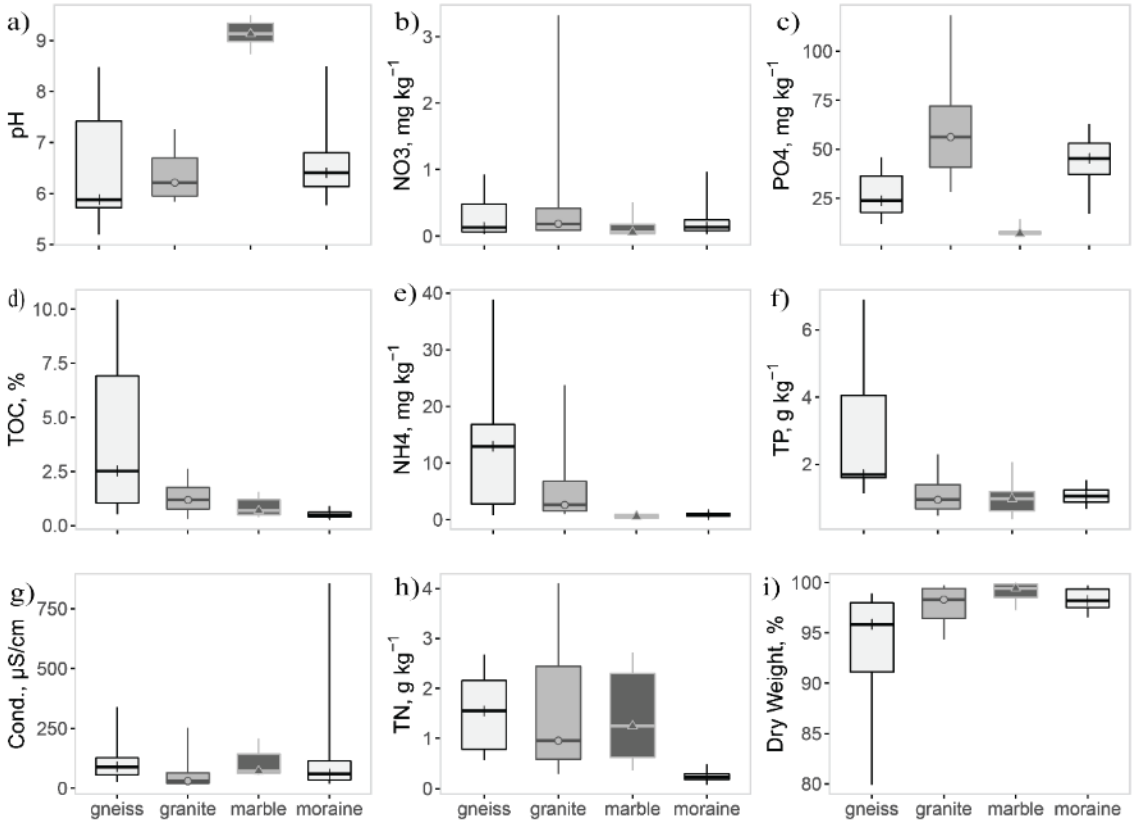


Fig. 4.3.1 Comparison of abiotic variables between bedrock types (gneiss, granite, marble and moraine): a) pH, b) Nitrate (NO_3), c) Orthophosphate (PO_4), d) Total Organic Carbon (TOC), e) Ammonia (NH_4), f) Total Phosphorus (TP), g) Conductivity, h) Total Nitrogen (TN), i) Soil dry weight. Shades indicate the bedrock types.

The amplicon sequencing and subsequent bioinformatic analysis of the 142 samples taken during the 2017-2019 campaigns resulted in 4 314 307 and 10 584 305 amplicon sequences of the 16S rRNA and 18S rRNA genes, respectively. These data were processed using our bioinformatics pipelines and subsequently analysed using multivariate statistics. Preliminary analyses suggest that pH and bedrock type are the main abiotic factors structuring microbial communities in these ice-free regions.

We identified 27 430 OTUs of Bacteria, mainly classified as Actinobacteria, Acidobacteria and Cyanobacteria. Bacteroidetes and Chloroflexi were also particularly abundant while the remaining abundant phyla consisted of Proteobacteria, Abditibacteriota (formerly known as candidate division FBP), Deinococcus-Thermus and Patescibacteria. Interestingly, a large portion (around 2%) of all reads remained unclassified at the phylum level and thus might represent a significant fraction of novel diversity (Fig. 4.3.2a and Fig. 4.3.2b).

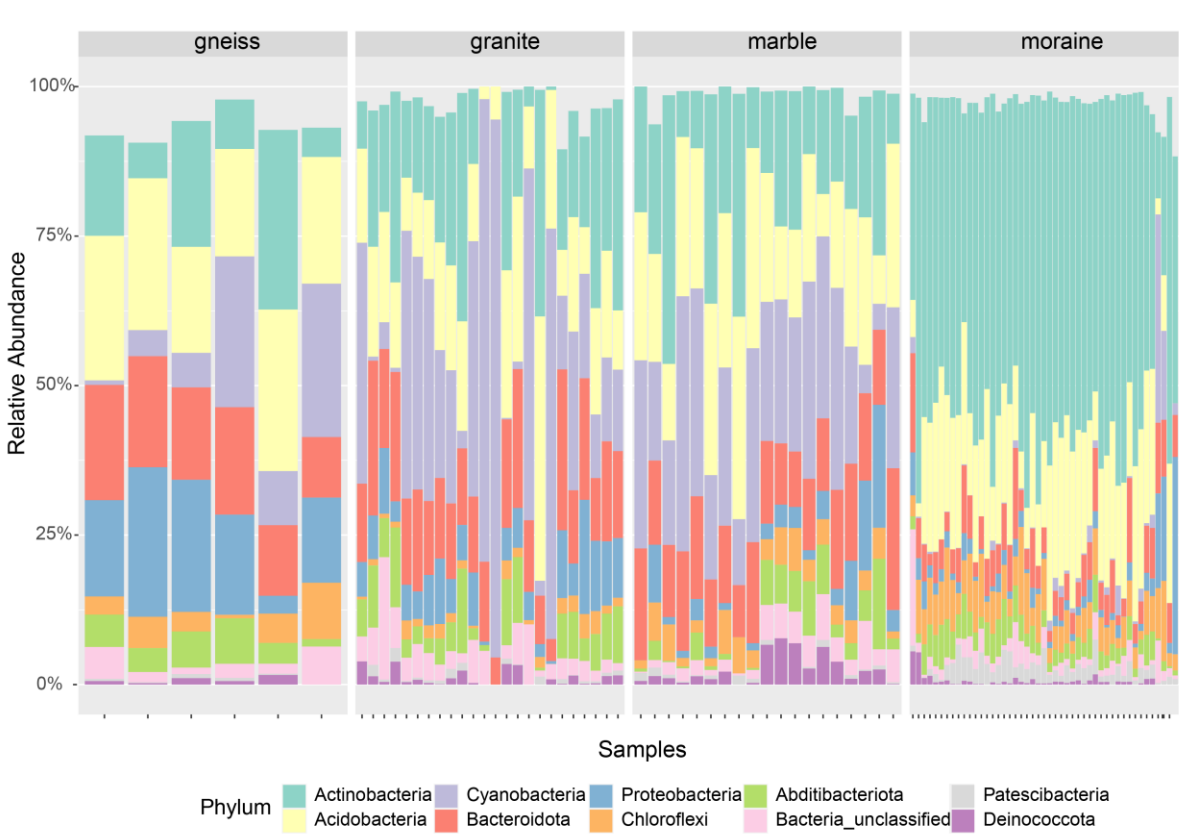


Fig. 4.3.2a Barplot of the relative abundances of the 10 most represented phyla of Bacteria. The empty space is occupied by the “others” Phyla.

Actinobacteria and Cyanobacteria often seem to be inversely correlated. For example, in moraine samples, Cyanobacterial reads are less abundant, while Actinobacteria is the most represented Phylum. On the contrary, Cyanobacteria were well represented in marble and granitic samples, whilst absent in moraine samples, and especially from ‘Dry Valleys’ (Fig. 4.3.2). Nonetheless, they represented an important portion of the Yûboku Valley diversity. Acidobacteria, instead, were found to be well represented in all kind of soils (Fig. 4.3.2).

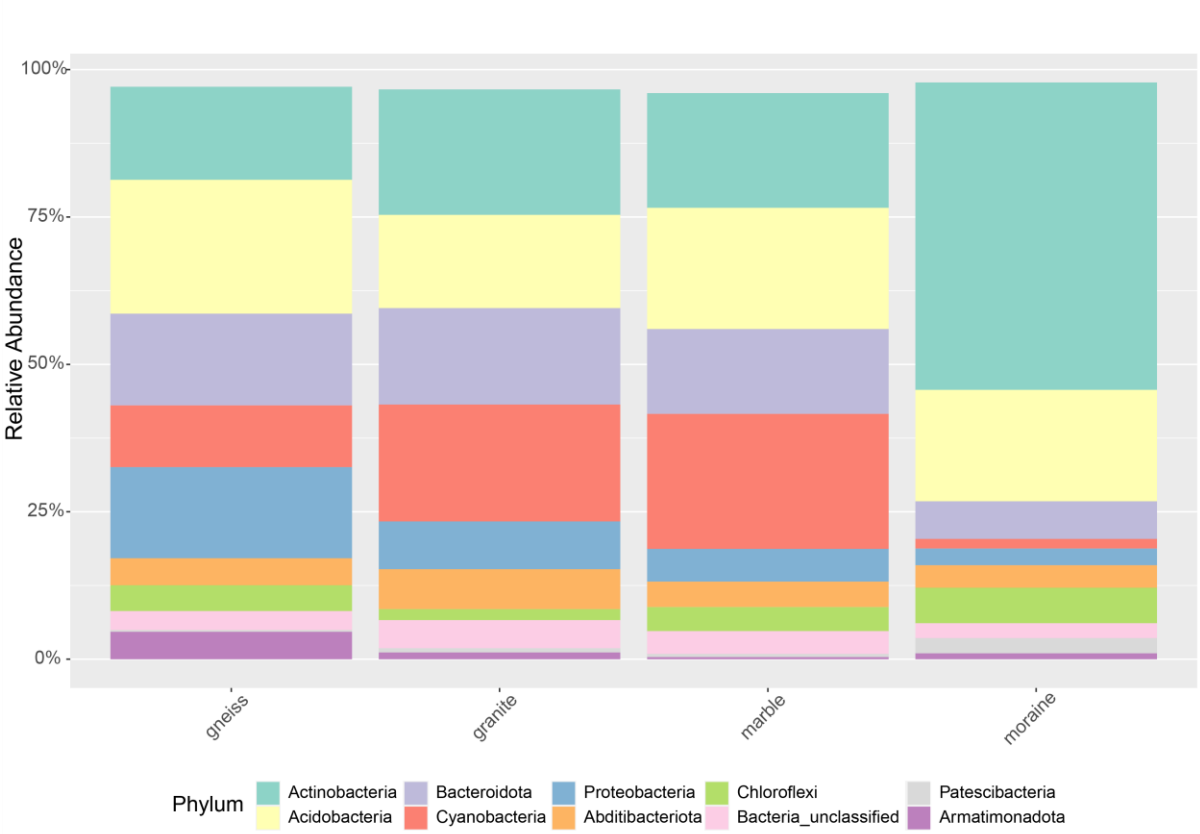


Fig. 4.3.2b: Barplot of the relative abundances of the 10 most represented phyla of Bacteria for each bedrock type. The empty space is occupied by the remaining Phyla.

