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"CLIMATE CHANGE AND ANTARCTIC MICROBIAL BIODIVERSITY"

«CCAMBIO»

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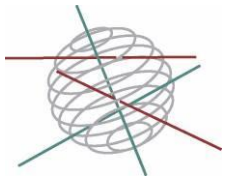

THEMATIC BIODIVERSITY

FINAL REPORT

CLIMATE CHANGE AND ANTARCTIC MICROBIAL BIODIVERSITY "CCAMBIO"

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ACRONYMS AND ABBREVIATIONS

APM: Agentschap Plantentuin Meise
CIP: Centre for Protein Engineering, Dept. Life Sciences, ULiège
LM: Laboratory of Microbiology, Dept. Biochemistry and Microbiology, UGent
PAE: Protistology and Aquatic Ecology, Dept. Biology, UGent
RBINS: Royal Belgian Institute of Natural Sciences
SCAR: Scientific Committee on Antarctic Research
SRP: Scientific Research Programme

ACBR: Antarctic Conservation Biogeographic Region
AR: Antarctic Realm
ARISA: Automated Ribosomal Intergenic Spacer Analysis
DGGE: Denaturating Gradient Gel Electrophoresis
HTS: High Throughput Sequencing
ITS: Internal Transcribed Spacer
LSU: Large Subunit (of the ribosome)
MALDI TOF MS: Matrix Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry
MID: Multiplex Identifier
NGS: Next Generation Sequencing
NRPS: Non Ribosomal Protein Synthase
OTU: Operational Taxonomic Unit
PCR: Polymerase Chain Reaction
Peg: protein encoding gene
PKS: Polyketide Synthase
rbcL: ribulose 1-5 biphosphate carboxylase Large subunit-gene
rRNA/rDNA: ribosomal RNA/DNA (ribonucleic acid/deoxyribonucleic acid)

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A. Context

Magnified climate change in the polar regions predicted by global circulation models may have a profound impact on terrestrial and aquatic ecosystems in Antarctica and Sub-Antarctica. Polar lacustrine ecosystems appear to be particularly responsive to climate changes. In high latitude lakes, eukaryote and bacterial microorganisms are the main sources of primary production and elemental cycling. These ecosystem functions are frequently concentrated in microbial mats growing in the illuminated benthic zone due to the combined effects of low nutrient content and relative stable water-columns caused by prolonged periods of ice cover. Changes in these environmental conditions can provoke pronounced shifts in community structure. As such, lacustrine microbiomes are especially relevant as early warning systems of environmental stress induced by climate change and anthropogenic impacts. However, this requires integration of data on their diversity, biogeographic zoning and evolutionary history with information on their functional attributes underlying their response and resilience to environmental change.

In macroscopic organisms, three main biogeographic regions are traditionally recognized (i.e. sub-Antarctica, Maritime Antarctica and Continental Antarctica). This observation suggests an ancient and common biogeographic and evolutionary origin of the terrestrial and freshwater biota in the southern hemisphere. In contrast to macroscopic organisms, however, relatively little is known on the biodiversity, biogeographic distribution patterns and functional potential of microbial taxa, largely due to the technological difficulties associated with studying the vast diversity of microorganisms and the large number of (characterized) samples required to cover the whole Antarctic Realm (AR).

In the last two decennia, the advent of high throughput sequencing (HTS) has marked an important turning point in the study of microbial biodiversity. HTS of taxonomic marker genes results in a high amount of sequences, including those of rare taxa, at a relative low effort and cost per sequence. Bioinformatic tools have also been developed to detect and try to correct potential artifacts that could create spurious taxonomic units and inflate the biodiversity.

In the Antarctic Realm (AR), it is becoming evident that, similar to multicellular taxa, endemism among microorganisms does exist and the Southern Ocean thus acted as an efficient dispersal barrier for most microbes. Formal tests of bioregionalization patterns in microbial organisms in the AR are however still lacking due to the paucity of region-wide taxonomic inventories using a standardized sampling and taxonomic identification strategy.

Recently, the Antarctic Peninsula and Continental Antarctica have been subdivided into 16 biologically distinct smaller regions, based on physical parameters of ice-free areas,

expert consultation, and multivariate analyses on occurrence patterns in macro-organisms. Named "Antarctic Conservation Biogeographic Regions" (ACBRs), these zones provide an important tool for conservation, management and policy. However, microbes were poorly integrated in this framework due to the limited availability of high resolution and spatially extensive data. It therefore remains unclear to what degree microbial communities differ between ACBRs and if the latter could also be used to conserve the unique microbial assemblages. In addition, testing the hypothesis that microbes show a high level of provincialism, comparable to that observed in multicellular biota in the AR, would provide further significant insights into the common evolutionary origin and history of Antarctic biota.

An unexpected opportunity for the CCAMBIO project was the possibility to include samples from 85 Arctic lakes (Greenland, Svalbard and Northern Norway). While both Polar Regions are characterized by similar climatic features, they have a different tectonic history and contrasting levels of isolation to neighboring land masses. Their particular evolutionary history has thus led to biological differences between habitats in Antarctica and their comparable counterparts in the Arctic. Therefore, it appeared interesting to test whether interhemispheric differences could be observed in microbial diversity and microbial biogeographic patterns.

B. Objectives

The overall objective of CCAMBIO was to study the diversity, biogeographic zoning, evolutionary history, and genomic make-up of lacustrine microbial mat communities in the Antarctic Realm (AR) in order to assess their resilience and local and regional responses to global change. The specific objectives were:

1. To extend and improve existing sample collections of lacustrine microbial communities by conducting field campaigns in understudied regions.
2. To quantify the degree and nature of microbial bioregionalisation in the AR using in-depth inventories of microbial biodiversity (cyanobacteria, bacteria, and protists) based on state-of-the-art techniques, including HTS, microscopy and analysis of biogeochemical markers along major geographical, climatic and environmental gradients. Biomass partitioning among the major groups of photosynthetic microorganisms was performed using high performance liquid chromatography (HPLC) of photosynthetic pigments. This was complemented by in depth analyses of microbial community composition using HTS analyses of the 16S or 18S rRNA genes for cyanobacteria, bacteria and microeukaryotes, which were processed using custom-made bioinformatic pipelines that were validated using laboratory-generated communities.

3. To test evolutionary hypotheses on the origin, diversification rate and range dynamics of selected taxa. For a selection of taxa, multi-gene molecular phylogenies were developed to study their diversity and evolutionary history within the AR. Due to problems in primer design, this work was replaced by genome sequencing for cyanobacteria.
4. To study the overall genomic make-up and biochemical properties of a selected microbial mat community along a depth gradient to assess the contribution of the different taxonomic/functional groups to the functioning of the consortium in response to differences in lake water depth and the light climate. Functional data of microbial communities were obtained by a metagenomic inventory.
5. To explore the potential of microorganisms and functional genes/groups as early warning indicators for global change through modelling the distribution of focal taxa and functional groups in response to climate and environmental variables. These can provide the baseline data to define bioregions and identify areas with an unusual diversity or harboring a relict microflora. Moreover, these data can be integrated with climate models to predict the distribution of taxa and functional groups under different scenarios of climate change.
6. To valorize and present the biodiversity data at various meeting and workshops attended by specialists in the field and the general public. The datasets and their inclusion in models will be useful as support to environmental policies in Antarctica. After publication in international peer-reviewed journals, all data will be made public in an Open Access data system (the microbial Antarctic Resource System at mars.biodiversity.aq and the Global Biodiversity Information Facility at gbif.org).

C. Conclusions

This study was the first survey of the benthic microbial mats in lakes from the whole Antarctic Realm. Based on HTS, the dominant eukaryotes in the benthic microbial mats were Metazoa, Chlorophyta and Stramenopila, followed by Fungi, Ciliophora and Cercozoa. Among bacteria, Proteobacteria and Cyanobacteria dominated the reads of 16S rRNA genes. Distinct biogeographic zones could be recognized in the distribution patterns of both eukaryotes and bacteria based on multivariate ordination and clustering techniques. This bioregionalisation is in agreement with the classical subdivision of the Antarctic Realm into Maritime Antarctica, Continental Antarctica and the sub-Antarctic Islands generally observed in plants and animals. Indeed, Stramenopiles, Ciliophora, Cercozoa, Chlorophytes and most Metazoa showed a clear bioregionalisation, while Dinophyta and the Metazoan group Rotifera exhibited no clear patterns. Moreover, microbial communities were also highly different from similar lakes in the Arctic, suggesting a strong imprint of historical factors such as dispersal limitation, extinction,

speciation and colonization. The Antarctic food-webs appeared to be less complex and truncated, with particular functional groups being absent (e.g. Annelida) while others were relatively diverse (e.g. ciliates) and comparable between sub-Antarctic and Arctic lakes. Moreover, local OTU-richness of both eukaryotes and bacteria was significantly lower in Antarctica compared with Sub-Antarctica and the Arctic, and decreased with increasing latitude in the Southern Hemisphere but not in the North. Generalized linear models revealed that this interhemispheric diversity-asymmetry in bacteria could be significantly explained by environmental properties of the lakes and differences in energy availability, while in eukaryotes, the lack of connectivity appeared to put additional constraints on OTU-richness. Focusing on cyanobacteria, the conductivity and pH appear to contribute to the structuring of the lake communities on the Antarctic continent. Combined, these results show that, as with plants and animals, contemporary distributions of microbes and their diversity in polar and sub-polar regions are highly impacted by historical processes.

The pigment study by HPLC showed that in Antarctic lakes, microbial mats were dominated by cyanobacteria whereas in sub-Antarctic lakes, photoautotrophs were dominated by mosses or higher plants. Interestingly, diatom-dominated biofilms appear widespread in high Arctic lakes.

The study of the diatom biogeography in the Antarctic Realm also showed strong bioregionalisation patterns at multiple spatial scales and supported the delineation of the Continental and Maritime Antarctic lakes into existing ACBRs, while the floras in Sub-Antarctica group into the three oceanic provinces. The diatom floras in each of the biogeographic entities appeared to be unique and different. In Maritime Antarctica, more than 120 new species were described.

D. Contribution of the project in a context of scientific support to a sustainable development policy

The rather high ratio of endemic and novel microbial phylotypes observed on the Antarctic continent shows that a significant fraction of the microbial diversity may have evolved *in situ* over larger temporal scales. Combined, the strong bioregionalization and macroecological patterns point to past and present dispersal limitation, evolution in isolation and persistence of Eukarya and Bacteria on the continent in glacial refugia during ice ages. This is largely in agreement with patterns for macroscopic organisms, and calls for stringent measures to avoid the introduction of alien microbial species into the Antarctic Biogeographic Realm, and to prevent the homogenisation of microbial communities between terrestrial ice-free regions. The loss of this Antarctic microbial diversity and its replacement by cosmopolitan invasive taxa would impair the scientific understanding of the functioning of these native communities and the study of their evolutionary history, specific adaptations and properties.

The conservation and management measures would include:

1) Management plans for terrestrial ice-free regions and their lakes should include measures to prevent the introduction of non-native microbes into the AR, as exotic taxa might potentially affect local communities and competitively exclude endemic and sometimes rare species.

2) Second, the unintentional transportation of microorganisms from one region to another should be avoided in order to protect regions against increased homogenization of their microbial floras. This evidently requires more stringent measures than those currently taken by national scientific program managers and tourist operators. Moreover, the awareness of scientists of other disciplines working in Antarctica would also need to be raised, as they also might disperse microorganisms to and from different local and regional sources during field work. This is particularly important for areas of Antarctica that are still pristine. Considering the steady increase in tourism and scientific activities in the AR as well as forecasted climate and environmental changes render this issue a high priority on the international conservation agenda. A special attention to this point could be integrated into the Environmental Impact Assessments that are mandatory to carry out activities in Antarctica following the Protocol on Environmental Protection of the Antarctic Treaty. In the sub-Antarctic Islands, the national authorities are responsible for the environmental management.

3) Antarctic Specially Protected Areas (ASPA) should be designated in areas of unique microbial diversity, that is currently undervalued and rarely considered as being worth protection. Of the 72 ASPAs that existed in 2015, 19 and 7 were mentionning algae or cyanobacteria, respectively. These ASPA would also include'inviolate areas' that would be closed to human presence for long periods and serve as 'reference areas' for future studies with methods that will be even more sensitive and sophisticated that those available today.

Though it is not possible to protect microbial habitats from the impact of climate change *per se*, and there will be climatic changes resulting in larger deglaciated areas, we advocate to work with the SCAR and CEP to avoid that anthropogenic dissemination and homogenization (including by tourism) would destroy the legacy of a unique and fascinating evolution.

E. Keywords

Antarctica, Arctic, lakes, microorganisms, biodiversity, biogeography, bacteria, cyanobacteria, green algae, diatoms, eukaryotes, climate change, metagenomics, cultivation, HTS, conservation, environmental protection

1. INTRODUCTION

1.1 Context

Global circulation models predict magnified climate change in the Polar Regions (e.g. Arctic Council, 2004; Turner et al., 2009), which will have a profound impact on terrestrial ecosystems if the current rates of change persist (Walther et al., 2002). In addition to changes in local ecosystem structure and functioning induced by climate change, invasions by alien species represent a potentially even greater threat to Antarctic and sub-Antarctic biota (Convey, 2011; Frenot et al., 2005; Lee et al., 2017) and result in range contraction or even extinction of local taxa. This is important because recent taxonomic inventories revealed clear bioregionalization patterns (Convey et al., 2014) and that the incidence of endemism in biota from the Antarctic Realm (AR) is high (Convey et al., 2007), which reflect their long history of isolation and low rates of migration and colonization within the region since the onset of the Mid-Miocene cooling of the continent (Convey et al., 2008; De Wever et al., 2009; Mortimer et al., 2011; Stevens et al., 2006; Van der Putten et al., 2010). Hence, magnified global warming and range reductions/expansions have the potential to strongly affect the evolutionary future of regional endemics at a rate far beyond the natural variability of at least the past 14 Ma. This was recognized by SCAR and urged scientists to improve the conservation of Antarctic life and ecosystems and obtain science-based evidences for the delineation of Antarctic Specially Protected Areas, as defined by the Antarctic Treaty (https://www.ats.aq/e/ep.htm). Recently, the Antarctic Peninsula and Continental Antarctica have been subdivided into 16 biologically distinct smaller regions, based on physical parameters of ice-free areas, expert consultation, and multivariate analyses on occurrence patterns in macro-organisms (Terauds et al., 2012, Terauds & Lee 2016). Named Antarctic Conservation Biogeographic Regions (ACBRs), these zones provide an important tool for conservation, management and policy (Terauds et al., 2012). These ACBRs are thought to result from long-term isolation of biota, which may in some cases reflect the presence of glacial refugia, i.e. small pockets of ice-free land where life was able to persist through glacial maxima (Fraser et al., 2014).