A total of 1696 ASVs were identified as belonging to Eukaryotes. Chlorophyta was the most important Phylum followed by Metazoa, Cercozoa and Ciliophora. Metazoa seemed to be well represented in all kind of soils but less in moraine ones, whilst Cercozoa were more abundant in marble and moraine soils. Similarly to Bacteria, many reads remained unclassified which might as well represent a significant fraction of novel diversity (Fig. 4.3.3 in orange).

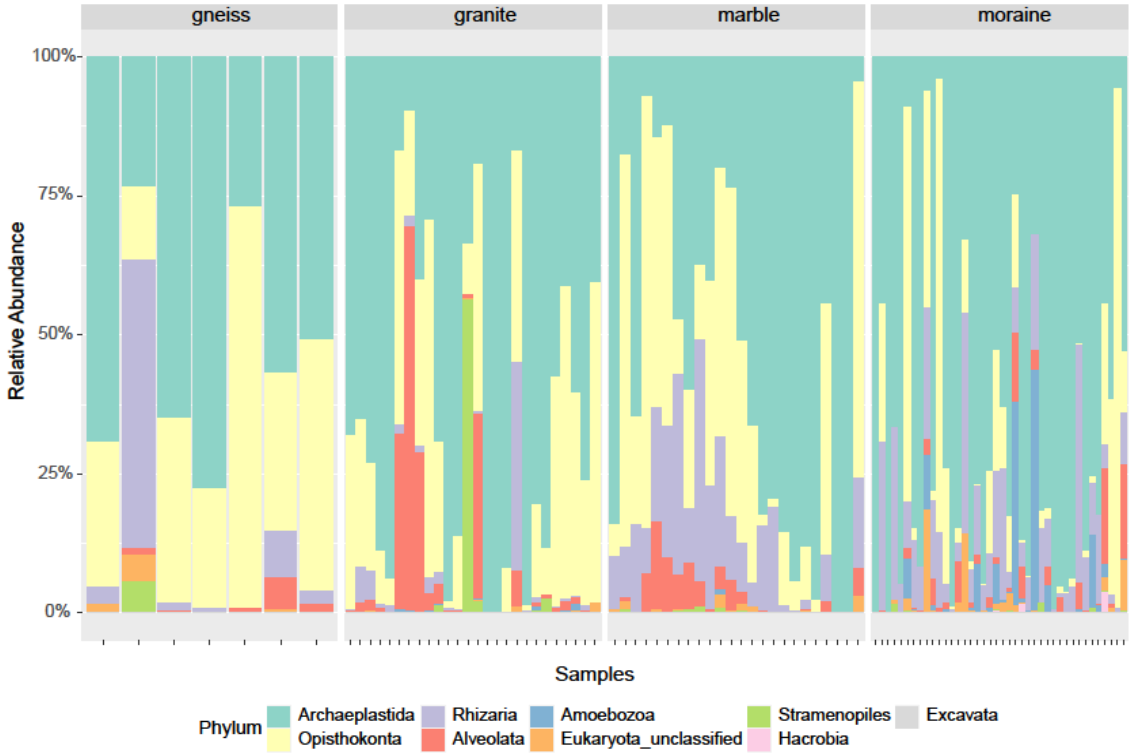


Fig. 4.3.3a Barplots of the relative abundances of the 9 Phyla of Eukaryotes in each sample.

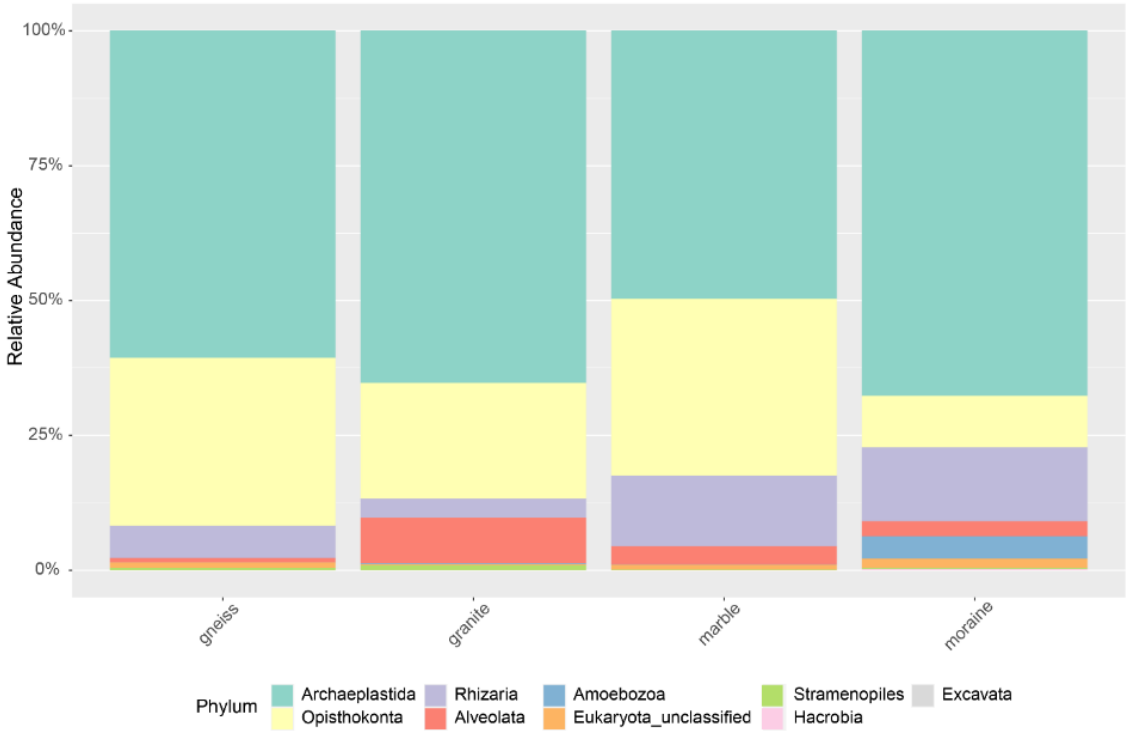


Fig. 4.3.3b Bar plots of the relative abundances of the different Phyla of Eukaryotes per bedrock type.

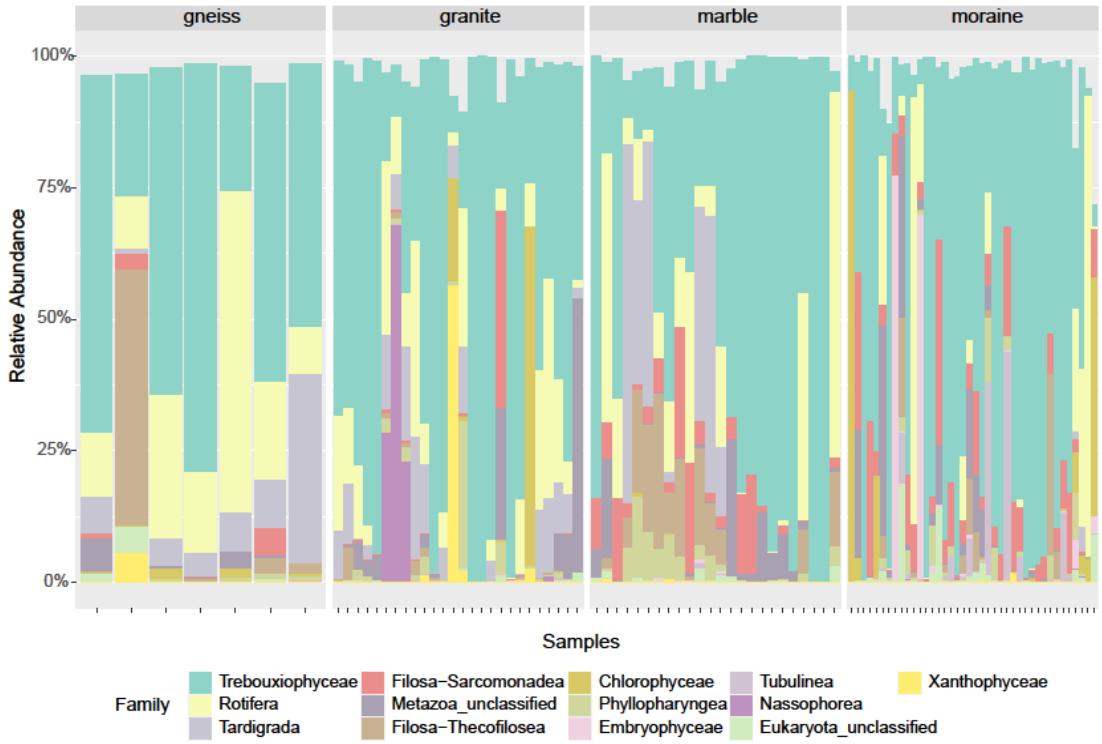


Fig. 4.3.4a Bar plots of the relative abundances of the top 13 families of Eukaryotes in each sample.