For life in Polar regions, liquid water is a severely limited resource. Hence lakes, ponds and seepage areas are particularly significant hot-spots of biological activity and biodiversity. Importantly, these lacustrine systems appear to be very responsive to climate changes (Quayle et al., 2002; Smol et al., 2007b; Verleyen et al., 2011) in different ways. Climate warming can lead to an increase in the number of ice-free days and to increased nutrient import as a result of the deglaciation of the catchment area, which, in turn, stimulate primary production (Quayle et al., 2002; Verleyen et al., 2011). Temperature rise can also lead to changes in the moisture balance (Verleyen et

al., 2011). In particular, lakes with a high surface area to volume ratio were shown to respond quickly to changes in the precipitation-evaporation balance and are prone to salinization and even complete desiccation (Hodgson et al., 2006; Smol et al., 2007a). These lacustrine systems are dominated by microorganisms, while more complex organisms are generally sparse or even absent (Cavicchioli, 2015; Priscu et al., 1998). In Antarctic coastal ice-free regions and some inland mountains, lakes are present and sometimes relatively widespread, representing a range of environmental parameters that enable to study the biogeographic patterns in microbial communities. Most primary production and nutrient cycling are generally concentrated in dense microbial mats thriving in the illuminated benthic zone (Quesada et al., 2009; Sabbe et al., 2004). Cyanobacteria are the dominant photosynthetic producers in these mats, and some taxa are also able to fix atmospheric nitrogen, which is a scarce resource in these (mostly) oligotrophic lakes (Quesada et al., 2012; Vincent, 2000). As such, benthic microbial mats are the first level of the foodweb in Antarctic lakes that are affected by climate induced environmental changes (Jungblut et al., 2010; Verleyen et al., 2010; Vincent, 2000). The responsiveness of microorganisms and their dominating effects on ecosystem functioning make them suitable indicators for the early detection of climate related environmental changes (Vincent, 2000; Yergeau et al., 2012). However, this requires integration of data on their diversity, biogeographic zoning and evolutionary history with information on their functional attributes underlying their response and resilience to change. Compared to macroscopic organisms, progress in this integration has been lagging behind, largely because of technological difficulties associated with studying the vast diversity of microorganisms. Moreover, little is known about how different taxa contribute to the functioning of these ecosystems.

In the last two decennia, the advent of high throughput sequencing (HTS) has marked an important turning point in the study of microbial biodiversity. HTS of taxonomic marker genes results in a high amount of sequences, including those of rare taxa, at a relative low effort and cost per sequence (Xu et al., 2014; Zeglin, 2015). Bioinformatic tools have also been developed to detect and try to correct potential artifacts that could create spurious taxonomic units and inflate the biodiversity (Edgar, 2013). These methodological break-troughs and revised species concepts based on genetic information revealed that many microorganisms occur in discrete ranges bordered by dispersal barriers (Foissner, 2008; Martiny et al., 2006), while only few are capable of long-distance dispersal (Cox et al., 2016). This contradicts the "ubiquity hypothesis" (Finlay, 2002) stating that, due to their high abundance and dispersal capabilities, microorganisms do not show the same degree of bioregionalisation as multicellular organisms do, and hence that most microbial species should be virtually cosmopolitan. The ubiquity hypothesis is further refuted by an increasing number of large-scale studies which reported that similar, yet distant, habitats contain different microbial assemblages,

thus indicating microbial provincialism (Nemergut et al., 2011; Vyverman et al., 2007). These differences can be caused by adaptations to past and present environments or genetic drift, but must be maintained by genetic isolation and hence dispersal limitation (Martiny et al., 2006; Mittelbach et al., 2015). However, the relative contributions of historical and environmental factors in explaining microbial biogeography seem to vary with the investigated spatial scale and the type of ecosystem studied (Green et al., 2006; Martiny et al., 2006; Verleyen et al., 2009).

In the Antarctic Realm (AR) it is becoming evident that, similar to multicellular taxa, endemism among microorganisms does exist (Esposito et al., 2006; Taton et al., 2006a, 2006b; Vyverman et al., 2010) and the Southern Ocean thus acted as an efficient dispersal barrier for most microbes. Formal tests of bioregionalization patterns in microbial organisms in the AR are however still lacking due to the paucity of region-wide taxonomic inventories using a standardized sampling and taxonomic approach. Moreover, although ACBRs serve as a tool for science, policy and conservation, microbes were poorly integrated in this framework due to the limited availability on high resolution and spatially extensive data, and it remains unclear to what degree microbial communities differ between ACBRs and if the latter could also be used to conserve the unique microbial assemblages. In addition, testing the hypothesis that microbes show a high level of provincialism comparable to that observed in multicellular biota in the AR (Chown et al., 2007; Van der Putten et al., 2010) would provide further significant insights into the common evolutionary origin and history of Antarctic biota.

An unexpected opportunity for the CCAMBIO project was the possibility to include samples from 85 Arctic lakes (Greenland, Svalbard and Northern Norway). While both Polar Regions are characterized by similar climatic features, they have a different tectonic history and contrasting levels of isolation to neighboring land masses. This had major consequences for the potential size range contractions and expansions of biota in response to past glaciations and climatic changes. This particular evolutionary history has thus led to biological differences between habitats in Antarctica and their comparable counterparts in the Arctic (Fraser et al., 2012; Pointing et al., 2015). Therefore, it appeared interesting to test whether interhemispheric differences could be observed in microbial diversity and in their biogeographic patterns.

1.2 Objectives and expected outcomes

The overall objective of CCAMBIO was to study the diversity, biogeographic zoning, evolutionary history, and genomic make-up of lacustrine microbial mat communities in the Antarctic Realm (AR) in order to assess their resilience and local and regional responses to global change. The specific objectives were:

1. To extend and improve existing sample collections of lacustrine microbial communities by conducting field campaigns in understudied regions.
2. To quantify the degree and nature of microbial bioregionalisation in the AR using in-depth inventories of microbial biodiversity (cyanobacteria, bacteria, and protists) based on state-of-the-art techniques, including HTS, microscopy and biogeochemical markers along major geographical, climatic and environmental gradients. Biomass partitioning among the major groups of photosynthetic microorganisms (green algae, cyanobacteria, diatoms/chrysophytes and cryptophytes, photosynthetic bacteria) was done using high performance liquid chromatography (HPLC) of photosynthetic pigments. The marker-pigment based inventory was complemented by in depth analyses of microbial community composition using HTS analyses of the 16S or 18S rRNA genes for cyanobacteria, bacteria and microeukaryotes, which were processed using custom-made bioinformatic pipelines that were validated using artificial communities.
3. To test evolutionary hypotheses on the origin, diversification rate and range dynamics of selected taxa. For a selection of taxa, multi-gene molecular phylogenies were developed to study their evolutionary history within the AR. Due to problems in primer design, this work was replaced by genome sequencing for cyanobacteria. The aim was to assess the importance of adaptive radiations and local population differentiation.
4. To study the overall genomic make-up and biochemical properties of a selected microbial mat community along a depth gradient to assess the contribution of the different taxonomic/functional groups to the functioning of the consortium in response to differences in lake water depth and the light climate. Functional data of microbial communities were obtained by a metagenomic inventory.
5. To explore the potential of microorganisms and functional genes/groups as early warning indicators for global change through modelling the distribution of focal taxa and functional groups in response to climate and environmental variables. These can provide the baseline data to define bioregions and identify areas with an unusual diversity or harboring a relict flora. Moreover, these data can be integrated with climate models to predict the distribution of taxa and functional groups under different scenarios of climate change.
6. The biodiversity data were valorized and presented at various meeting and workshops attended by specialists in the field and the general public. The datasets and and their inclusion in models will be useful as support to environmental policies in Antarctica. After publication of the results and findings in international peer-reviewed journals, all data will be made public in Open Access data systems (the microbial Antarctic Resource System at mars.biodiversity.aq and the Global Biodiversity Information Facility at gbif.org).

2. METHODOLOGY

2.1. Sampling

2.1.1. Samples

We used a large set of 193 and 439 samples for HTS and morphological identifications of diatoms, respectively. The sample set consisted of samples obtained from previous research collaborations, exchange with international partners and during new samplings campaigns (Figure 1).

The joint sample selection (by PAE, LM and CIP), ensured that the most important environmental gradients (lake water salinity, lake depth and pH) (Verleyen et al., 2012) were included in the eight different Antarctic Biogeographic Regions of Conservation (ACBRs) (Terauds et al., 2012) where lakes occur. The diatom dataset additionally included samples from the three biogeographic oceanic provinces in the Sub-Antarctic (Figure 1). These primary sample sets were further extended with strategically selected lake and soil samples from Antarctica, and (sub-) Arctic (Greenland, Svalbard and northern Norway; Figure 2), alpine (e.g., Chilean Patagonia, European Alps) and temperate regions for meta-analysis, to assess the bipolar distribution of taxa, and to develop molecular phylogenies of focal taxa (e.g. *Pinnularia borealis*).

2.1.2. Sampling procedures

Benthic microbial mats were sampled using a UWITEC Glew corer in the deepest part in deep lakes and using a spatula in terrestrial habitats and in the littoral zone (20 cm) of deep lakes and shallow lakes (lake depth $<$2m).

2.1.3. Sample preservation

The majority of the samples consist of sediment and microbial mat samples transported and stored at -20°C. A few samples were preserved in ethanol. Some recent samples were also kept cool for the isolation of micro-algae.

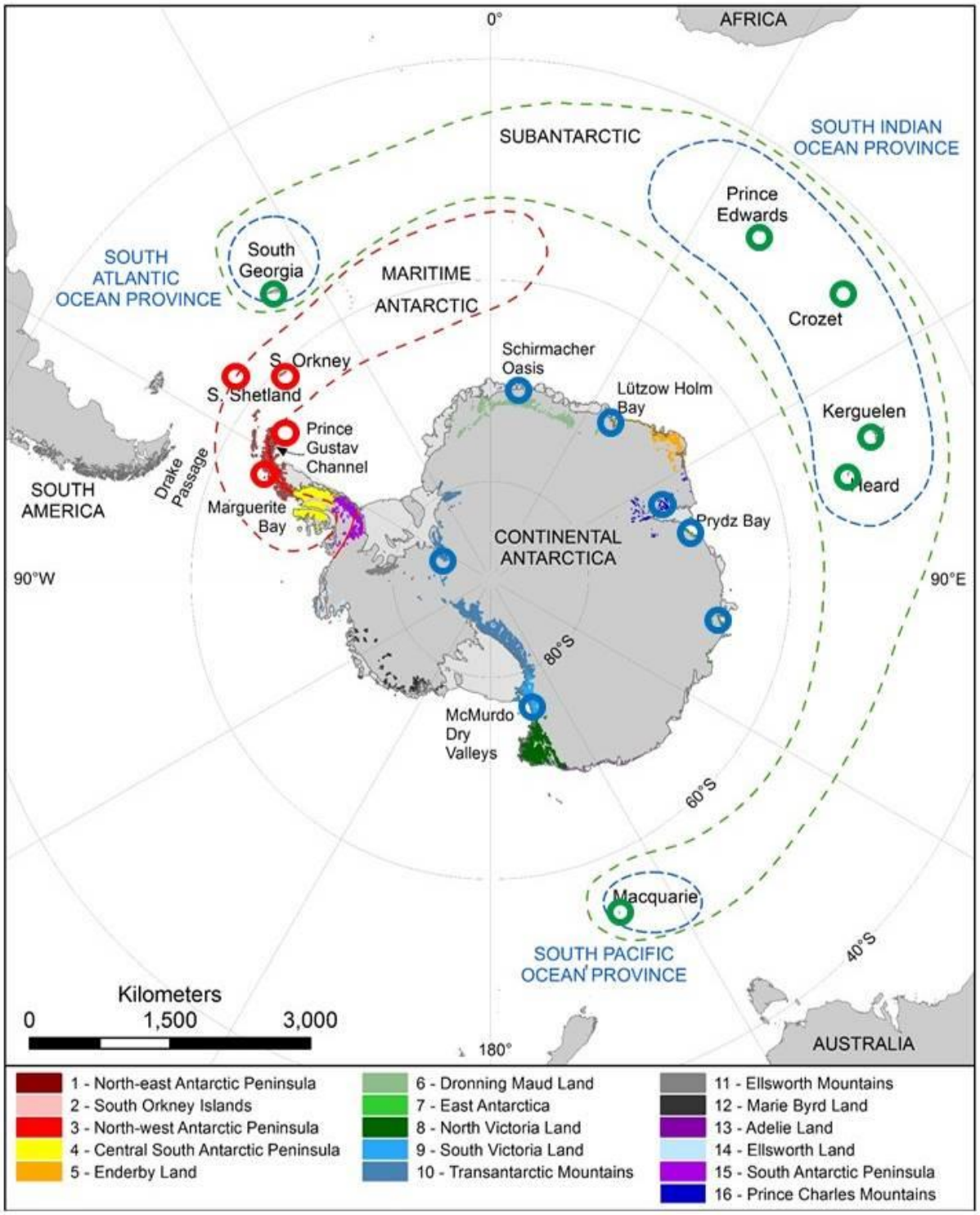

Figure 1: Islands and ice-free regions included in the sample set of the CCAMBIO project with an indication of the three main biogeographic regions in the Antarctic Realm, namely Continental Antarctica (blue circles), Maritime Antarctica (red circles) and the sub-Antarctic Islands (green circles). The latter is traditionally subdivided into three biogeographic provinces, namely the South Pacific, the South Atlantic and the South Indian Provinces. The Antarctic Conservation Biogeographic Regions (ACBR) are indicated in different colors. Map modified from Chown & Convey (2007), Terauds et al. (2012) and Terauds & Lee (2016). For different studies, subsets of this sample set were selected (see description of the separate tasks below).

2.1.4. Supporting environmental data

For samples taken by members of the CCAMBIO team, the instruments and protocols used to measure the limnological variables can be found in Verleyen et al. (2011). Specific conductance and pH were measured using calibrated field meters (a YSI 600 or YSI 33 meter and a Hanna pH meter). The concentration of the main ions was analysed in certified labs by using ion chromatography following ISO 10304-1(2007). For other samples, data were obtained from other projects or from our international partners. In summary, for the majority of the Antarctic, sub-Antarctic and Arctic samples, we have pH and measurements of specific conductance. For a subset of the Antarctic and sub-Antarctic lake samples (n = 213), additional data on the concentration of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, NH_4^+ and PO_4^{3+} are available.

2.2. Bioregionalisation of microbial biota and functional groups

2.2.1. Structural-functional characterization of phototrophic community composition

A total of 80 lakes from the three main biogeographic regions (i.e., Sub-Antarctica, Maritime Antarctica and Continental Antarctica) as well as North and South Greenland (Figure 2) were selected for a high performance liquid chromatography analysis of their photosynthetic pigments. The samples were freeze-dried, followed by immediate pigment extraction by sonication in 2 to 5 mL HPLC-grade acetone (90%), and filtration of the extracts through a nylon 0.20 μm filter. Pigments were separated and quantified using an Agilent technologies 1100 series HPLC system, calibrated using authentic pigment standards and compounds isolated from reference cultures (Jeffrey et al., 1997). A detailed description of the methods used is given in Tavernier et al. (2014).

2.2.2. Distribution patterns of diatoms based on the morphology

Diatom samples (n = 439) were collected from the uppermost 5 mm of sediment cores in deep lakes (depth > 0.5 m). In shallow lakes, which have little or no ice cover around the edges during summer, sediment samples were collected at a depth of 0.2 m in the littoral zone. Subsamples for diatom analysis were subsequently digested with H_2O_2 (30 %). For light microscopy, a subsample was dried onto a glass coverslip, mounted in Naphrax® and studied using an Olympus BX53 microscope equipped with differential interference contrast optics and a Zeiss Axiophot microscope equipped with differential interference contrast. For scanning electron microscopy, oxidized sample material was directly air-dried onto specimen stubs and sputter-coated, and examined with a Jeol JSM-840 operated at 15 kV and a Zeiss Ultra field emission SEM operated at 5 kV. For transmission electron microscopy, 10 μl aliquots of oxidized material were placed on formvar-coated copper slot grids. Grids were examined with a Jeol JEM 1010 operating at 60 kV.

The diatom floras of the Maritime Antarctic locations, James Ross Island and the South Shetland Islands, were revised. Our existing datasets were also extended with additional samples from Marion Island (Sub-Antarctica) and Schirmacher Oasis (Dronning Maud Land) and analysed using multivariate statistics to reveal biogeographic patterns.

2.2.3. High-Throughput Sequencing targeting Bacteria

2.2.3.1. Amplicon sequencing of environmental 16S rRNA genes for the Bacteria

Per microbial mat sample, 1 g sediment was used for DNA extraction. After extracellular DNA was removed following Corinaldesi et al. (2005), DNA extraction was performed according to (Zwart et al., 1998). Extracted DNA was subsequently stored in TE-buffer at -80°C until further processing.

Technical specifications and protocols described by Tytgat et al (2016) were followed. Briefly, duplicate PCRs were performed, targeting the V1–V3 hypervariable regions of the 16S rRNA genes. Modified primers were used to allow tagging of each sample using the Nextera XT index kit (Illumina, USA). Products were purified, quantified and pooled for sequencing by using Illumina MiSeq technology yielding 2x 300bp paired end reads. To check the overall run quality and benchmark processing variables, a blank sample and two artificial communities (composed of a set of known organisms) as well as several replicate samples were included (Tytgat et al., 2016).

In total 193 aquatic samples, originating from 8 Antarctic and 2 sub-Antarctic regions as well as 39 Arctic samples (Figure 2), were analysed and compared using a range of analysis tools (see 2.2.5 below). For all samples pH and specific conductance were available. Monthly averaged (1990-2013) air temperature measurement were obtained from the CRUTEM4 database version 4-2015-06 (Jones et al., 2012; Osborn et al., 2014), using records for the nearest station within a 500 km radius. When no temperature data were available (Transantarctic mountains), an approximation was made based on monthly averaged satellite data (1985-2005) from the NASA Earth Observations database (NEO, NEO-team), which used data provided by the MODIS Land Science Team (MODIS Land Science Team, 2017). Monthly averaged (1983-2005) insolation incident on a horizontal surface data (solar irradiance, kWh $m^{-2}day^{-1}$) were obtained from NASA Surface meteorology and Solar Energy (SSE, release 6.0) (Stackhouse et al., 2008) at a one by one degree resolution. The percentage of surface area covered by sea/ocean and ice sheets in a 200 km radius circle was used as a proxy measure for lake isolation, and was calculated following Vyverman et al. (2007). Altitude and approximate shortest distance to the sea measurements were taken using the Google Earth measuring tool and satellite image maps (15 February 2015; Google Inc.).

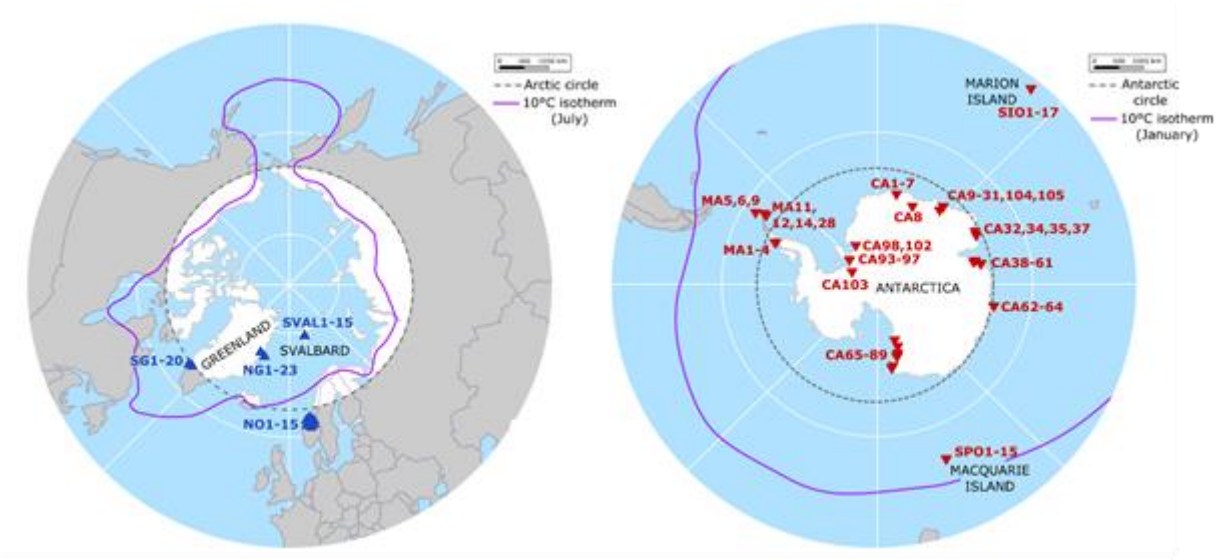

Figure 2: Sample locations (depicted by triangles) displayed on a map of the Northern (left) and Southern (right) hemispheres. Dashed line: Arctic/Antarctic circle (defined as the latitude where the sun is above the horizon for 24h/day at least once a year); purple line: 10°C summer isotherm.

Results were compared with the 18S eukaryote sequencing data from the same samples (2.2.4 below) and are grouped in several manuscripts that are in preparation.

2.2.3.2. Amplicon sequencing of environmental 16S rRNA genes for the order *Deinococcales*

For amplicon sequencing of 16S rRNA genes of the focal group *Deinococcales*, DNA were extracted as described above and a specific amplicon sequencing was attempted as this group was previously found to comprise mostly isolates that are potentially restricted to Antarctica. We hypothesized that some of these could represent endemic species adapted to harsh environmental conditions and therefore particularly sensitive to climate change. However, as it proved not possible to design primers of sufficient specificity, we tested a published primer set (Theodorakopoulos et al., 2013) for Illumina sequencing. We used DNA from 5 environmental samples (3 from the site of the Princess Elisabeth Station, 1 from Schirmacher Oasis and 1 from Pourquoi-Pas Island) and DNA extraction, PCR and quality controls were done as before. Also here, a modified artificial community was included to optimize subsequent data analysis. Our results indicate that also the Theodorakopoulos-primers lack specificity and pick up other taxa (i.a. Phylum Firmicutes) besides *Deinococcus*. We therefore abandoned our attempts to target *Deinococcus* in the Illumina sequencing.

2.2.3.3. Amplicon sequencing of environmental 16S rRNA genes for the Cyanobacteria

As very little work had been published for cyanobacteria when the CCAMBIO project started, a pilot study was carried out to optimize the use of HTS for the study of Antarctic cyanobacterial diversity (Pessi et al., 2016). Artificial communities were used to evaluate the performance of different bioinformatics pipelines. These were assembled using twenty-two cyanobacterial strains from the BCCM/ULC Cyanobacteria Collection (http://bccm.belspo.be/about-us/bccm-ulc) (TABLE I). Furthermore, the effect of different DNA extraction methods, the use of longer barcoded primers and the reproducibility of the results were determined using environmental samples.

TABLE I. List of reference strains used in the artificial communities Art1 and Art2.

ID[a]	Strain name	Accession number	Relative abundance (%)	
			Art1	Art2
ULC022	*Phormidesmis priestleyi* ANT.L61.2	AY493582	4.5	8.9
ULC026	*Phormidesmis priestleyi* ANT.L52.6	AY493579	4.5	8.9
ULC049	*Phormidesmis priestleyi* ANT.L66.1	AY493581	4.5	8.9
ULC009	*Plectolyngbya hodgsonii* ANT.LPR2.2	AY493583	4.5	8.9
ULC001	*Leptolyngbya frigida* ANT.L53B.1	AY493608	4.5	8.9
ULC029	*Leptolyngbya frigida* ANT.L52B.3	AY493612	4.5	8.9
ULC043	*Leptolyngbya antarctica* ANT.LWAV6.1	AY493602	4.5	8.9
S141	*Leptolyngbya* sp. 'Doroninskoye'	KT753316	4.5	8.9
ULC041	*Leptolyngbya antarctica* ANT.LAC.1	AY493588	4.5	8.9
ULC065	*Cyanobium* sp. 'Bylot Island'	KT753317	4.5	1.5
S082	*Cyanobium* sp. 'Chester Cone'	KT753318	4.5	1.5
ULC084	*Cyanobium* sp. 'Laguna Chica'	KT753319	4.5	1.5
ULC004	*Leptolyngbya* cf. *fragilis* ANT.L52.1	AY493584	4.5	1.5
S111	*Microcoleus vaginatus* JR6	KT753320	4.5	1.5
S120	*Microcoleus favosus* JR20	KT753321	4.5	1.5
ULC117	*Leptolyngbya* sp. ANT07.JR16	KT753322	4.5	1.5
ULC123	*Leptolyngbya* sp. ANT07.JR23	KT753323	4.5	1.5
ULC080	*Anabaena* sp. CY-036	KT753324	4.5	1.5
S133	*Nodularia* sp. 'khil 06-sten'	KT753325	4.5	1.5
ULC050	*Nostoc* sp. ANT.L34.1	AY493591	4.5	1.5
ULC069	*Pseudanabaena frigida* O-302	KT753326	4.5	1.5
S075	Oscillatoriaceae Toolik Lake O-103	KT753327	4.5	1.5

[a]A ULCXXX number indicates that the strain is available at the BCCM/ULC Cyanobacteria Collection (http://bccm.belspo.be/about-us/bccm-ulc)

Sequences of the 22 reference strains were aligned using MUSCLE (Edgar, 2004) and trimmed to the expected amplicon product of the V3-V4 variable region – hereafter referred to as "reference sequences". For the artificial community, genomic DNA was extracted from the reference strains using the DNeasy Plant Mini Kit (Qiagen, Venlo, the Netherlands). DNA extracts were quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA) and pooled at known abundances: equal amounts for library Art1 ("even") and different ones for library Art2 ("staggered")(TABLE I). The latter was assembled to represent a more realistic scenario of taxa distribution in Antarctic environments, where some species are more abundant than others.

The PCR reactions were carried out with the cyanobacteria-specific primer set CYA359F and CYA781R(a)/CYA781R(b)as described by Pessi et al. (2016). Negative controls (PCR mixes with either no DNA or the blank DNA extracts) were always included during PCR amplifications. To minimize stochastic PCR bias, amplification was carried out as six independent PCR reactions (three for each reverse primer) that were pooled before purification. Pooled PCR reactions were purified using the GeneJet PCR Purification Kit (Thermo Scientific, Waltham, MA, USA). Purified amplicons were quantified as described above, pooled in equimolar concentrations and sent to Beckman Coulter Genomics (Danvers, MA, USA), where smaller amplicons were removed using the Agencourt AMPure XP Kit (Beckman Coulter, Brea, CA, USA) and sequencing adapters were ligated to the amplicons. Sequences were obtained using the 454 GS FLX+ Titanium platform (454 Life Sciences, Branford, CT, USA) or the Illumina Hiseq2500 platform (Illumina, San Diego, CA, USA) because the first technology was discontinued by Roche during the course of the project. The Figure 3 shows the location of samples studied by one or the other technology.

A first 454 pyrosequencing study was carried out on microbial mats from 13 Antarctic lakes (Figure 3). The studied lakes are distributed across eight Antarctic regions encompassing four distinct Antarctic Conservation Biogeographic Regions (ACBRs): ACBR1 "NW Antarctic Peninsula" (AP), ACBR5 "Enderby Land" (EL), ACBR7 "East Antarctica" (EA) and ACBR10 "Transantarctic Mountains" (TM). DNA was extracted from the mats using the PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions with some modifications. Tubes were agitated on a vortex for 20 extra min to ensure a good disintegration of the mats and, if not completely disintegrated, a sterile pestle was used to crush the remaining pieces. DNA concentration and quality were determined using a NanoVue spectrophotometer (GE Healthcare Life Sciences, Little Chalfont, UK), and double stranded DNA was quantified as described above. A blank DNA extraction consisting of sterile Milli-Q

water was carried out in parallel. PCR and sequencing were performed as described for the pilot study above.

Finally, using Illumina Sequencing, additional analyses were performed for 89 microbial mat samples from 84 lakes or ponds, distributed in 8 ACBRs (ACBR 1 NE Antarctic Peninsula, ACBR 3 NW Antarctic Peninsula, ACBR 5 Enderby Land, ACBR6 Dronning Maud Land, ACBR 7 East Antarctica, ACBR8 North Victoria Land, ACBR 9 South Victoria Land, ACBR10 Transantarctic Mountains) and the sub-Antarctic Marion and Macquarie Islands (Figure 3). Five samples were run in duplicate, leading to a total of 94 samples. DNA extractions and PCR were carried out as described above for the 454 pyrosequencing study. Sequencing was performed on the Illumina Hiseq2500 platform (Illumina, San Diego, CA, USA) using 2x100 bp paired end libraries.

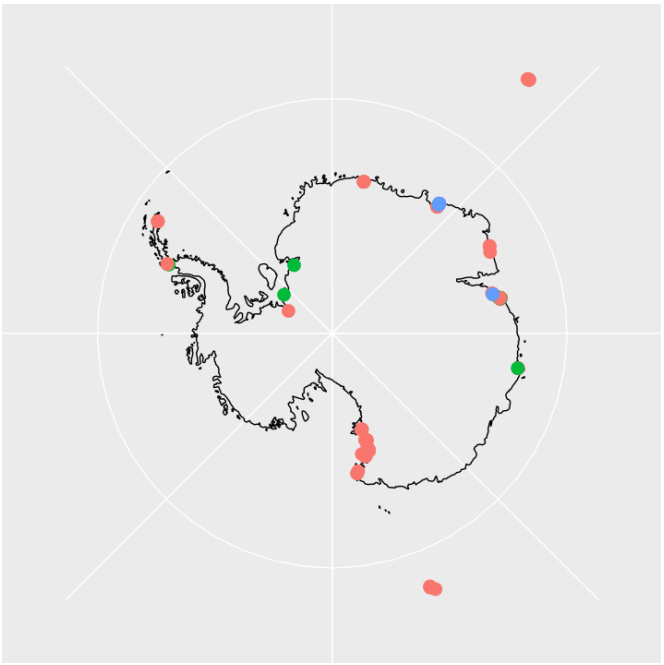

Figure 3. Map of Antarctica showing the lakes investigated in the Amplicon sequencing of environmental 16S rRNA genes studies using R package "ggplot2" and "mapproj". Blue: unique to the 454 pyrosequencing study, Green: common to the two studies, Red: unique to the Illumina sequencing study.