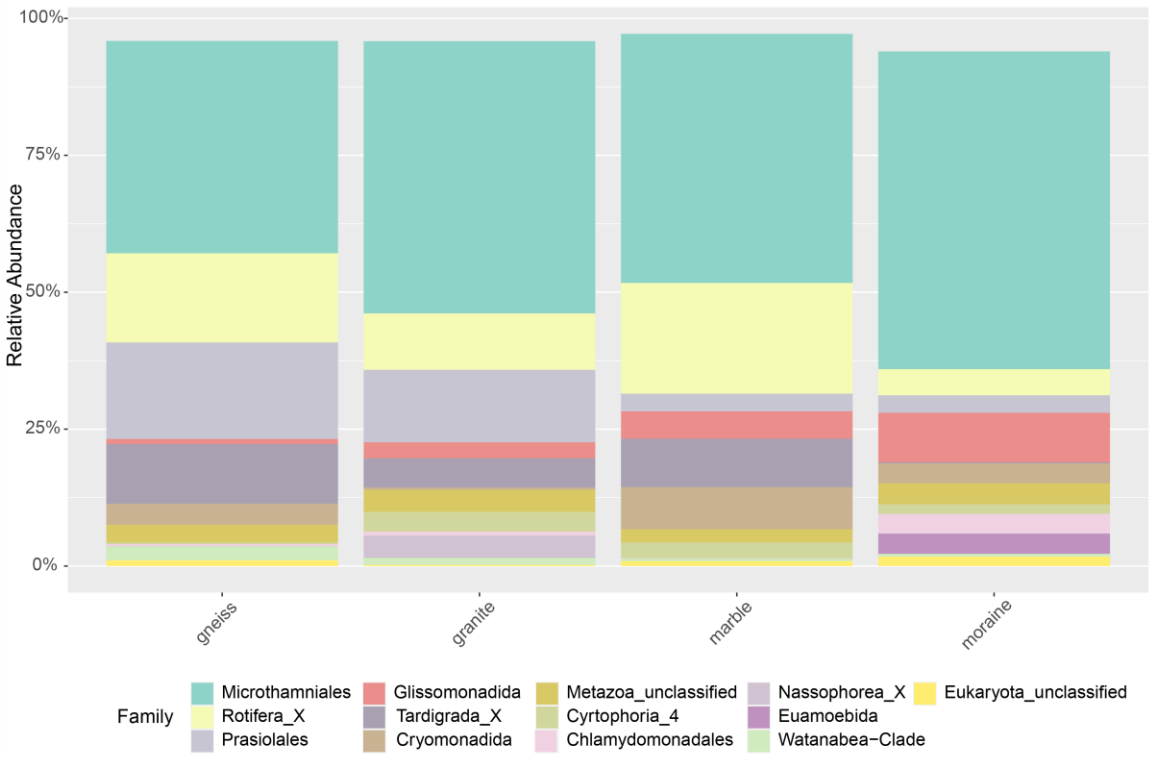


Fig. 4.3.4b Bar plots of the relative abundances of the top 13 Families of Eukaryotes per bedrock type. The empty space is occupied by the remaining Families.

The PCoA and RDA plots of both Bacteria and Eukaryotes show that different sampling locations within the same bedrock type tended to have similar soil chemical characteristics (Fig. 4.3.5 and Fig. 4.3.6). Marble samples tended to cluster together in both Bacteria and Eukaryotes. Granitic and gneiss soils from different regions were also clustering together in the Bacteria PCoA plot, although different clusters were observed between the granitic Utsteinen and Perlebandet samples compared to the Pingvinane and Petrellnuten ones as observed in the Eukaryotes PCoA plot, indicating that those pairs of sites are similar in soil characteristics and/or microhabitat conditions. The Dry Valleys region (DV) appeared to be unique in terms of the soil characteristics measured in this study. Even if considered as "moraine samples", very often samples labeled as moraine from other sites (Austkampane and Yûboku Valley) were not found to be clustering together, probably because of the very peculiar soil and microclimatic conditions characteristic of each of these very different and apart sites.

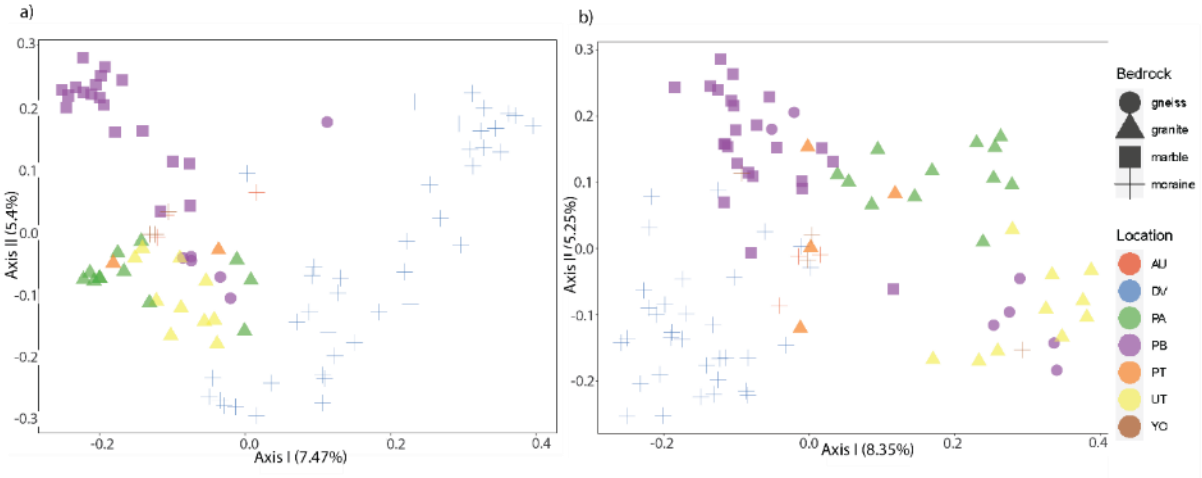


Fig. 4.3.5 Principal coordinates analysis (PCoA) plot of the 96 Illumina sequenced samples studied using the V1-V3 region of the 16S rRNA gene (a) and of the 97 Illumina sequenced samples studied using the V4 region of the 18S rRNA gene (b).

To estimate the amount of variation in the dissimilarity matrices (Bacteria and Eukaryotes) that could be explained by environmental variables, we used distance-based redundancy analysis (dbRDA). As such, a forward step-wise model selection based on permutation tests was used to identify the most parsimonious set of environmental factors that significantly explained the variation in the dissimilarity matrices. pH and bedrock type were found to be one of the most important structuring factors for both Bacteria and Eukaryotes (see Tables 4.3.1 and 4.3.2).

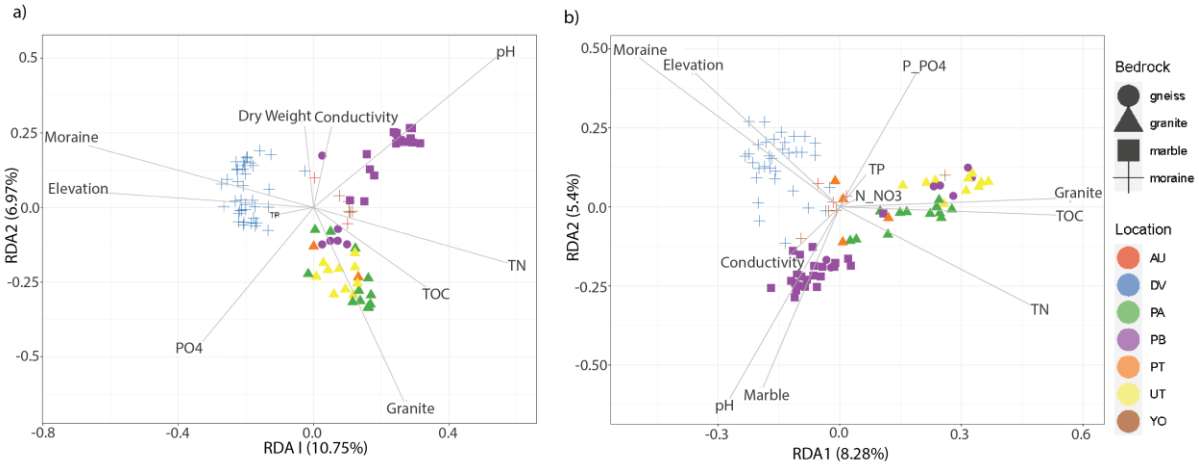


Fig. 4.3.6: Redundancy analysis (RDA) plot of the 96 Illumina sequenced samples characterising the V1-V3 region of the 16S rRNA gene (a) and of the 97 Illumina sequenced samples characterising the V4 region of the 18S rRNA gene (b).

Table 4.3.1: Significant environmental parameters derived from the best-fit linear model (dbRDA) for Bacteria.

Environmental variables	Adj. R2	F	df	P
pH	0.065	7.59	1	0.002
Granite	0.123	7.27	1	0.002
TN	0.152	4.16	1	0.002
Marble	0.169	2.87	1	0.002
Gneiss	0.183	2.54	1	0.002
Conductivity	0.191	1.87	1	0.004
PO4	0.196	1.63	1	0.008
TP	0.196	1.56	1	0.018
TOC	0.206	1.53	1	0.006
Dry weight	0.211	1.53	1	0.016
All	21.110	NA	NA	NA

Table 4.3.2: Significant environmental parameters derived from the best-fit linear model (dbRDA) for Eukaryotes.

Environmental variables	Adj. R2	F	df	P
Moraine	0.076	8.77	1	0.002
pH	0.144	8.50	1	0.002
Elevation	0.178	4.83	1	0.002
granite	0.200	3.72	1	0.002
TOC	0.218	2.92	1	0.002
marble	0.234	2.86	1	0.002

Conductivity	0.241	1.83	1	0.002
TP	0.247	1.69	1	0.01
NO3	0.253	1.62	1	0.012
PO4	0.258	1.53	1	0.032
TN	0.262	1.44	1	0.032
All	0.264	NA	NA	NA

Integration of the biotic and abiotic data with the DEM is ongoing, as well as the species distribution modelling.