2.2.4. Amplicon sequencing of environmental 18S rRNA genes for Eukarya

In parallel with the library preparation of the bacterial 16S rRNA (2.2.3.1), the V4 region of the eukaryotic 18S rRNA gene was targeted with the universal primers TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Stoeck et al., 2010). Amplicon libraries were also prepared as described in Tytgat et al. (2016). In short, PCRs were performed in duplicate, after which, products were purified with Agencourt AMPure XP beads (Beckman Coulter Inc.). The amplicon libraries were barcoded using the NEXTERA DNA

kit (Illumina Inc.) according to manufacturer's instructions, and were pooled in equimolar concentrations. Sequencing was done on a 300bp paired-end Illumina MiSeq machine, where cluster density was reduced by spiking with 20% PhiX DNA (Illumina Inc.), which has been shown to increase to overall quality of the sequencing run (Kozich et al., 2013). Two artificial communities (composed of pooled cultures) as well as several replicate samples were also included in each run.

For the 18S rRNA amplicon sequencing, similar as for the 16S rRNA gene (2.2.3.1), 193 samples from benthic microbial mats were sequenced, as well as well as 39 samples from four Arctic regions to allow a broader comparison of polar microbial diversity (Figure 2). The results of the 16S and 18S rRNA sequencing were combined, and will be discussed in two manuscripts that are currently at the final stages of preparation.

2.2.5. Data processing and analysis of amplicon sequences

For Eukaryotes and Bacteria, paired end reads were assembled using PEAR (version 0.9.1; Zhang et al., 2014) and were further processed and quality filtered using USEARCH (Edgar, 2013). The processing parameters were optimized using the artificial community data as described by Tytgat et al. (2016). OTU clustering was done with UPARSE (Edgar, 2013) and de novo chimera filtering with UCHIME (Edgar et al., 2011) with a cut-off of 97% used as threshold to cluster sequences. The sequence with the most identical reads in each OTU was chosen as representative, to which a taxonomy was annotated with Mothur's (Schloss et al., 2009) implementation the Wang naive Bayesian classifier algorithm (Wang et al., 2007), using the PR2 database (version gb_203) as template for eukaryotes (Guillou et al., 2013) and the Greengenes database version 13_5 (DeSantis et al., 2006) for bacteria.

For Cyanobacteria, **the pilot study** on artificial communities was first realized to select the most correct settings for the bioinformatic pipeline. Quality control of reads, removal of chimeric sequences and Operational Taxonomic Unit (OTU) clustering were first performed on the data obtained for the artificial communities, with five independent bioinformatics pipelines using MOTHUR and UPARSE as described in Pessi et al. (2016). For each pipeline, sequences were clustered into OTUs using a cut-off similarity value of 97.5% according to Taton et al. (2003).

The correspondence between the OTUs retrieved at the end of each pipeline and the reference strains used to assemble the artificial communities was determined by mapping the OTU representative sequences to the 22 reference sequences using the "uparse_ref" command in USEARCH with default parameters (Edgar, 2013). OTUs were classified as "Perfect" (identical to a reference sequence), "Good" (≥99% similarity), "Noisy" (≥97.5% to <99% similarity), "Other" (<97.5% similarity), and "Chimeric" (composed of two or more parent reference sequences). The relative abundance of each reference strain was inferred considering only "Perfect" and "Good" OTUs, i.e., OTUs

with ≥99% similarity to a reference sequence. To avoid biases due to uneven sequencing depths, data sets were subsampled without replacement to 15,820 sequences per sample.

For the environmental samples studies of Cyanobacteria (454 pyrosequencing and Illumina sequencing runs), based on the results obtained for the artificial communities, pipeline (IV) "fastq_maxee" was selected for the analysis. OTUs were classified using the lowest common ancestor (LCA) algorithm implemented in CREST (Lanzén et al., 2012) based on two databases: Greengenes (McDonald et al., 2012) for the 454 pyrosequencing run and RDP (Wang et al., 2007) for the Illumina sequencing run, and non-cyanobacterial OTUs were removed from downstream analyses. For each OTU, all closely related (≥99% similarity) cultured and uncultured sequences were retrieved from GenBank using BLAST, and information regarding the geographic origin of each hit was obtained using in-house UNIX scripts. OTUs were then classified as "Novel" (if no related sequences were found in GenBank at the 99% similarity threshold), "Endemic" (when only hits from Antarctica were retrieved), "Polar" (when hits were limited to Arctic and Antarctic biotopes), "Polar/Alpine" (when hits from high altitude biotopes were also included) and "Cosmopolitan" (when hits located outside Polar and alpine biotopes were found). For the phylogenetic analysis, a 'backbone' tree was first built using full 16S rRNA gene sequences from 141 cyanobacterial genomes, as well as sequences from 125 cyanobacterial strains from the BCCM/ULC Cyanobacteria Collection and their closest relatives in GenBank. Sequences were aligned using MUSCLE and a maximum likelihood tree was built using the RAxML algorithm based on the GTRGAMMA model and 1,000 bootstrap replicates (Stamatakis, 2006). OTU representative sequences were then added to the alignment and another RAxML tree was computed as above but using the previous tree as a stable backbone. In addition, for the Illumina sequencing study, closely related sequences (>= 94.0% similarity) of the representative sequence of each OTU were retrieved from GenBank using BLAST in order to improve the taxonomic identification. Home made bash/perl scripts were used for the final identification. OTUs matching with sequences named "uncultured bacterium" or "uncultured cyanobacterium" were checked manually in order to remove non cyanobacterial ones.

Several multivariate statistical techniques were applied on the different datasets. These included the calculation of pairwise distance measures, clustering techniques and direct and indirect ordinations. The specific methods used for each dataset are given in the results section**.**

2.3. Phylogeographic studies of focal taxa

2.3.1. Isolation and characterization of bacterial strains

Isolations have been performed, with a focus on the presence of colored pigments in the bacterial colonies. Red to yellow pigments may reflect adaptation to UV-radiation and light stress and more generally, exposure to stress. Such stress tolerant organisms might be more tolerant to impacts of climate change. Isolates were grouped by MALDI-TOF MS and representatives were identified by partial 16S rRNA gene sequence analysis.

In view of the particular resistance of Deinococci to exposure to desiccation and radiation stress, and because we have previously obtained numerous novel isolates of this group from Antarctic samples, we have focused on this group to develop a multi-locus sequence scheme for phylogenetic analysis. Therefore, the available annotated genomes of *Deinococcus* type strains and relatives were compared to select 6 housekeeping genes and to design specific primers for their amplification. PCR protocols were optimized and six genes were sequenced. Our goal was to end up with 2 or 3 genes with robust PCRs that can be used to assess the *Deinococcus* diversity and its biogeographic distribution.

2.3.2. Isolation and characterization of cyanobacterial strains

a) In order to isolate new strains from regions from which we do not have yet isolates, three samples from North Victoria Land and three samples from South Victoria Land were inoculated on BG11 1/3 diluted medium with 500 $\mu g/mL^{-1}$ cycloheximide to inhibit the growth of eukaryotic organisms.

b) Molecular phylogenies based on *rpoC1* gene and ITS sequences from 43 thin filamentous cyanobacterial strains difficult to identify and one Nostocales were constructed with RAXML and the MEGA 5 software. Seventy *rpoC1* sequences from published genomes were aligned with our sequences to perform a maximum likelihood phylogeny. Among the selected strains, our dataset contained 13 strains of the cosmopolitan genus *Nodosilinea* (Perkerson et al., 2011), 13 Antarctic strains from the lineage of *Phormidesmis priestleyi* (Taton et al., 2006a) and six *Leptolyngbya antarctica*.

c) Detection of *nifH/D* genes was carried by PCR with the *nifH* primers of Olson et al. (1998) and Roeselers et al. (2007) on the above mentioned strains.

d) As preliminary experiments with Multiple Locus Sequence Analysis have shown that the primers designed on certain taxa did not work well for the whole cyanobacterial phylum and that this resulted in many missing data and impaired analyses, the whole genome sequencing of several Antarctic strains was started. Ten unicyanobacterial strains from six different lineages and four regions (West Peninsula, Transantarctic Mountains, East Antarctica, Dronning Maud Land) were selected for genome sequencing. They represent genotypes that are very frequently observed in our diversity

studies. The strain *Phormidesmis priestleyi* ULC007 was purified to axenic status through multiple liquid/solid media transfers as described by Rippka et al. (1979). The genomic DNAs were extracted using the GenElute Bacterial genomic DNA kit (Sigma-Aldrich, St Louis, Missouri, USA).

For ULC007, two Illumina runs generating 100-paired and 250-paired end reads were performed. Sequences from both Illumina runs were assembled using the SPAdes and Velvet software packages. Resulting contigs were assembled using the CISA3 software. The genome sequences were annotated using glimmer3, GeneMark, Prodigal by RAST taking the SEED approach with the Figfams technology. The genome comparative analysis was performed on a database of 72 curated cyanobacterial genomes. The 16S rRNA sequences were extracted using RNAmmer with same genomes' dataset. After aligning the sequences, the BEAST software was used to construct a calibrated relaxed molecular clock. Root prior was set at 3.8 Ga and akinete fossils were used as a calibration prior to support the Nostocales clades at 2.1 Ga. In order to screen for sequences encoding secondary metabolites biosynthesis enzymes, assembled contigs were analyzed by the online version of antiSMASH software.

The other nine genomes were in fact kinds of metagenomes because they still included the sequences of accompanying bacteria and were also analyzed during a collaboration with Luc Cornet, Prof. Denis Baurain (Phylogenomics, ULiege) and Prof. Emmanuelle J. Javaux (Evolution and Astrobiology Lab, ULiege) . After observing that a number of the publically available cyanobacterial genomes were contaminated by bacterial sequences, these public genomes were cleaned and made available (more details in Cornet et al., 2018b). Dedicated bioinformatic pipelines were designed to treat the metagenomes of 7 Antarctic strains: ULC027 *Phormidium priestleyi* ANT.PROGRESS2.5, ULC041 *Leptolyngbya antarctica* ANT.ACE.1, ULC073 *L. glacialis* TM1FOS73, ULC082 *Cyanobium* sp. Chester Cone, ULC084 *Cyanobium* sp. Laguna Chica, ULC129 *L. foveolarum* TM2FOS129, ULC165 *Leptolyngbya* sp. OTC1/1 (more details in Cornet et al., 2018a)

2.3.3. Isolation and characterization of diatom strains

About 300 monoclonal diatom cultures of *Pinnularia borealis*, a cosmopolitan species complex, were established from various Maritime Antarctic, Arctic and Alpine regions, including Spitsbergen, James Ross Island and the South Shetland Islands, and added to the existing dataset (Souffreau et al., 2013).

A maximum parsimony phylogeny of the D1-D3 LSU rDNA gene has been developed for all newly obtained strains.

2.3.4. Isolation and characterization of green algal strains

Strains from the 18S type EO2-14, II-11, VPL6-4, B6-6, WO1L-3 were isolated from lakes in Maritime and Continental Antarctica (De Wever et al., 2009) and coupled to the dataset of Dr. K. Sciuto, Dr. I Moro, and Dr. N. La Rocca (University of Padova, Italy) which contained the 18S rRNA sequences of the Gondwana strain stored in the International Nucleotide Sequence Database (INSD) with the accession number AM419228.

Molecular phylogenies were developed based on the ITS2 spacer, *rbc*L and *tuf*A gene in collaboration with the team from the University of Padova and combined with morphological (light microscopy and scanning electron microscopy) and ultrastructural observations.

2.4. Metagenomics and metatranscriptomics of Antarctic lake samples

This work package was aimed to study the genetic make-up of lacustrine microbial communities and their adaptations to the extreme Antarctic environment in East Antarctica. Six samples were taken in February 2015 at 0.1 m, 1 m, 2 m, 4 m, 5 m and 10 m depth in Lake Naga (Naga Ike, SK5, Syowa Oasis, 69°S, 39°E; maximum depth 10.8 m), in Skarvsnes, an ice-free peninsula in Lützow Holm Bay. The samples for metatranscriptomics were immediately preserved with LifeGuard® solution (MoBio) to deactivate RNAse proteins and frozen to further prevent the breakdown of RNA.

DNA and RNA were co-extracted using the MoBio RNA PowerSoil® Total RNA Isolation Kit and MoBio DNA Elution Accessory Kit. Care was taken to avoid moss material, to focus only on the microbial and viroid communities.

The RNA processing involved ribosomal RNA depletion in all but one replicate of each transcriptomics sample using the Ribo-Zero Magnetic Gold (Epidemiology) kit (Epicentre) to increase the mRNA recovery. The non-rRNA depleted replicates were used to identify the active members of the community. cDNA synthesis was finally performed using the Bio Scientific NEXTflex Directional RNA-Seq Kit V2, which enabled us to track strand-specific transcription. However, the extracted RNA proved not to be of sufficient quality to continue the processing pipeline up to sequencing. This degraded RNA was likely due to fluctuations in temperature during different transportation stages.

Hence, we only applied metagenomics sequencing. For this, extracted DNA of three samples (naga1 (0.1 m depth), naga5 (5 m depth) and naga6 (10 m depth)) was sent to Baseclear B.V. (Leiden, Netherlands), where metagenome shotgun libraries were prepared following the standard protocol of the Nextera XT library preparation kit (Illumina), and 125bp paired-end sequenced on an Illumina HiSeq 2500 machine. Reads were processed using an in-house developed pipeline. Quality trimming was done using Trimmomatic, first removing any remaining Illumina adapter sequences, followed by a 4 bp sliding window set to an average Q score of 20, and finally

removing trimmed reads shorter than 60 bp. Next, 2 pipelines were run in parallel. The first one applied a direct annotation approach, while the second one involved an assembly using both Megahit (Li et al., 2015) and (Nurk et al., 2017) for comparison. For both pipelines, ribosomal reads were extracted using SortMeRNA (Kopylova et al., 2012) and Kraken2 (Wood et al., 2014) was subsequently used to assign a taxonomy to these reads. The non-ribosomal reads were subjected to Diamond BLAST (Buchfink et al., 2014) and the output was consequently imported in MEGAN (Huson et al., 2007) to deduce taxonomical associations and gene annotations using several databases (EggNOG, SEED, InterPro2GO). An independent functional annotation was done using InterProScan (Jones et al., 2014). Contigs were binned using CONCOCT (Alneberg et al., 2014) to try and reconstruct genomes.

3. RESULTS

3.1. Phototrophic communities studied using marker pigments

The following chlorophyll and carotenoid pigments could be identified in the 80 Arctic, Antarctic and sub-Antarctic lakes: chlorophyll a, chlorophyll b, chlorophyll C2, phaeophytin a, fucoxanthin, neoxanthin, hexa-fucoxanthin, violaxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, lutein, canthaxanthin, ß-cryptoxanthin, echinenone, α-carotene, ß-carotene, Chlorophillide a. Among these, a number of major phototrophic groups (specific marker pigments in brackets) (Hodgson et al., 2004a) could be distinguished, including, Bacillariophyta (chlorophyll c2), Ochrophyta (fucoxanthine, diadinoxanthine), Cyanobacteria (canthaxanthin, echinenone), Chlorophyta (chlorophyll b, lutein, α-carotene).

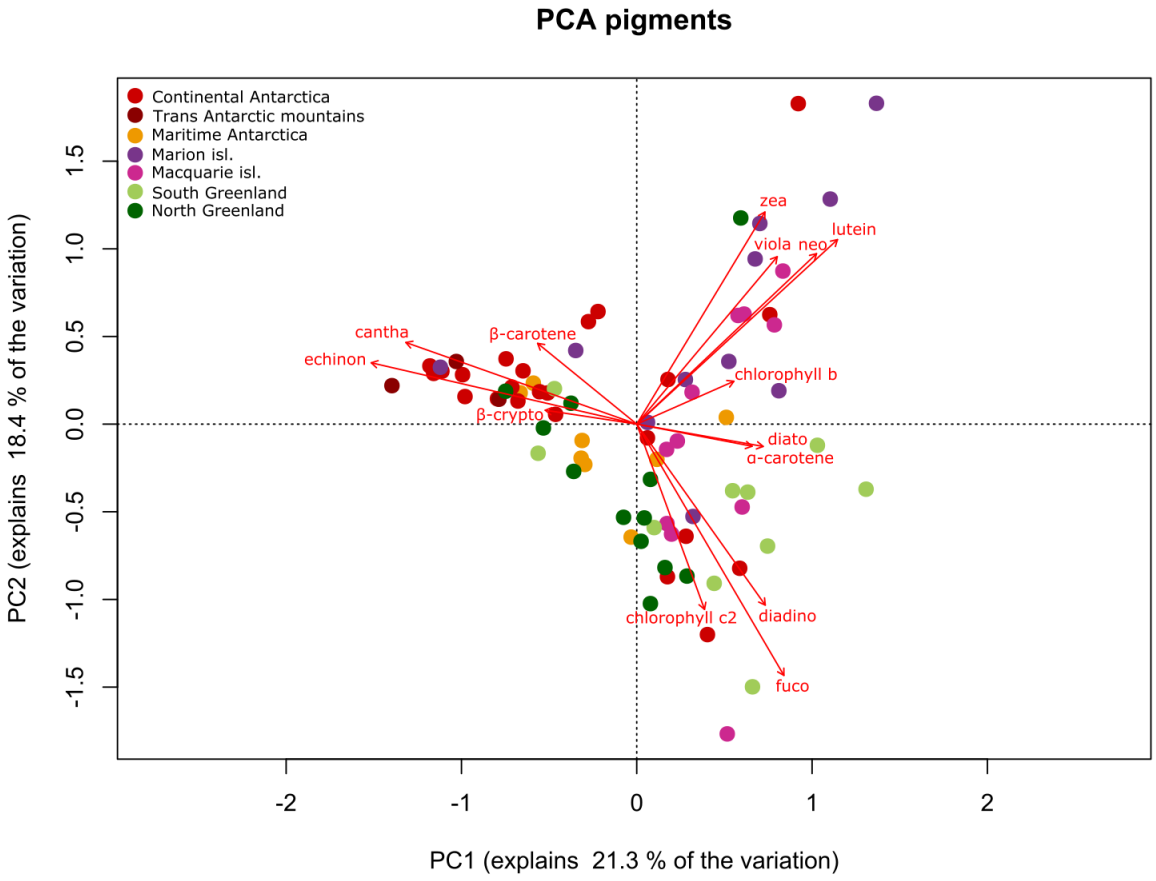

Figure 4. PCA of the group-specific HPLC marker pigments. Chlorophyll and carotenoid pigment concentrations (*μ*g/g dry-weight sediment) were normalized to their respective totals

PCA revealed a separation along the first axis between samples dominated by cyanobacteria (echinenone, canthaxanthin), most of which were situated in Antarctica, from those in which the most abundant pigments were predominately affiliated with chlorophytes (including higher plants) (mostly sub-Antarctic lakes) (Figure 4). The second PC axis, allowed to discriminate a third group that included mainly Arctic lakes, which were dominated by ochrophyte or diatom specific pigments (chlorophyll c2, fucoxanthine). This separation is in agreement with previous studies which showed that in Antarctic lakes microbial mats dominated by Cyanobacteria are widespread as a result of the lack of large grazers and hence bioturbation as well as the lack of competitors (macrophytes….) (Vincent, 2000). By contrast, in sub-Antarctic lakes, photoautotrophic benthic communities are dominated by mosses or higher plants, while diatom-dominated biofilms are widespread in high Arctic lakes.

3.2. Bioregionalisation patterns in diatoms

The integration of newly developed and taxonomically harmonized existing diatom datasets resulted in a circum-Antarctic wide diatom database containing 439 lakes. For this, the freshwater and terrestrial diatom flora of the Maritime Antarctic Region (James Ross Island and the South Shetland Island) needed to be entirely revised, based on the currently accepted species concepts. The analysed sampling set contained, besides lake samples, also samples collected from streams, moss vegetations and soils. Every diatom genus present in these samples was analysed, resulting in the description of more than 120 new species, mainly in the genera *Muelleria*, *Hantzschia*, *Nitzschia*, *Pinnularia*, *Luticola* and *Humidophila*. A new iconographic guide for the Maritime Antarctic Region presenting all these new species was published recently (Zidarova et al, 2016). Additionally, in the sub-Antarctic Region, samples from Macquarie Island were analysed in order to obtain composition data from the Pacific Province of this region.

Strong bioregionalisation patterns emerged at multiple spatial scales. Distinct and differently sized diatom floras characterized each of the three main biogeographic regions, with only 4% of the species being shared between Maritime Antarctica, Continental Antarctica and the sub-Antarctic islands. Biogeographical provincialism within the different ice-free regions largely followed previous delineations based on macroscopic organisms. More in particular, there is a general support for the delineation of the Continental and Maritime Antarctic lakes into ACBRs (Terauds et al., 2012), while the floras in Sub-Antarctica group into the three oceanic provinces (Figures 1, 5). These beta diversity patterns were underlain by species turnover rather than nestedness (calculated following Basegla (2010)), which underscores the uniqueness of the diatom floras in each of these biogeographic entities.

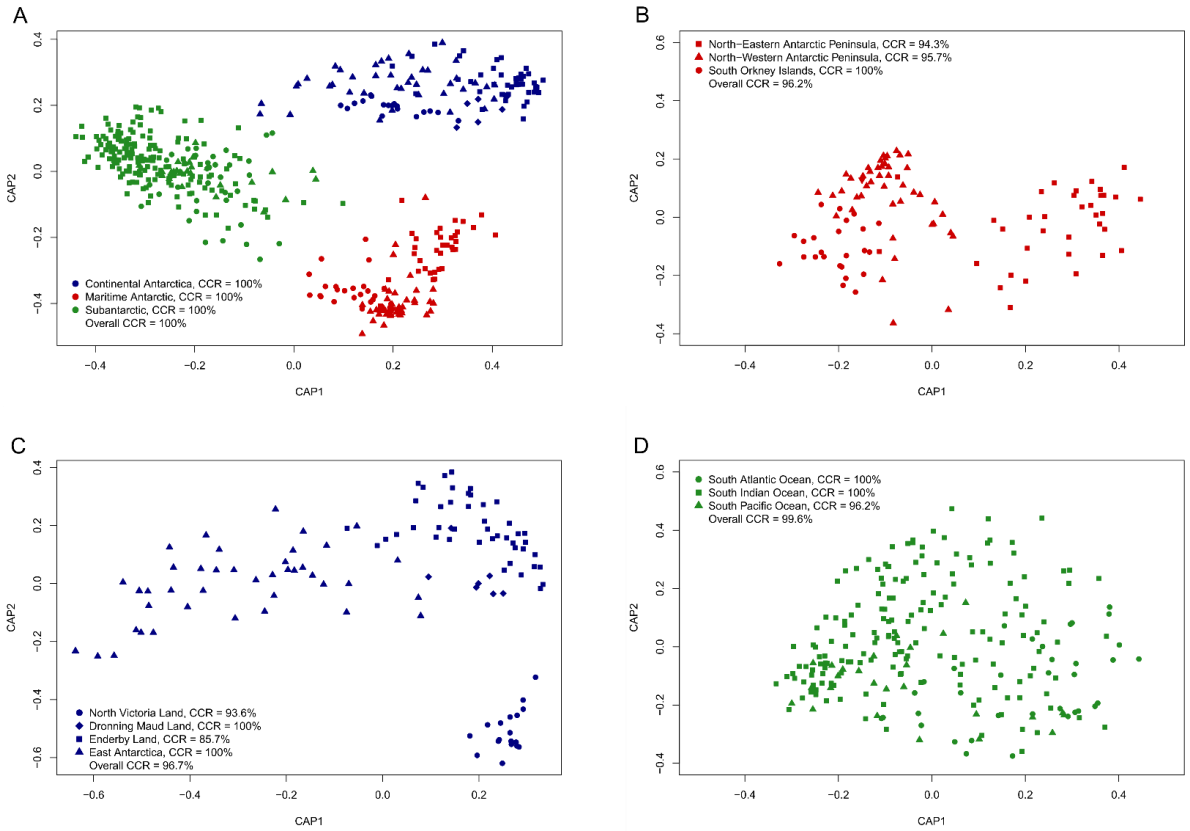

Figure 5: Biplots of canonical analyses of principal coordinates showing the site scores of the 457 samples from (A) the entire dataset and (B-D) the three regions analysed separately, namely Maritime Antarctica (in red, B), Continental Antarctica (in blue, C), and Sub-Antarctica (in green, D). CCR is the correct classification rate, or classification success, and denotes the percentage of lakes that are grouped in their respective *a priori* defined biogeographic entities.

Redundancy analysis and variation partitioning of a subset of lakes (n = 213) for which a common set of environmental data was available, revealed that variation in local diatom community structure is significantly ($P < 0.05$) explained by both (i) environmental, as well as (ii) historical and geographic factors. Combined, geographic and historical factors explain 44.4% of the total variation in local diatom community structure. The significant local and climatic variables are the difference between mean summer and winter temperature, pH, specific conductance, and the concentrations of Cl^-, Na^+, Ca^{2+}, PO_4^{2+}, K^+, NO_3^-, Mg^{2+} and NH_4^+. These factors jointly explain 33.6% of the variation in diatom turnover between the lakes. As expected, the overlap between both sets of predictors is relatively large (24.8%), given that much of the variation in environmental conditions is spatially structured at such a large scale. Additionally, however, geographic and historical variables uniquely explain 10.8% of the total variation in diatom data, while the unique contribution of local environmental and climatic variables amounts to only 8.8%.

Standardized estimates of regional richness to an equal number of samples (n = 105) increased from 58 diatom species, to 120, and 232 in Continental Antarctica, Maritime Antarctica and the sub-Antarctic islands, respectively. Local species richness increased linearly with decreasing latitude. A total of 221 out of the 470 species is only known from the Antarctic Realm, with the proportion of regionally restricted species decreasing with latitude (74 ± 16% in Continental Antarctica, 61 ± 9% in Maritime Antarctica, and 46 ± 13% in the sub-Antarctic Islands). The endemism appeared particularly high in terrestrial genera such as *Luticola*, *Muelleria*, *Humidophila* and *Hantzschia*. These latitudinal gradients in species richness and the level of endemism could be significantly explained by geographical isolation, regional differences in the deglaciation history, as well as by geographic variation in environmental and climatic conditions.
Combined, the strong bioregionalization and macroecological patterns point to past and present dispersal limitation, evolution in isolation and persistence of diatoms on the continent in glacial refugia during ice ages. This is largely in agreement with macroscopic organisms, and calls for stringent measure to avoid the introduction of alien microbial species into the Antarctic Biogeographic Realm, and to prevent the homogenisation of microbial communities between terrestrial ice-free regions

3.3. Amplicon sequencing of environmental 16S rRNA genes for Bacteria and 18S rRNA genes for Eukarya

On average, 94.824 ± 64.888 and 22.033 ± 27.776 quality controled sequences per sample for Eukaryotes and Bacteria, respectively, were obtained. This resulted in 9,403 and 8,871 OTUs for Eukarya and Bacteria, respectively in the Antarctic, sub-Antarctic and Arctic samples (Figures 1,2), after removal of singleton and doubleton OTUs.
Among eukaryotes, Metazoa, Chlorophyta, Stramenopila, Fungi, Ciliophora and Cercozoa dominated the assemblages, while among Bacteria, Proteobacteria and Cyanobacteria were most abundant (Figure 6). Interestingly, sub-Antarctic assemblages harbored more complex food webs, with arthropods, nematodes, rotifers, flatworms and annelids as main metazoan groups. Lakes on the continent, however, were characterized by fewer metazoan groups, the almost complete absence of Platyhelminthes, Annelida and Gastrotricha, and a dominance of microbial herbivores and secondary consumers, including a relative high diversity of ciliates and tardigrades. This is in agreement with the depauperate pelagic food webs reported in the small number of Antarctic lakes studied so far (Laybourn-Parry et al., 2007). In addition to the lack of competition by higher plants, the absence of large metazoan grazers in the Antarctic lakes, might favor the dominance of Cyanobacteria, because the lack of bioturbation is one of the factors resulting in the presence of perennial microbial mats in these systems (Vincent, 2000).