Isolation experiments for slow-growing bacteria were performed and some incubations are still ongoing. Long-term incubations (16 months) at 4 °C yielded an interesting diversity of 6 new Actinobacteria species or genera which were absent in short-term incubations (12 weeks) at 4 °C and at incubations at 15 °C. Strains have been preserved and identified based on 16S rRNA genes. Further study of these strains might prove interesting, because it has been shown that Actinobacteria often represent the primary producers in these Antarctic desert soils and generate biomass by consuming atmospheric H_2, CO_2 and CO. In addition, we obtained isolates of the phyla Bacteroidetes, Proteobacteria and Deinococcus-Thermus that, from preliminary identifications, may represent new species. Isolation experiments for Chloracidobacteria (i.a. the alleged Ellin6075) were initiated and incubations were followed up over more than 6 months. Despite the use of a range of different cultivation media, these were not successful for the growth of this group.
Next to new isolation experiments, we also continued the characterization of recently isolated strains that were found to represent novel groups. One of these was the first cultivated representative of the FBP phylum. It was isolated from a terrestrial surface sample from Utsteinen and is a very slow-growing Gram-negative, aerobic, oligotrophic chemoheterotrophic bacterium, metabolically adapted for survival in low-nutrient habitats. We named it *Abditibacterium utsteinense* and the FBP phylum was named Abditibacteriota (Tahon et al. 2018). Furthermore, genomic and phenotypic characterization of other novel isolates allowed us to describe the new species *Spirosoma utsteinense* in the family Cytophagaceae, phylum Bacteroidetes (Tahon et al 2021b) and the new genus *Chioneia* (with four new species: *C. frigida, C. hiemis, C. brumae* and *C. algoris*) in the family *Sphingomonadaceae*, phylum Proteobacteria (Tahon et al. 2021a). Type strains for new taxa were deposited in the BCCM/LMG Bacteria collection.

Isolation of Cyanobacteria, too, was initiated. Three *Nostoc* spp. were successfully isolated from samples collected in 2018 in Yûboku Valley and Pingvinane III nunatak (Pingvinane North) and other samples have been plated and incubated at 4, 12 and 18 °C, with different media dilutions to mimic oligotrophic Antarctic conditions. These three strains were deposited in the BCCM/ULC Cyanobacteria Collection and are publicly available as ULC606, ULC608 and ULC609.

Assessment of the diatom diversity resulted in an improved knowledge of the present diatom flora in the Antarctic region. Samples from the Vestfold Hills published in 1999 by Roberts & McMinn revealed a much higher diversity than previously assessed, mainly due to the application of a fine-grained taxonomy (Bischop et al. 2020). This led to the publication of a new diatom genus, *Sabbea* for the Antarctic Continent (Bischop et al. 2019, Kusber et al. 2020). A second taxon, identified as the presumably cosmopolitan *Navicula phyllepta* proved to be endemic for the Antarctic Continent and has been revised (in preparation). Additionally, the revision of the sub-Antarctic and maritime Antarctic diatom flora yielded several new species described in the genera *Cyclotella* (1 species), *Ferocia* (1 species), *Angusticopula* (1 species), *Melosira* (1 species), *Staurosira* (1 species) and *Hantzschia* (3 species), *Planothidium* (2 species), *Psammothidium* (4 species) (Van de Vijver & Dessein 2018, Van de Vijver et al. 2018, Van de Vijver 2019, Van de Vijver & Houk 2019, Van de Vijver & Crawford 2020, Van de Vijver et al. 2020). One new species was published as *Microcostatus elisabethianus* (Van de Vijver & Ector, 2019), named after crown princess Elisabeth on the occasion of the 10th anniversary of the opening of the Belgian Antarctic Station.

Work package 4: Functional diversity

Forty-three polar desert soil samples were selected for further analysis. A total of 125 high-quality and 248 medium-quality draft genomes have been reconstructed, resulting in a dereplicated genome catalogue of 373 draft genomes from 14 phyla with an average completeness of 86.29% and an average contamination of 4.45% (Fig. 4.4.1).

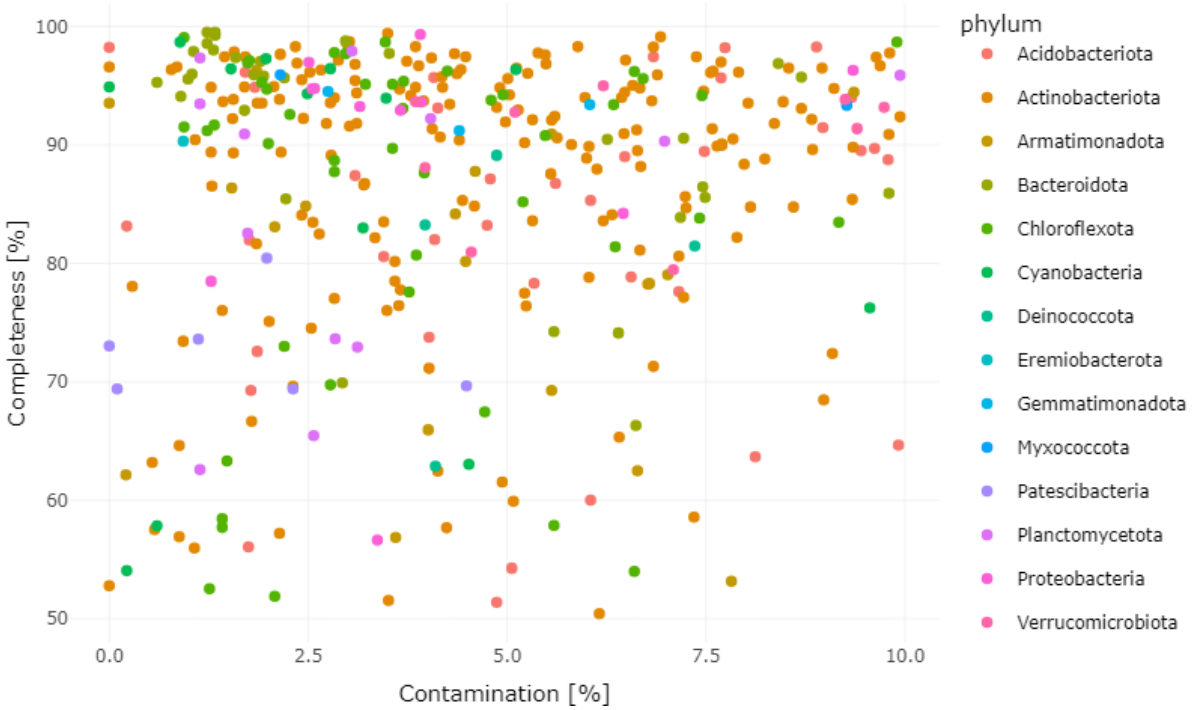


Fig. 4.4.1 dereplicated genome catalogue of 373 draft genomes.

To analyse the metabolic capabilities that support the microbial communities in these 43 extremely oligotrophic soil samples, the distribution of 52 marker genes representing various energy production and carbon fixation pathways in both the metagenomics raw reads and

MAGs were estimated. As expected, genes for aerobic organotrophic respiration were among the most abundant, while genes for anaerobic respiration and fermentation were low across all samples. Interestingly, however, the second most-abundant marker gene across all samples was the catalytic subunit of group 1 [NiFe]-hydrogenases (present on average in 93.52% of community members). Among the genes that are not involved in aerobic respiration, the two other most abundant genes are RuBisCO (RbcL; 47.6%) and form I carbon monoxide dehydrogenases (CoxL; 23.3%) respectively (Fig. 4.4.2). Genes involved in phototrophy were relatively rare (PsaA; 8.21%, PsbA; 12.4%, RHO; 6.7%). Our results suggest that atmospheric trace gas oxidation and chemosynthesis might be an important process in the Sør Rondane Mountains, supporting primary production independently of photoautotrophy.

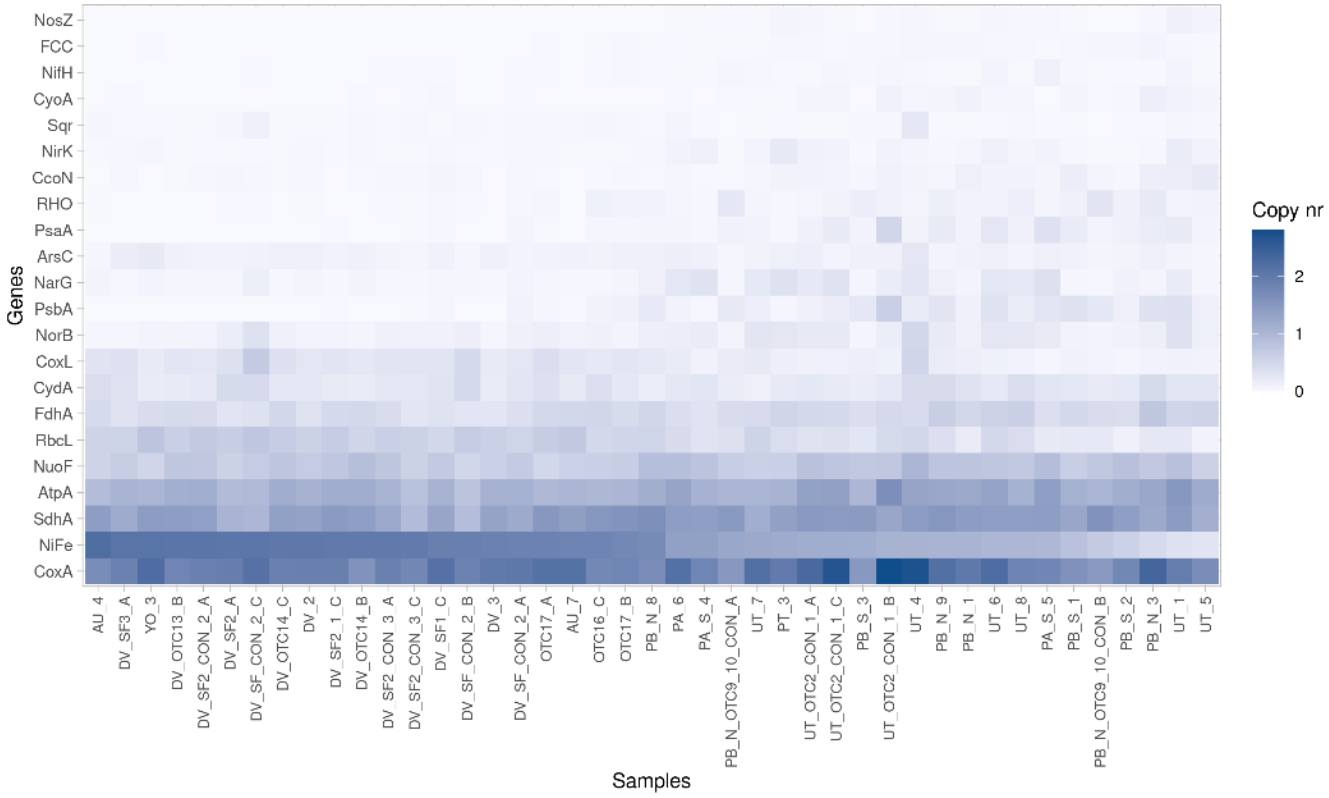


Fig. 4.4.2: RecA normalized copy number estimation of the most abundant metabolic marker genes. Ranked based on the abundance across all samples, and sorted based on the abundance of the NiFe hydrogenase genes.