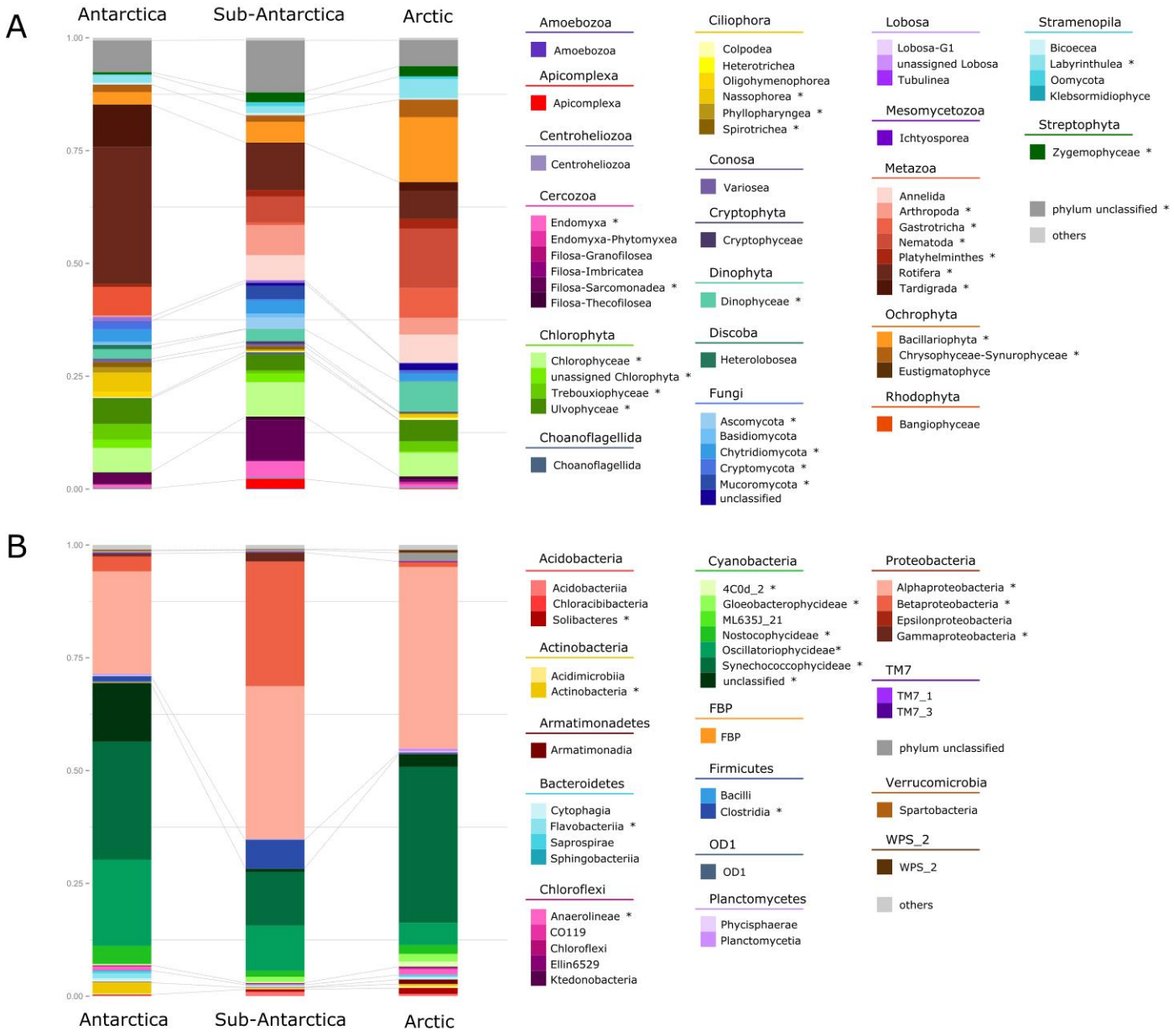

Figure 6. Regional community composition, based on the summed relative abundances per sample. The averaged composition of the Antarctic, Sub-Antarctic and Arctic regions for eukaryotes (A) and bacteria (B) are shown at the class level (bars), and are alphabetically grouped and colour coded per phylum. Only classes that represent at least 0.1% of the sequences in at least one region are shown. Classes representing over 1% of the sequences in one region are indicated with (*).

Moreover, in both eukaryotes and bacteria, local OTU-richness was significantly lower in Antarctica compared to the Sub-Antarctic and the Arctic, and decreased with increasing latitude in the Southern Hemisphere but not in the North (Figure 7). We used

generalized linear models (assuming Poisson-distributed count data) to examine possible drivers of this interhemispheric diversity-asymmetry. This analysis revealed that in bacteria, environmental properties of the lakes and differences in mean temperature could significantly explain the observed patterns, while in eukaryotes, the lack of connectivity between the ice-free regions appeared to put additional constraints on OTU-richness.

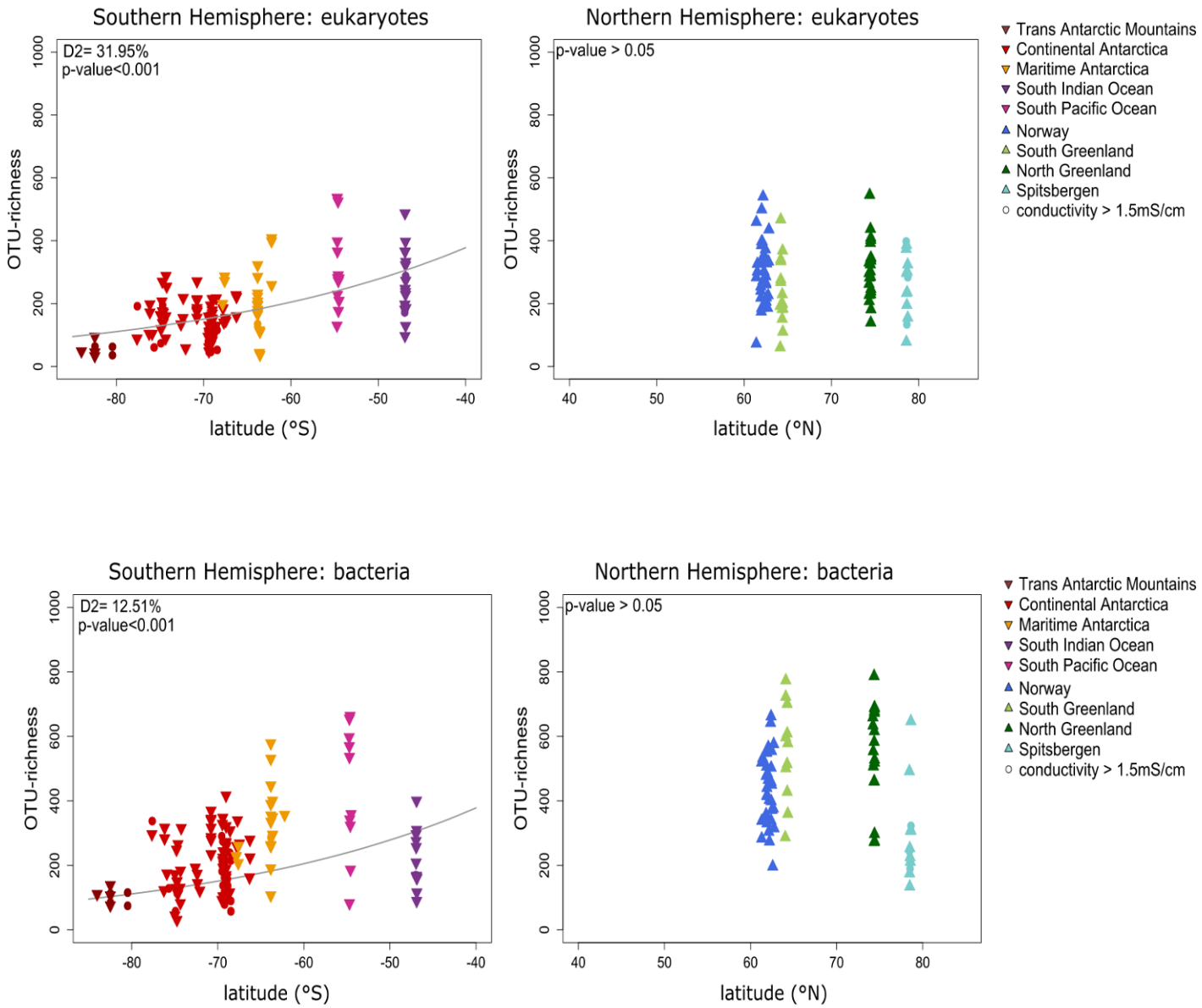

Figure 7: Local OTU richness after standardization for the number of sequences per sample against latitude.

In addition to this pattern in microbial diversity, distinct biogeographic zones could be recognized in the distribution patterns of both eukaryotes and bacteria, based on multivariate ordination and clustering techniques (Figure 8). Northern and southern

hemisphere communities were clearly distinct in eukaryotes, while the bioregionalisation within the southern hemisphere was largely in agreement with the classical subdivision of the Antarctic Realm into Maritime Antarctica, Continental Antarctica and the sub-Antarctic Islands generally observed in plants and animals. For bacteria, however, northern and southern hemisphere communities were still distinct, but interhemispheric segregation was less clear than in eukaryotes.

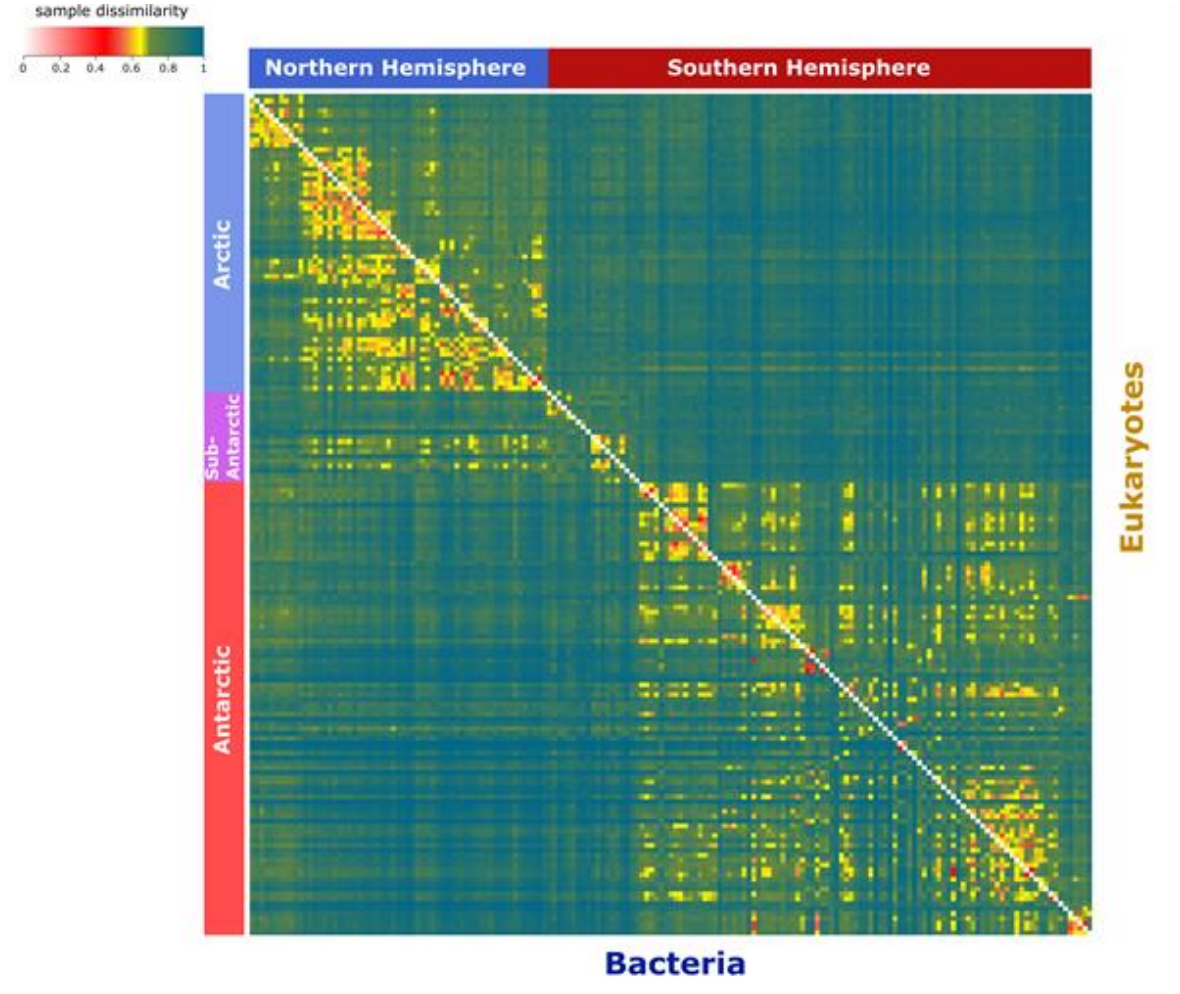

Figure 8**.** Heatmap showing a sample by sample matrix, ordered following their geographical location and proximity, while intersecting cells were colour-coded according to the ecological dissimilarities between individual lakes (Bray-Curtis, based on presence-absence data). Only samples with sufficient sampling depth (>4,500 sequences) for both eukaryotes (upper triangle) and bacteria (lower triangle) are shown. In eukaryotes, community differences between the Arctic, Antarctic and Sub-Antarctic clearly surpass those within regions. Also in Bacteria, there is a major geographic grouping, although Sub-Antarctic lakes resemble Northern Hemisphere sites more closely than Antarctic ones.

In total, 19% of the bacterial communities did not cluster according to hemisphere, while this was only 5% for the eukaryotes. This was mainly due to the relative high similarities of bacterial assemblages in Svalbard to Continental Antarctic communities, while several Macquarie lakes (Sub-Antarctica) also clustered together with microbial

mats from Norway. These biogeographic patterns were found using both presence-absence and abundance data, suggesting that the relative large timeframe in which samples were collected did not significantly affect the relative abundances of taxa, which could have shifted due to degradation of DNA or continued growth.

The proportion of bipolar OTUs and those restricted to one of the three biogeographic regions varied considerably between the different phylogenetic groups. The fraction of bipolar OTUs at the 97% sequence similarity was 34.9% for eukaryotes and 43.4% for bacteria, respectively. While for Bacteria these numbers are similar to recently reported estimates (Kleinteich et al., 2017), the proportion of OTUs restricted to each of the three regions varied. In Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria, the number of OTUs restricted to Antarctica was 1.3 to 15.3 times higher than for the other two regions. For Chloroflexi and Cyanobacteria, and all eukaryote clades (except Cercozoa), the number of OTUs restricted to the Arctic was highest. In Cercozoa, more OTUs uniquely occurred in Sub-Antarctica than in the two other regions.

Detailed analysis of the major phyla in the dataset showed that the two sub-Antarctic islands were generally highly differentiated, except for Dinophyta, Streptophyta, Chloroflexi and Cyanobacteria. This may be the result of high dispersal or low phylogenetic divergences in these groups, but could also be related to the relatively low diversity (e.g. few Cyanobacteria on Marion Island). Community composition differences between maritime and continental Antarctic lakes were not larger than differences between communities within these regions (except for Metazoa and Streptophyta) at the 97% similarity level. Moreover, at a geographically fine-grained level, biogeographic zoning along the Antarctic Conservation Biogeographic zones (ACRBs) proposed by Terauds et al. (2012) was not well-defined. This may indicate that the community turnover along the Gressitt line boundary (Chown et al., 2007) or within continental Antarctica (Terauds et al., 2012) is less strong for microorganisms, for instance because of local wind transportation. Alternatively, the pronounced biogeographic divergences in species or OTU composition documented by other studies may here be obscured by insufficient taxonomic resolution at the 18S and 16S rRNA gene barcodes. For instance, the degree of endemism of nematodes in Victoria land (South-west Antarctic continent) should be extremely high (Adams et al., 2014) but several OTUs clustered at 97% sequence similarities had global distribution, which indicates lumping of closely related species (Bik et al., 2010).

3.4. Amplicon sequencing of environmental 16S rRNA genes for the Cyanobacteria

3.4.1. Evaluation of bioinformatics pipelines using artificial communities

A total of 61,419 reads were obtained for the two artificial communities (34,213 and 27,206 reads for Art1 and Art2, respectively). After applying the bioinformatics pipelines between 15,820 and 25,105 reads remained, representing a decrease of up to 47% from the original number of reads. Observed relative abundances differed from the theoretical expectations and were similar in communities Art1 and Art2, despite differences in the initial proportions of each template DNA (Figure 9). Recovered relative abundances were also consistent across the different pipelines. In general, a number of strains were three to six times more abundant than expected (e.g. *Phormidesmis priestleyi* ANT.L52.6 (ULC026), whereas others were underrepresented (e.g. *L. frigida* ANT.L53B.1 (ULC001)).