When looking at the distribution of these genes across the samples, preliminary analysis suggests that these 3 genes are correlated to elevation, and inversely correlated to moisture and TOC. Highest abundance was estimated in the more extreme oligotrophic samples from Austkampane and the Dry Valley (Figs. 4.4.3 and 4.4.4).

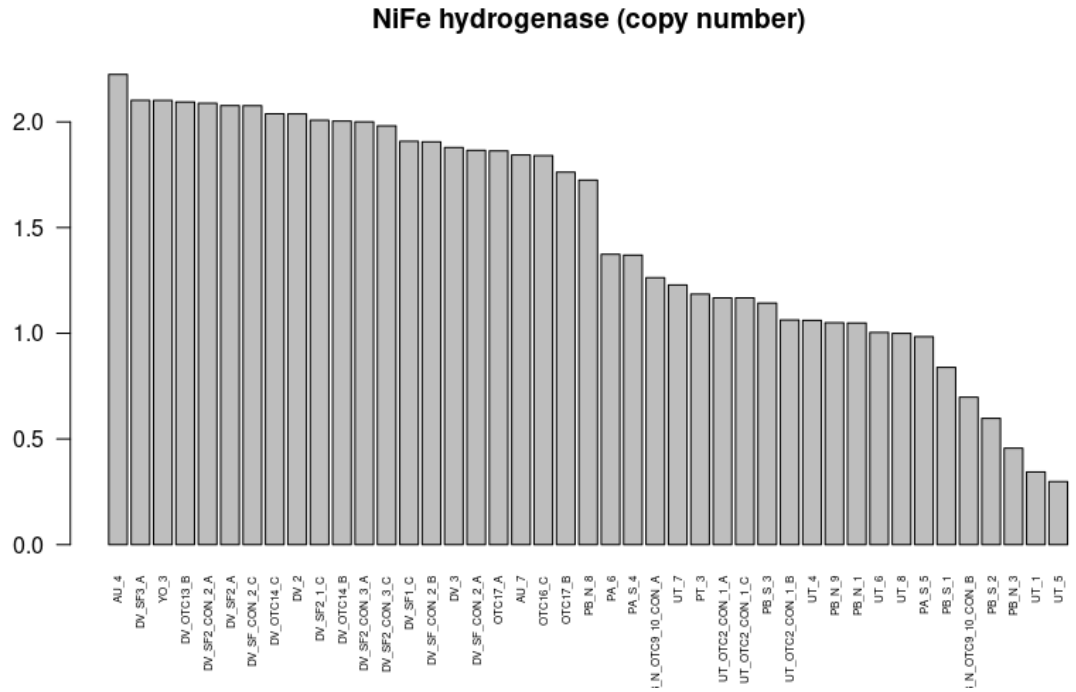


Fig. 4.4.3: Per sample, copy number of NiFE hydrogenase genes.

The samples that contain relatively fewer copies per genome of these 3 genes, are characterised by a high variety of versatile mixotrophs with specialist capabilities for energy and carbon metabolism. Among these are multiple chemolithoautotrophs.

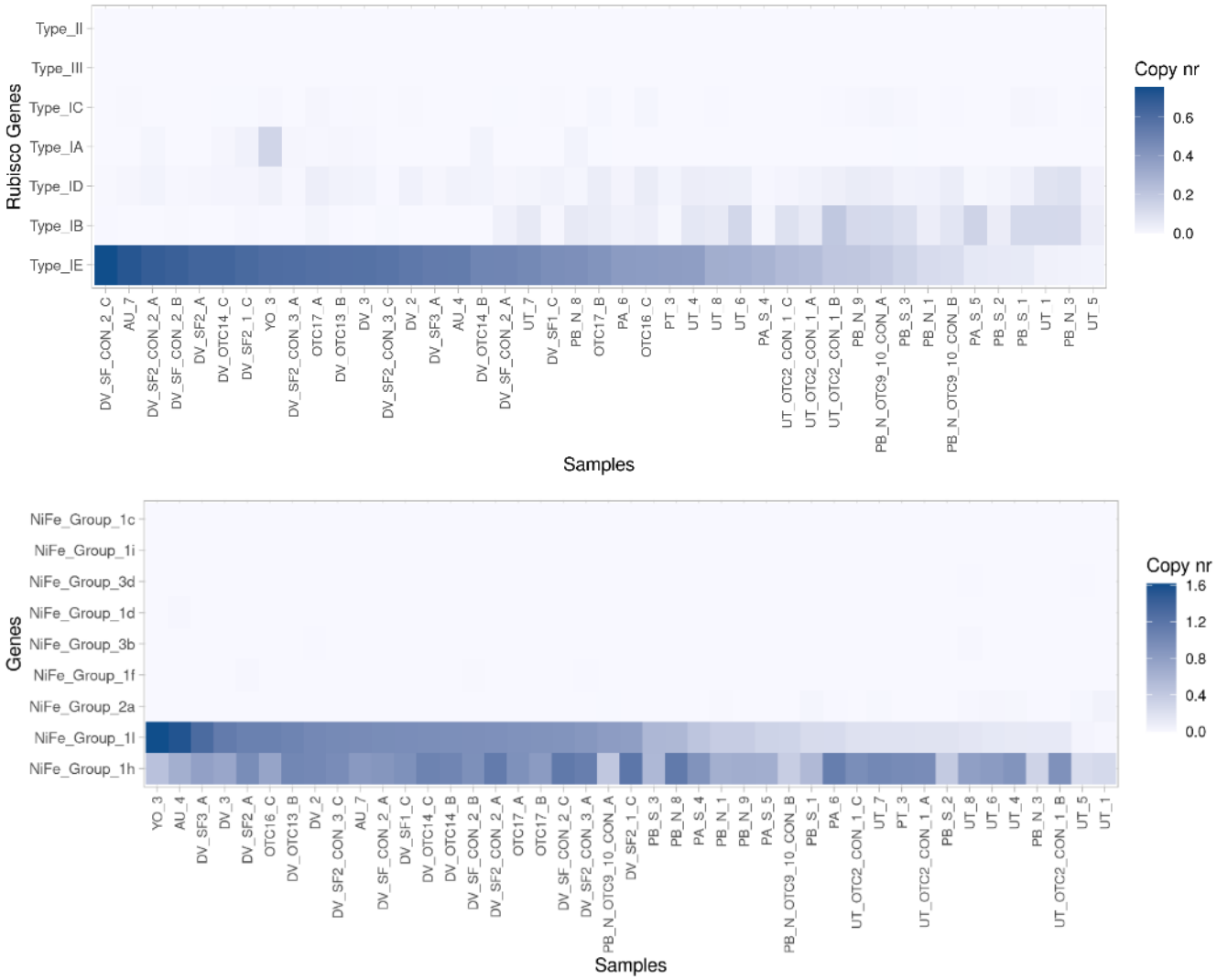


Fig. 4.4.4: Per sample, copy number of Rubisco and NiFe gene variants.

We reconstructed cyanobacterial MAGs for 34 of the 56 samples (60,7%). Among these samples, 14 contain cyanobacterial MAGs with a completeness of >= 95% (Tab.4.4.1). We then focused on the Nostocales bins for further analyses. Phylogenomic analysis was done on the 7 Nostocales bins (Tab.4.4.1) and the 23 Nostocales reference genomes retrieved from Genbank. We also added a cyanobacterial outgroup (*Cyanobium* sp.). The phylogenomic tree based on an alignment of sequences of 23 concatenated ribosomal proteins showed that 6 bins clustered with *Nostoc flageliforme* CCNUN1, and the last bin branched between *Brasilonema sennae* CENA114 and the main Nostocales cluster (Fig.4.4.5).

Table 4.4.1: the 34 samples containing cyanobacterial MAGs and the details of binning of MAG with a completeness of at least 95%.