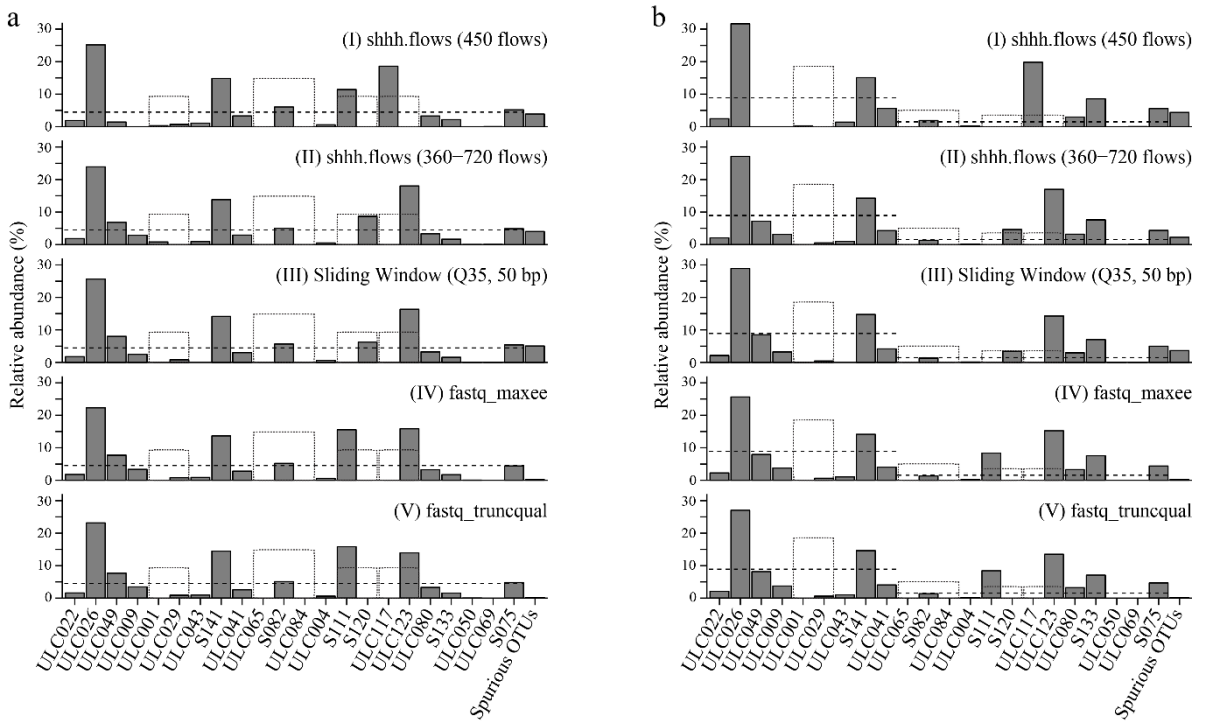

Figure 9. Recovered relative abundances of reference strains in artificial communities Art1 (a) and Art2 (b) after application of each bioinformatics pipeline. Relative abundances were computed taking into account only OTUs classified as "Perfect" or "Good" (i.e., with ≥99% similarity to a reference sequence). Remaining OTUs ("Noisy," "Other" and "Chimeric") are grouped under "Spurious OTUs."

Inferred phylotype richness varied considerably between pipelines (Figure 10). Phylotype richness obtained with the mothur-based pipelines (pipelines I-III) was surprisingly high (98–261 OTUs), and rarefaction curves suggested that it would be even

higher with increased sequencing depth. OTU richness was much lower (16–21 OTUs) with the UPARSE-based protocols (pipelines IV-V), with rarefaction curves reaching a plateau at around 1,000 sequences. Even at this lower sequencing depth, OTU richness reported by the mothur-based pipelines was 2–3 times higher than expected. The number of biologically relevant OTUs ("Perfect" and "Good," therefore with ≥99% similarity to a reference sequence) was similar for all pipelines (15–24 OTUs), meaning that all of them were able to identify the real taxa). However, the mothur-based protocols reported a high number (82–237 OTUs) of additional spurious phylotypes ("Noisy," "Other" and "Chimeric" OTUs). Although these spurious phylotypes accounted for a small proportion of the dataset (0.2%–5.1% of the reads; Figure 9), they contributed significantly to the overestimation of phylotype richness since their divergent sequences are perceived as new OTUs (Figure 10). In contrast, phylotype richness reported by the UPARSE-based protocols was in line with expected results, consisting of 15–16 OTUs with ≥99% similarity to a reference sequence and only 1–5 additional spurious OTUs. Thus, the latter protocol was selected for future analyses.

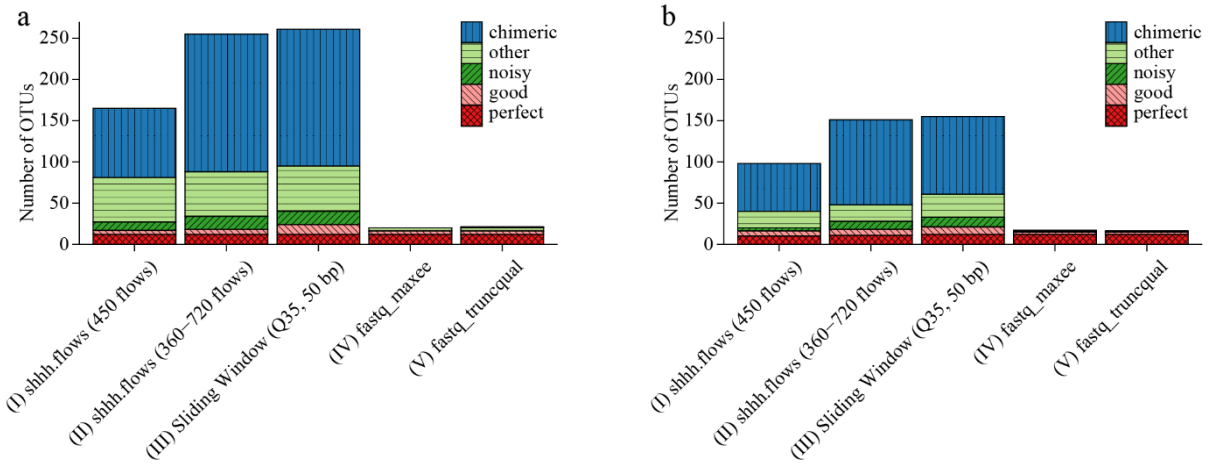

Figure 10. Classification of OTUs in artificial communities Art1 (a) and Art2 (b) after application of each bioinformatics pipeline (see Pessi et al.2016). OTUs were classified as "Perfect" (identical to a reference sequence), "Good" (≥99% similarity), "Noisy" (≥97.5% to <99% similarity), "Other" (<97.5% similarity) and "Chimeric" (composed of two or more parent reference sequences).

3.4.2. Spatial patterns of Antarctic lacustrine cyanobacterial communities

For the 454 pyrosequencing run, a total of 249,191 reads with an average length of 410 bp were obtained for 13 microbial mat samples. After removal of low-quality and chimeric sequences 177,482 sequences (71.2%) remained. From these, 578 sequences (0.3%) were assigned to plastid sequences of eukaryotes, and 788 sequences (0.4%) to other bacterial phyla such as Acidobacteria, Chloroflexi, Planctomycetes, TM7 and

Verrucomicrobia. Remaining cyanobacterial sequences (176,116 reads, 99.3% of the quality-filtered reads) were grouped into 112 OTUs at 97.5% similarity. Pseudanabaenales comprised the majority of the OTUs (61 OTUs, 54.5%), followed by Oscillatoriales, Synechococcales (11 OTUs each, 9.8%), Nostocales (10 OTUs, 8.9%) and Chroococcales (8 OTUs, 7.2%). Eleven OTUs (9.8%) were not classified at the order level. In general, EL land lakes were highly dominated by Pseudanabaenales OTUs, which made up an average of 95.9% of the quality-filtered reads in these lakes (Figure 11). Nostocales and Chroococcales OTUs were only observed in EA lakes (average of 1.8 and 1.0% of the reads, respectively).

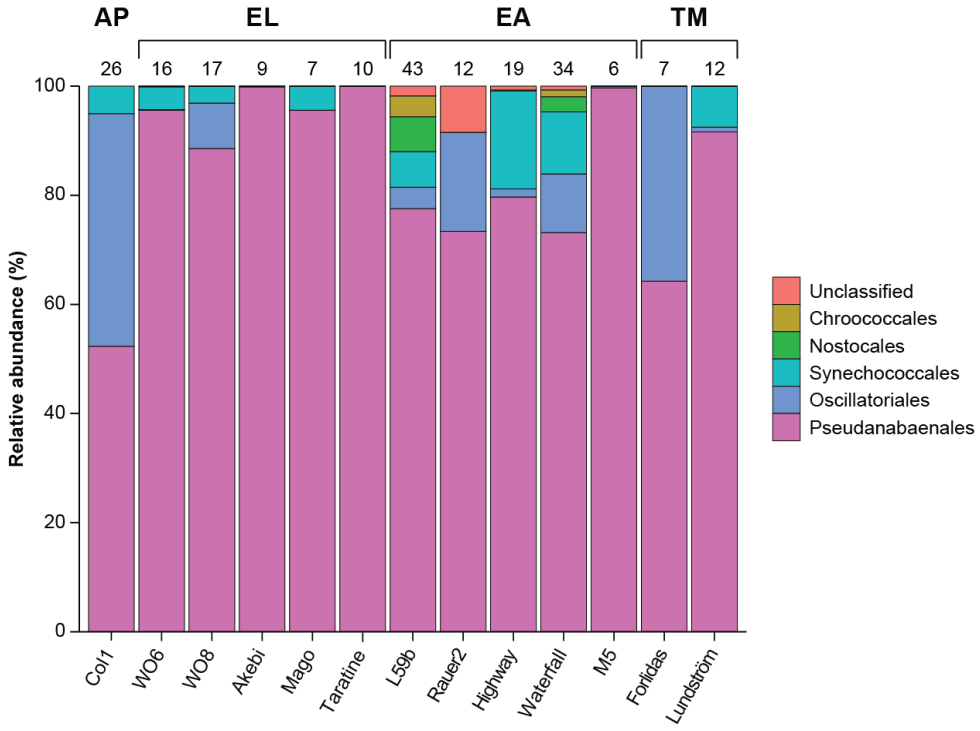

Figure 11. Cyanobacterial community structure summarized at the order level. Numbers above bars represent the total phylotype richness in each sample.

In order to perform beta diversity analyses, pairwise lake physicochemical distance were calculated after log(x+1) transformation (except pH) and standardization, pairwise geographic distance between lakes was computed based on GPS coordinates using the Geographic Distance Matrix Generator (available in http://biodiversityinformatics.amnh.org/ open_source/gdmg) and pairwise cyanobacterial community similarities were assessed based on Bray-Curtis distances after square root transformation of OTU abundance data. Analyze of variation in community structure (UPGMA) discriminated between three community groups, each comprising lakes from different ACBRs (Figure 12). Cluster I was composed by communities from higher conductivity lakes, cluster II consisted of communities from lakes with enriched DOC content, and cluster III included the remaining (freshwater and oligotrophic) lakes. As

suggested by the UPGMA clustering, community structure was unrelated to geographic distance but was strongly correlated with overall lake physicochemical composition (RELATE (non-parametric version of Mantel test); $\rho = 0.19$, $p > 0.05$ and $\rho = 0.61$, $p = 0.001$, respectively). Distance-based linear models (distLM) with forward selection based on the adjusted R^2 criterion were further applied in order to investigate the importance of individual physicochemical parameters. Conductivity significantly explained 24.1% of the variation in community structure between lakes (distLM, $p = 0.001$). The effect of DOC was marginally significant (17.4% of the variation, $p = 0.09$) and remaining physicochemical parameters had no influence in community structure ($p > 0.05$). Overall, community structure was best explained by conductivity, NO_3, SiO_4, pH, TOC and DOC, which explained together 78.6% of the variation between lakes. Other studies already suggested that conductivity/salinity is an important factor structuring the communities, not only of cyanobacteria (Fernandez-Carazo et al., 2011; Jungblut et al., 2005; Taton et al., 2006a) but also of diatoms and other microeucaryotes (Sabbe et al., 2004; Verleyen et al., 2010).

From the 112 OTUs found, 37 OTUs (33.0%) were related (≥99% similarity) to sequences with a wide global distribution and were thus classified as "Cosmopolitan" OTUs. The remaining phylotypes (79 OTUs, 77.0%) appeared restricted to the cold biosphere. More specifically, 42 OTUs (37.5%) had no related sequences in GenBank at a 99% similarity threshold ("Novel" OTUs) and are thus considered as potentially endemic; 20 OTUs (17.9%) were only related to sequences coming from Antarctic biotopes (potentially "Endemic" OTUs); 6 OTUs (5.4%) have a bipolar distribution ("Polar" OTUs); and 7 OTUs (6.3%) also included hits from high altitude regions such as the Alps, the Andes and the Himalaya ("Polar/Alpine" OTUs). The 10 most abundant OTUs (OTUs 1–9 and OTU16, comprising together 83.1% of the quality-filtered reads) had cosmopolitan distributions. Cosmopolitan OTUs were generally more abundant (average of 2.4% of the quality-filtered reads) and appeared in more samples (4.1 samples in average) than endemic (0.2% of the reads, 2.6 samples in average) and novel OTUs (0.1% of the reads, 2 samples in average).

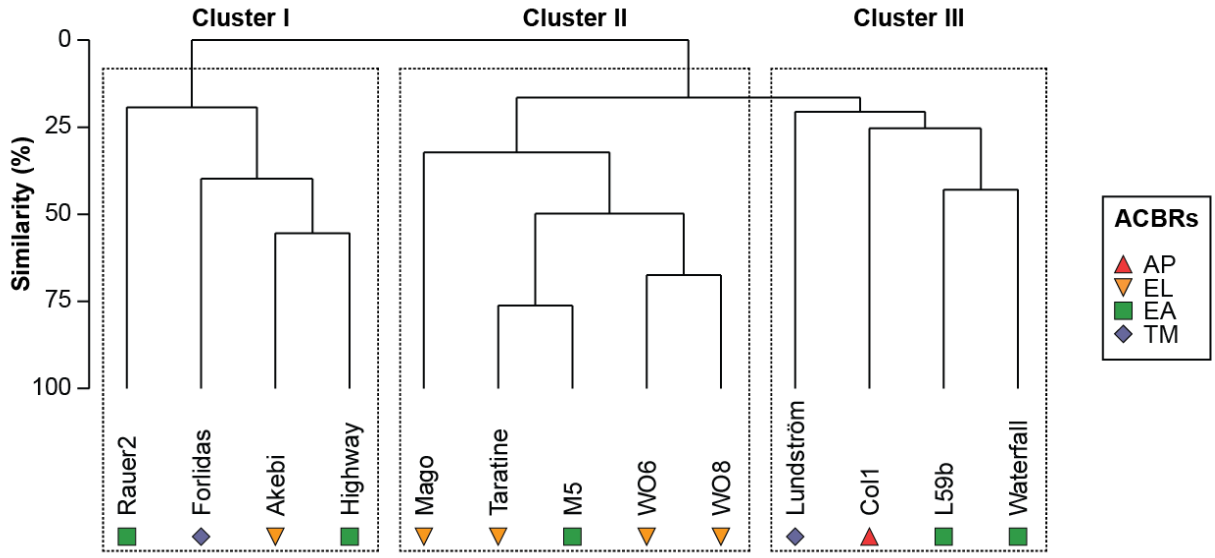

Figure 12. Unweighted pair group method with arithmetic mean (UPGMA) analysis based on pairwise Bray Curtis distances between cyanobacterial communities.