Samples	**# cyano bins**	**# bins > 95 %**	**Taxonomy (completedness, contamination, heterogeneity)**
OTC11_2018_B	2	1	Cyanobacteria (bin4 : 96.44 1.00 83.33)
AU_2018_10	3	0	

AU_2018_11	1	1	Oscillatoria (bin17 : 99.34 0.44 50.00)
OTC9_10_2018_CON_A	2	0	
YO_2018_2	5	2	Cyanobacteria (bin71 : 96.46 4.48 0.00) Pseudanabaena (bin3 : 95.87 0.83 75.00)
YO_2018_4	9	2	Cyanobacteria (bin60 : 96.70 0.24 0.00) Pseudanabaena (bin31 : 95.87 2.12 77.78)
PB_N_2018_2	2	0	
PB_N_2018_3	2	0	
UT_2018_1	4	1	Cyanobacteria (bin41 : 96.20 1.96 58.33)
UT_2018_4	4	0	
UT_2018_5	1	0	
UT_2018_6	4	1	Nostocales (bin28 : 99.22 1.56 0.00)
UT_2018_7	3	1	Nostocales (bin14 : 97.11 0.89 0.00)
PA_2018_1	8	1	Nostocales (bin9 : 96.30 11.64 27.40)
PA_2018_3	9	3	Nostocales (bin5 : 97.47 11.57 49.09) Cyanobacteria (bin31 : 96.34 1.06 0.00) Nostocales (bin25 : 96.07 2.22 33.33)
PB_N_2018_4	3	0	
PB_N_2018_11	2	0	
PA_2018_6	1	0	
OTC2_2018_CON_1_A	1	0	
OTC2_2018_CON_1_C	1	0	
PB_S_2018_1	3	0	
PB_S_2018_3	5	0	
DV_SF_2018_CON_2_A	1	0	
DV_SF2_2018_1_C	1	0	
PA_S_2019_1_p4	2	1	Nostocales (bin21 : 97.37 1.01 25.00)
PA_S_2019_2	3	1	Nostocales (bin14 : 96.22 2.22 23.08)
PA_S_2019_5	1	0	
YO_2019_1	9	3	Cyanobacteria (bin16 : 96.46 0.55 33.33) Oscillatoria (bin34 : 95.77 0.22 0.00) Cyanobacteria (bin14 : 95.28 0.59 0.00)
YO_2019_2	9	3	Cyanobacteria (bin17 : 98.63 0.63 25.00) Oscillatoria (bin16 : 97.01 0.66 75.00)

			Cyanobacteria (bin31 : 95.99 1.30 0.00)
PT_2019_1b	1	1	Cyanobacteria (bin39 : 99.70 0.00 0.00)
PT_2019_4	6	0	
OTC16_2019_C	2	0	
OTC17_2019_A	1	0	
OTC17_2019_B	1	0	

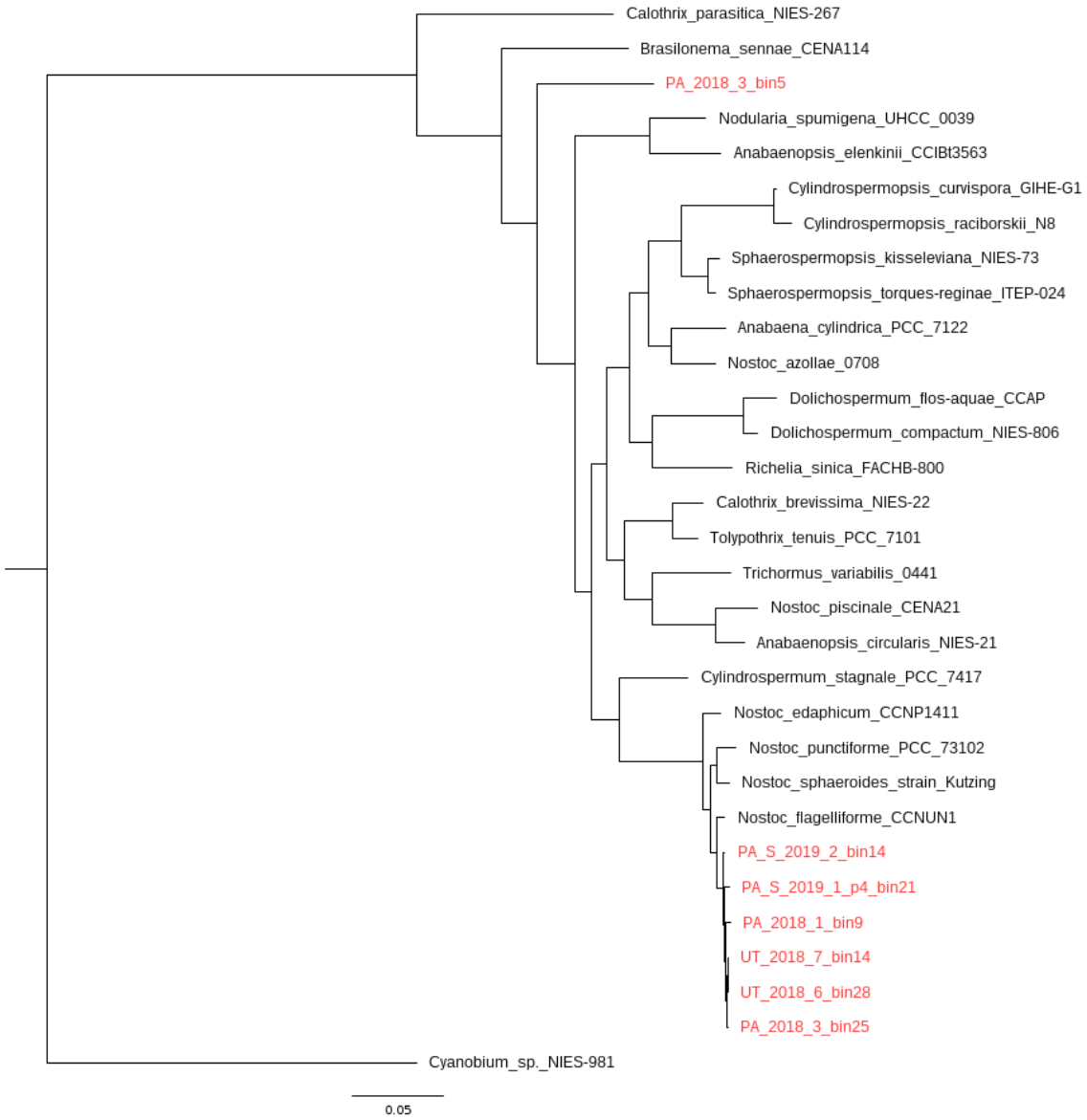


Fig.4.4.5 : Phylogenomic tree of the 7 Antarctic Nostocales bins (red), the 23 Nostocales reference genomes and the outgroup (black), based on the concatenation of the amino acid sequences of 23 ribosomal proteins.

Interestingly, the pan-genomic analysis revealed that the 6 bins that are phylogenetically closely related (Fig.4.4.5) cluster together. Indeed, they share more genes than with other genomes (Fig.4.4.6). At least 145 genes are unique to these 6 bins ("*Antarctic_1*", on Fig.4.4.6). Further analyses are in progress to characterize these genes.

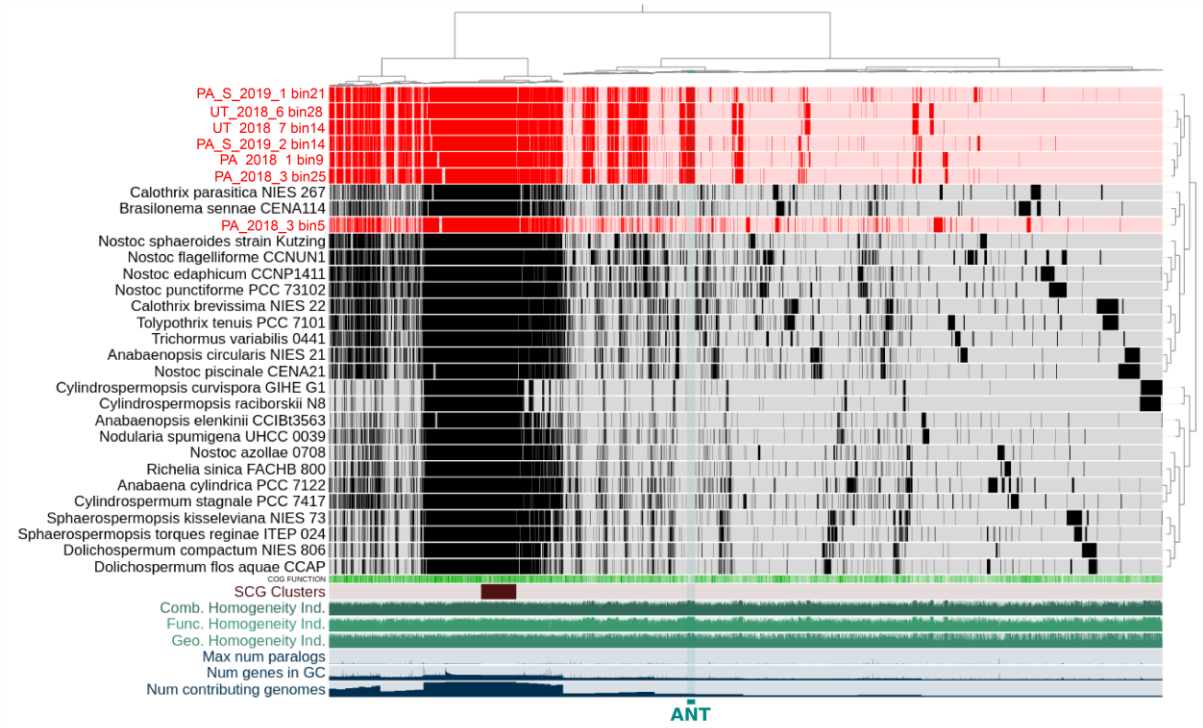


Fig. 4.4.6: Phylogram resulting from the pan-genomic analysis of the 30 Nostocales genomes.

Work package 5: Policy support activities

Task 5.1: Assessment of cross contamination risks between nunataks and contamination of the environment (P2, P3)

Experiments to investigate the possible cross-contamination due to sampling of microbial communities in the soil were carried out in the Utsteinen moraine and Dry Valleys sites during the second and third field campaigns. Sampling soil communities requires scientists to kneel for long periods, on the snow or on the ice-free soil. If biomass becomes attached to the knees and is transported to another site, it could introduce a diversity not yet present and homogenize the diversity. The risk of such a phenomenon is unknown and this pilot study was designed to answer this question for the first time. To perform the assessment, we went to the Utsteinen moraine in January 2019. The bedrock there is composed of fractured granite with underneath gravel and sand, but no visible growth of biocrusts. Two types of experiments were performed: one only occurring within two ice-free sampling places and another one occurring first in a snow patch and afterwards on an ice-free spot, while simulating a sampling period on the knees of 2 minutes each time. The experiment was performed in triplicates for each sampling spot and the protocol was tested in three different places of the Utsteinen

moraines. Surgical masks were used to retain the biological material on the knees and T_0 masks were also sampled in order to assess the potential initial bacterial composition. A total of 9 samples were taken. Human-mediated contamination may also occur while going to one site to another one on skidoo. The material collected from under the sole's boots may bring 'alien' microorganisms into the new sites as well, hence into another ecosystem. This matter was investigated in the Dry Valleys in January 2020, where swabs samples were taken as controls, and then after walking onto (i) the snow and (ii) the snow followed by the gravel. A final test after walking onto the snow was also sampled in order to assess whether the material might have been removed after walking onto the snow. A total of 40 swab samples were taken. During both samplings, the collected material was immediately stored into the cool box filled with ice packs upon arrival to the station where samples were stored at – 80 °C. Samples were then brought to Belgium at – 20 °C and are currently stored at the same temperature upon further processing.

In April 2022, the MICROBIAN partners wrote a final draft Management Plan that was sent to the 3 involved Ministries. Finally, the Management Plan was submitted to CEPXXIV as WP15 and recommended by CEP for review by the Subsidiary Group on Management Plans during the intersession. After revision, it should be ready to be accepted at the next CEP meeting.

Work package 6: Outreach and formation of junior scientists

A blog was created (www.bloggen.be/microbian) and was frequently updated during the first field campaign. As of January 2022 there have been 764 visitors. An additional blog was created (https://microbianantarctica.blogspot.com/) for the second and third campaigns and the field scientists (Valentina Savaglia, Beatriz Roncero-Ramos) provided information on the different sampling activities and field experiments. It also highlighted some publications related to microbial diversity and Polar research. As of June 2022, it has received 3731 views. A project website was constructed and can be visited at microbian.ugent.be.

Since 2016, every year, Anne Willems presents an overview of Antarctic microbial research results as part of a course on Molecular Microbial Ecology for students of the MSc program Biochemistry and Biotechnology at UGent.

PhD-students Valentina Savaglia (CPE, PAE) and Sam Lambrechts (LMG) have been involved in the preparation of the sampling campaign, and participated in all and one field campaigns, respectively. They have been trained in the wet lab for cultivation and isolation of microorganisms (P2, P3), and for the library preparation for the different sequencing approaches, as well as for the bioinformatics and statistical analysis of the resulting data (C, P2, P3). PhD student Valentina Savaglia was also the team leader of the last field campaign (2019-2020).

Both PhD students further followed courses within the doctoral schools program of their host universities and participated to the following workshops and training courses:

- "Resolving Microbial Communities At Strain-Level Resolution" workshop at the University of Exeter (Penryn, UK) (V. Savaglia, S. Lambrechts);
- "Microbial ecology: Hands-on training in prokaryotic and eukaryotic metagenomics", EMBL workshop at the Université Libre de Brussels (V. Savaglia);

- PAM training with Dr. Jana Kvíderova to measure in situ photosynthetic performance on cyanobacterial crusts (South Bohemia University, Czech Republic) (V. Savaglia);
- 'Freshwater and Terrestrial Cyanobacteria determination course' at the Department of Botany, University of South Bohemia, České Budějovice in July 2019 (V. Savaglia);
- "Introduction to multivariate analysis in R" course at KULeuven in September 2018 (V. Savaglia);
- Advanced Microbiome Data Analyses Workshop at Hasselt University, April 2021 (S. Lambrechts);
- Metagenome-Atlas course organized by the University of Oulu Graduate school (UniOGS, Finland), September – October 2021 (S. Lambrechts)

The shotgun metagenomic data is part of the PhD of Jill De Visscher (PAE).

In collaboration with the Charles University Prague (Czech Republic), a PhD student (Jordan Bishop, supervisor: Dr. K. Kopalová, co-supervisor APM) started a biogeographical, taxonomical and ecological analysis of the non-marine diatom flora from the Antarctic Continent (PhD successfully passed in 2020).

The following outreach and education activities were done:

- Results obtained within MICROBIAN and previous projects funded by BelSPO are used in courses taught within the Bachelor program in 'Biology' and the MSc programs in 'Biology', 'Biochemistry and Biotechnology' and 'Oceans and Lakes' at Ghent University, such as 'Biogeography', 'Microbial ecology', 'Molecular Microbial Ecology' and 'Aquatic Microbial Ecology'.
- The PAE laboratory trained two bachelor students (3th year biology) for five weeks within the MICROBIAN framework (bioinformatics and statistical analysis techniques of NGS amplicon data of both Eukarya and Bacteria)
- Two PhD theses are being prepared (Valentina Savaglia and Sam Lambrechts)

5. DISSEMINATION AND VALORISATION

In addition to the activities of WP6, the following actions were taken:

- Following the cancellation of SCAR2020/COMNAP, two MICROBIAN presentations were selected to be included in the SCAR2020 abstract book and were given the opportunity to be presented during a web-based oral session in the week before the planned meeting. The session was organized as an online meeting that was advertised on SCAR official channels and was recorded and uploaded to the YouTube SCAR official channel. It was followed by a text-based discussion planned for the OSC week.
- The information on the visible abundances of microorganisms in terrestrial and aquatic biotopes of the SRM is used for the drafting of the management plan of the proposed ASPA. The decision of Belgium to propose the creation was announced by public media : https://www.lalibre.be/planete/environnement/2022/05/06/la-belgique-propose-la-creation-dune-nouvelle-zone-specialement-protegee-en-antarctique-TM7SWMU2SBBGNOWZC6ESKM4IIE/
- Wilmotte is lead author of a White Paper to indicate the most pressing issues in Polar biology for the European Commission as part of an initiative of EU-POLARNET (https://www.eu-polarnet.eu/fileadmin/user_upload/www.eu-polarnet.eu/user_upload/White_Paper_2.pdf)
- The MICROBIAN project was presented (video, outreach during the 'Family Day' in the temporary Antarctica exhibition at the Royal Belgian Institute for Natural Sciences, Brussels (October 2019 – January 2021).
- 26/09/2018. Cours Collège Belgique at the Royal Academy of Belgium on 'Les cyanobactéries, microscopiques mais fondamentales' by E. Javaux, P. Jacques and A. Wilmotte
- Interview of E. Verleyen in the 'Kits jongerenkrant (www.kits.be)' regarding 'modern explorers'
- Interactive presentation about the MICROBIAN Antarctic field campaign to kids between 6 and 10 years old within the 'Printemps des Sciences' occurred at the 'Maison des Sciences' in Liège in March 2018 - V. Savaglia;
- MICROBIAN project presentation within the APECS (Association for Polar Early Career Scientists) Belgium open Day (18.09.2018) - V. Savaglia;
- Antarctic field campaign and cyanobacterial diversity presentation within an interactive workshop organized by APECS Belgium, Netherlands and Luxembourg in different primary schools for the Antarctica Day (01.12.2018) (https://apecsbelgium.wordpress.com/2018/10/24/antarctica-day-school-visit/) - V. Savaglia;
- APECS Poster presentation at the NWO (The Netherlands Organization for Scientific Research) in The Hague (Netherlands) for the Polar Symposium 2018 'Polar Impacts' (https://www.nwo.nl/en/news-and-events/news/2018/09/polar-symposium-2018.html) (07.12.2018) - V. Savaglia;
- APECS Poster presentation at the 51st International Liège Colloquium on Ocean Dynamics 2019 'Polar Ocean facing changes' in May 2019- V. Savaglia

- Outreach presentation regarding polar microbial communities by E. Verleyen for the staff of the Princess Elisabeth Station (January 2019)
- Antarctic field campaign and cyanobacterial diversity presentation within an interactive workshop organized by APECS Belgium at the Royal Belgian Institute for Natural Sciences in Brussels on the occasion of the Antarctica Day (01.12.2019) - V.Savaglia, B. Durieu
- Skype meeting with students (17yrs old) from the Antarctica Princess Elisabeth Station to the École Montjoie in Brussels (January 2020). – V.Savaglia
- A. Wilmotte was member of the Steering group of the ANTECO programme of the Scientific Committee on Antarctic Research.
- E. Verleyen is member of the board of the SCAR ANTOS working group.

Oral and poster presentations

- Willems A. 2018. Exploring hidden bacterial diversity in continental Antarctica. GGBN 2018 Conference Global Genome Biodiversity Network. 22-25 May 2018, Vienna, Austria. Invited lecture.
- Savaglia, V., Namsraev, Z., Mano, M.-J., & Wilmotte, A. (2018). Highest diversity of cyanobacterial on granite substrates in the Sør Rondane Mountains. Poster session presented at POLAR 2018 Conference - Where the Poles come together.
- Durieu, B., Baurain, D., Wilmotte, A., & Lara, Y. (2018, June 19). Cold Adaptation Strategy of the Antarctic Cyanobacterium P. priestleyi ULC007. Poster session presented at Polar 2018, Where the Poles come together, Open Science Conference,Davos, Switzerland.
- Hughes, K., Xavier, J. C., Liggett, D., Roldan, G., & Wilmotte, A. (2018, June). How Can My Research Data Be Useful for Conservation and Policy-making? Poster session presented at POLAR 2018 'Where the Poles come together', Davos, Switzerland.
- Vincent, W., & Wilmotte, A. (2018, June). Conservation Issues in the High Arctic and Pole-to-Pole Comparisons. Paper presented at POLAR 2018 'Where the Poles come together', Davos, Switzerland.
- Wilmotte, A., Beets, K., Simons, V., Lara, Y., Durieu, B., Cornet, L., Baurain, D., & Laughinghouse IV, H. (2018, June). Ex-situ Conservation of Polar Cyanobacteria in the BCCM/ULC Collection. Poster session presented at POLAR 2018 'Where the Poles come together', Davos, Switzerland.
- Tahon G., B. Tytgat, L. Lebbe, A. Carlier, A. Willems. 2018. Uncovering hidden diversity: Abditibacterium utsteinense sp. nov., the first cultivated member of candidate phylum FBP. International Symposium on Microbial Ecology - ISME17, 12-17 August 2018, Leipzig, Germany. Poster presentation.
- Santoro, M., Beets, K., Lara, Y., Durieu, B., Simons, V., & Wilmotte, A. (2018, September 13). BCCM/ULC: a unique Biological Resource Center of (sub)polar cyanobacteria. Poster session presented at XXXVII Annual Meeting of European Culture Collections' Organization - ECCO 2018, Moscou, Russia.
- Wilmotte, A., Santoro, M., Beets, K., Lara, Y., Durieu, B., Simons, V., Silva-Stenico, E., De Fiore, M., Cornet, L., & Baurain, D.(2018, October). Ex-situ conservation and exploration of polar cyanobacteria in the BCCM/ULC Collection. Poster session presented at BSM 2018 Annual Symposium "Microbes in the spotlight", Brussels, Belgium.

- Savaglia V, Lambrechts S, Tytgat B, Verleyen E, Willems A, Vyverman W, Wilmotte A: MICROBIAN: Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica (2019). Poster session presented at the Belgian Society for Microbiology Symposium 2019, Brussels.
- Willems A. 2019. Bacterial taxonomy in the age of high throughput sequencing. Federation of European Microbiological Societies (FEMS) 8th Congress of European Microbiologists, 7-11 July 2019, Glasgow, Scotland. Invited lecture using examples from our Antarctic projects.
- Valentina Savaglia, Sam Lambrechts, Bjorn Tygtat, Elie Verleyen, Anne Willems, Wim Vyverman, Annick Wilmotte. 2019. MICROBIAN: Microbiome diversity and function in the Sør Rondane Mountains, East Antarctica. Annual Symposium of the Belgian Society for Microbiology, Microbes without Frontiers. 18 October 2019, Brussels, Belgium. Poster presentation (poster B26)
- Savaglia V, Lambrechts S, Durieu B, Vanhellemont Q, Tytgat B, Verleyen E, Willems A, Vyverman W, Wilmotte A (2020): Understanding the microbiome diversity through a combination of remote sensing and close-range field observation techniques in the Sør Rondane Mountains, East Antarctica. Poster session during the Online SCAR 2020 meeting.
- Sweetlove, M., Wurzbacher, C., Nilsson, H., Tytgat, B., Sabbe, K., Verleyen, E. and Vyverman W. Diversity, biogeography and potential parasite-host interactions of aquatic fungi in (sub-)polar lakes, SCAR, 27th July (online presentation due to cancelled SCAR conference, 31st-11th August, Hobard, Tasmania, Australia. (presentation)
- Savaglia V, Lambrechts S, Durieu B, Vanhellemont Q, Tytgat B, Verleyen E, Willems A, Vyverman W, Wilmotte A (2021): Elucidating microbial community composition in the Sør Rondane Mountains, East Antarctica Poster session presented online at the World Microbe Forum 2021.

6. PUBLICATIONS

- Bulínová M., Kochman-Kędziora N., Kopalová K. & Van de Vijver B. (2018) Three new *Hantzschia* species (Bacillariophyta) from the Maritime Antarctic Region. Phytotaxa 371: 168–184. https://doi.org/10.11646/phytotaxa.371.3.2
- Pushkareva, E., Pessi, I. S., Namsaraev, Z., Mano, M.-J., Elster, J., & Wilmotte, A. (2018). Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica. Systematic and Applied Microbiology, 41, 363-373. http://hdl.handle.net/2268/218320.
- Hughes, K., Constable, A., Frenot, Y., Lopez-Martinez, J., McIvor, E., Njåstad, B., Terauds, A., Liggett, D., Roldan, G., Wilmotte,A., & Xavier, J. C. (2018). Antarctic environmental protection: strengthening the links between science and governance. Environmental Science and Policy, 83, 86-95. http://hdl.handle.net/2268/220382
- Van de Vijver B. & Kociolek J.P. (2018) A new species of *Nagumoea* (Bacillariophyta) from Antarctica, and a further consideration of the systematic position of the genus. Phytotaxa 349: 152-158. https://doi.org/10.11646/phytotaxa.349.2.5
- Van de Vijver B. & Kusber W.-H. (2018) Validation of *Navicula adminensis* D.Roberts & McMinn ("*Navicula adminii*" D.Roberts & McMinn) (Naviculaceae, Bacillariophyceae). Notulae algarum 65: 1. ISSN 2009-8987
- Tahon G., B. Tytgat, L. Lebbe, A. Carlier, A. Willems. (2018) *Abditibacterium utsteinense* sp. nov. the first cultivated member of candidate phylum FBP, isolated from ice-free Antarctic soil samples. Syst. Appl. Microbiol. 41:279-290. https://doi.org/10.1016/j.syapm.2018.01.009
- Van de Vijver B. & Dessein S. (2018) *Cyclotella deceusteriana*, a new centric diatom species (Bacillariophyta) from the sub-Antarctic Region. Phytotaxa 333: 108-116. https://doi.org/10.11646/phytotaxa.333.1.8
- Van de Vijver B., Wetzel C.E. & Ector L. (2018) Analysis of the type material of *Planothidium delicatulum* (Bacillariophyta) with the description of two new Planothidium species from the sub–Antarctic Region. Fottea 18: 200–211. https://doi.org/10.5507/fot.2018.006
- Lambrechts S., Willems A. and Tahon G. (2019) Uncovering the Uncultivated Majority in Antarctic Soils: Toward a Synergistic Approach. *Front. Microbiol.* 10:242. https://doi.org/10.3389/fmicb.2019.00242.
- Bishop J., Kopalová K., Darling J.P., Schulte N.O., Kohler T.J., McMinn A., Spaulding S.A., McKnight D.M. & Van de Vijver B. (2019) *Sabbea* gen. nov., a new diatom genus (Bacillariophyta) from continental Antarctica. Phytotaxa 418 (1): 042–056. https://doi.org/10.11646/phytotaxa.418.1.2
- Van de Vijver B. (2019) Revision of the *Psammothidium manguinii* complex (Bacillariophyta) in the sub-Antarctic Region with the description of four new taxa. Fottea 19: 90–106. https://doi.org/10.5507/fot.2019.001
- Van de Vijver B. & Ector L. (2019) *Microcostatus elisabethianus*, a new freshwater diatom species (Bacillariophyta) from the sub-Antarctic region. Plant Ecology & Evolution 152: 539–545. https://doi.org/10.5091/plecevo.2019.1600
- Van de Vijver B. & Houk V. (2019) Two new centric diatoms (Bacillariophyta) from the sub-Antarctic region. Phytotaxa 394: 050–058. https://doi.org/10.11646/phytotaxa.394.1.2

- Xavier, J. C., Mateev, D., Capper, L., Wilmotte, A., & Walton, D. W. H. (2019). Education and Outreach by the Antarctic Treaty Parties, Observers and Experts under the framework of the Antarctic Treaty Consultative meetings. Polar Record. http://hdl.handle.net/2268/220886.
- Bishop J., Kopalová K., Kohler T.J., Van de Vijver B., Roberts D., Mcminn A. & Gibson J. (2020) A re-investigation of lake sediment diatoms from the Vestfold Hills, Antarctica, using an updated, fine-grained taxonomy. Diatom Research 25: 231–254. https://doi.org/10.1080/0269249X.2020.1794982
- Majewska R.& Van de Vijver B. (2020) *Nagumoea serrata,* a new diatom species (Bacillariophyceae) found on seagrass from the south–eastern coast of Africa (Indian Ocean). Fottea 20: 98–103. https://doi.org/10.5507/fot.2019.019
- Vanhellemont Q. 2020 Automated water surface temperature retrieval from Landsat 8/TIRS Remote Sensing of Environment https://doi.org/10.1016/j.rse.2019.111518
- Van de Vijver B. & Crawford R.M. (2020) *Melosira jeanbertrandiana*, a new *Melosira* species (Bacillariophyceae) from the sub-Antarctic region. Botany Letters 167: 50–56. https://doi.org/10.1080/23818107.2019.1688677
- Van de Vijver B., Tusset E., Williams D.M. & Ector L. (2020) Analysis of the type of *Fragilaria alpestris* (Bacillariophyta) with the description of a new *Staurosira* species from the sub-Antarctic Region. Phytotaxa 471(1): 1–15. https://doi.org/10.11646/phytotaxa.471.1.1
- Van de Vijver B., Straub F., Wetzel C.E. & Ector L. (2020) Observations on and epitypification of *Synedra austriaca* Grunow (Fragilariaceae, Bacillariophyta). Notulae Algarum 130: 1–5.
- Tahon G., L. Lebbe, A. Willems. (2021). *Spirosoma utsteinenense* sp. nov. isolated from Antarctic ice-free soils from the Utsteinen region, East Antarctica. Int. J. Syst. Evol. Microbiol. 71:004754. https://doi.prg/10.1099/ijsem.0.004754
- Tahon G., D. Gök, L. Lebbe and A. Willems. (2021). Description and functional testing of four species of the novel phototrophic genus *Chioneia* gen. nov., isolated from different East Antarctic environments. Syst. Appl. Microbiol. 44:126250. https://doi.org/10.1016/j.syapm.2021.126250
- Vanhellemont Q (2020a), Automated water surface temperature retrieval from Landsat 8/TIRS (https://doi.org/10.1016/j.rse.2019.111518)
- Vanhellemont Q (2020b), Combined land surface emissivity and temperature estimation from Landsat 8 OLI and TIRS (https://doi.org/10.1016/j.isprsjprs.2020.06.007)
- Vanhellemont Q, Lambrechts S, Savaglia V, Tytgat B, Verleyen E,& Vyverman W (2021). Towards physical habitat characterisation in the Antarctic Sør Rondane Mountains using satellite remote sensing. (https://doi.org/10.1016/j.rsase.2021.100529)
- Vanhellemont Q, Brewin RJW, Bresnahan PJ & Cyronak T (2022). Validation of Landsat 8 high resolution Sea Surface Temperature using surfers (https://doi.org/10.1016/j.ecss.2021.107650)

7. ACKNOWLEDGEMENTS

The MICROBIAN team wishes to thank Maaike Vancauwenberghe and BELSPO, the personnel of the Princess Elisabeth station and the field guides, Josef Elster, Alex Terauds, Orthodrone and Juri Klusak, and the members of the follow-up committee.