Lakes from different, geographically distant ACBRs appear to harbor comparable cyanobacterial communities (Figure 12). For example, Col1 (AP) was clustered alongside with Lake L59b and Waterfall Lake (EA), located ca. 2,880 and 3,000 km away, respectively. In comparison with larger organisms, the low effect of distance on the continent supports the dispersal of cyanobacterial propagules between the different Antarctic ice-free regions (Jungblut et al., 2010). However, the presence of a large proportion of OTUs with a restricted distribution in Antarctica suggest that life may have persisted in glacial refugia such as inland nunataks and coastal oases, which have remained ice-free during past glaciations (Convey et al., 2008; Strunecký et al., 2012).

For the Illumina sequencing run, a total of 7.808,519 high quality reads (max error = 0.5; 370 bp) were obtained for the 94 samples. A total of 974 OTUs was affiliated to the phylum of Cyanobacteria including plastidial OTUs and OTUs affiliated to the Melainabacteria, using the RDP database. The total number of cyanobacterial OTUs (including Melainabacteria) was 796 (Figure 14A). Finally, the 534 OTUs having a minimum of 5 counts in all samples were further analyzed by BLAST and phylogeny analyses. Total numbers of OTUs were consistent between duplicates.

Pairwise cyanobacterial community similarities (OTU abundance matrix) and pairwise lake physicochemical distances (pH and conductivity) were calculated as described for the pyrosequencing. The geographic distance matrix were transformed (package geosphere, R) in spatial variables named Principal Coordinates of Neighborhood Matrix (PCNM) eigenvectors (Borcard et al., 2002) using R (package vegan). Each PCNM represents geographic information at a different scale (Figure 13). With these spatial

variables, the effect of pH and conductivity on cyanobacterial communities at different spatial scales was calculated.

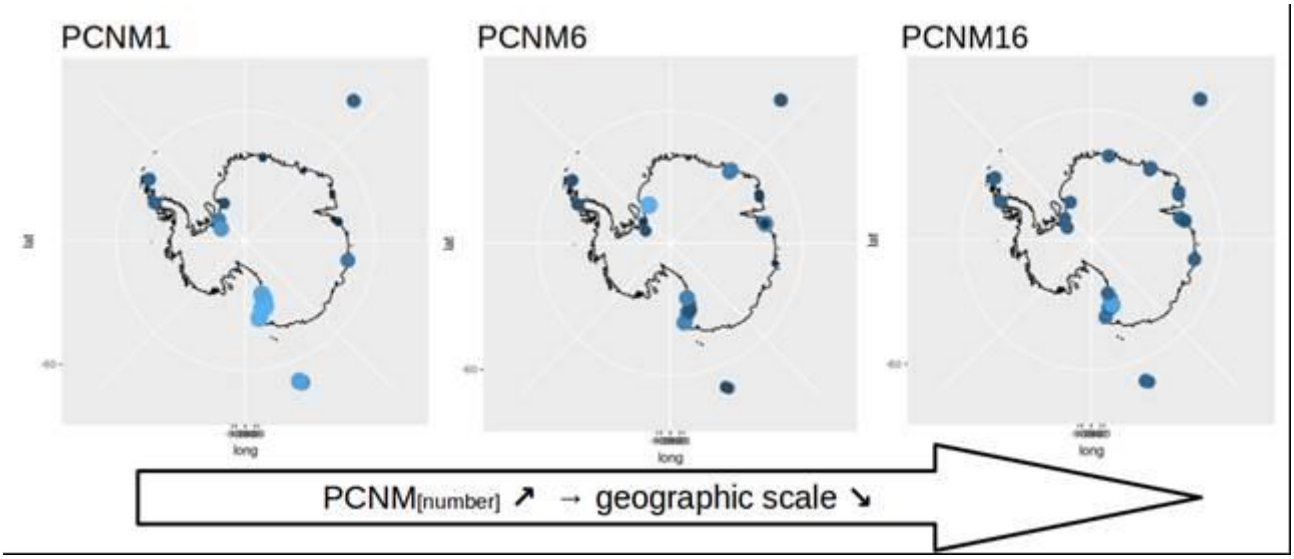

Figure 13: Visualisation of the PCNMs variation with geographic distance. Each PCNM represents a different scale of spatial variation. For example, PCNM1, PCNM6 and PCNM16 explain spatial variation at a scale of 2000 to 3000 kms, 100 to 1000 kms and 10 to 100 kms, respectively.

Correlation tests (permutation test on distance based redundancy analysis (McArdle et al., 2001)) between cyanobacterial community structure and geographic distance, pH and conductivity give different results in function of the geographic scales we focus on (TABLE II).

TABLE II. Mantel test (Mantel r and P-value) and variation portioning (Varpart) at two different spatial scales. Variation partitioning is calculated using adjusted R-squared from distance-based redundancy analysis in R (package "vegan").

Area scale	geographic distance			conductivity			pH		
	Mantel r	P-value	Varpart (%)	Mantel r	P-value	Varpart (%)	Mantel r	P-value	Varpart (%)
Antarct. + sub-Ant. Islands	0.33	< 0.001	17.5	0.1	0.035	1.7	0.14	< 0.001	1.4
Antarctica	0.27	< 0.001	12.2	0.14	0.014	3	0.09	0.016	2.5

At the largest scale (Antarctica and sub-Antarctic islands), the OTU richness shows a strong correlation with the geographic distance and a lower correlation with pH and conductivity. Indeed, 17,5% of the cyanobacterial community structure variation appears explained by the geographic distance. This variation is more important between distant cyanobacterial communities (from 1000 to 2000 kms) than between community separated by 10 to 100 kms or less. In contrast, conductivity only explains 1.7% of the variation and is not much correlated to geographic distance (29% shared with PCNM9).

Indeed, linear regression of the abundance of OTUs with conductivity reveals that 18 of the 534 OTUs (3.36 %) are significantly distributed in respect to the conductivity (p-value < 0.05, Fisher test). pH explains only 1.4% of the variation and is completely spatially structured (100% shared with PCNM1).

When the analyses are performed at the continental scale, the effect of geographic distance decreases whereas conductivity and pH shows an inverse trends (TABLE II).

By decreasing the spatial scale from the largest to regional scales, the effect of geographic distance is replaced progressively by the impact of environmental parameters.

By zooming even more, the analysis results are also different according to the regions. For Dronning Maud Land, North East Antarctic Peninsula, Macquarie Island and Marion Island, there is no significant correlation between the structure of cyanobacterial communities and the parameters tested (geographic distance, conductivity and pH). For North Victoria Land, geographic distance is explaining 12% of the abundance variations. For East Antarctica, Enderby Land and South Victoria Land, the conductivity appears to structure the cyanobacterial communities, explaining respectively 6.7%, 12 % and 18 % of the abundance variation. Analyses were not performed for the Transantarctic Mountains and North West Antarctic Peninsula because there were 2 and 3 samples, respectively, which is not sufficient. Unfortunately, the pH and salinity were the only lake parameters available for the 94 samples, and the analyses show that other factors that are not available for this study must play a role to explain the community structures.

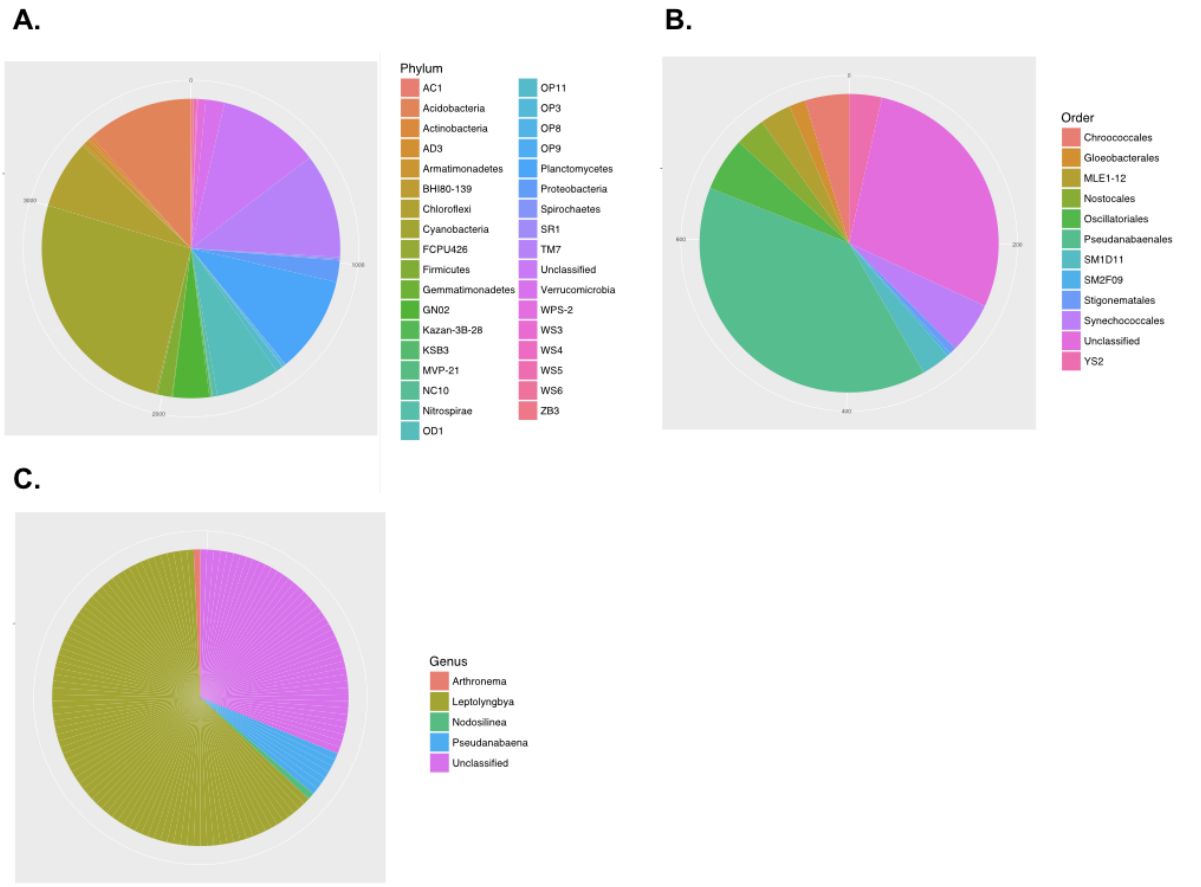

Figure 14. OTUs distribution according to RDP taxonomic affiliation. A. Number of OTUs per phylum (Total = 3768), B. Number of cyanobacterial OTUs per order (Total = 247), C. Number of OTUs per genus among Pseudanabaenales (Total = 139).

Looking at the distribution of the OTUs, 4 OTUs are only present in the sub-Antarctic islands (0.7% of the 534 OTUs). In contrast, 82 of the 534 OTUs (15.3%) appear only present on the Antarctic continent (potentially endemic).

The 247 most abundant cyanobacterial OTUs (including Melainabacteria) representing 99.03% of the reads were selected to perform more focused analyses. Briefly, a majority (56.28%) of these OTUs belongs to the order Pseudanabaenales and gathered 72.9% of the total number of cyanobacterial reads (Figure 14B). Besides, Pseudanabaenales were dominated by OTUs belonging to the genus *Leptolyngbya* (Figure 14C). More precisely, OTU 1, which was 99.7% similar to *Phormidesmis* sp. HOR_11_6 (KU219729) was represented by 490310 reads which was 1,7 times the total number of OTU 2 (100% similar to *Timaviella* sp MH688850). OTU 1 appeared to be present in all our samples regardless of the region, pH, or conductivity.

For these 247 most abundant OTUs, preliminary multivariate analysis confirmed a significant difference between cyanobacterial community structures from sub Antarctic islands and the Continent (Figure 15). This observation seems in agreement with observations made with the heatmap analysis.

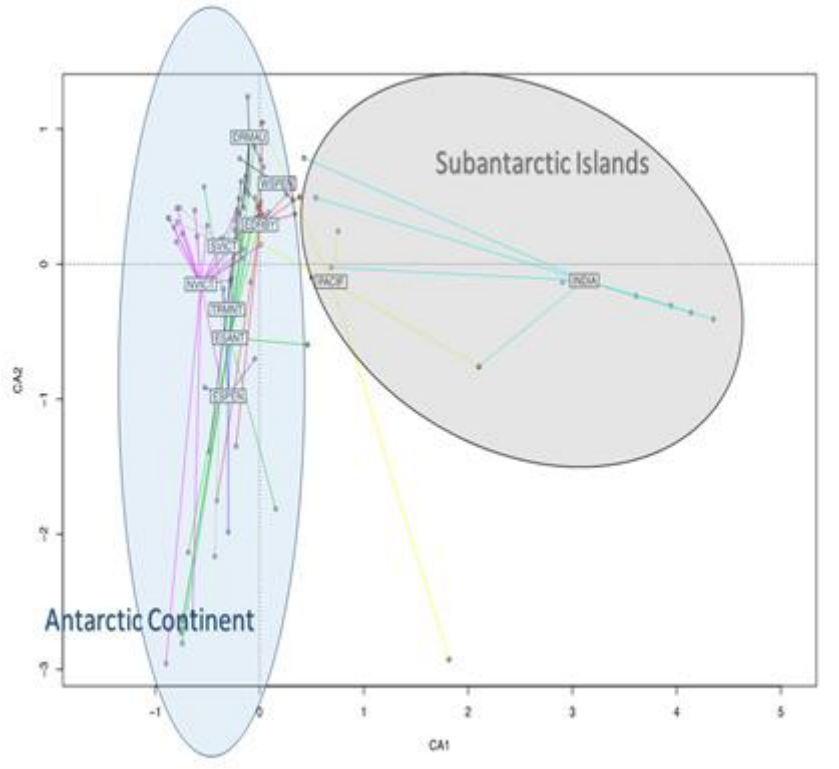

Figure 15. Representation of the first two axis of the Correspondance Analysis using the cyanobacterial community structure (247 most abundant OTUs) showing the distinction between the Antarctic continent and the sub-Antarctic islands.

A heatmap analysis was performed on the matrix of the relative abundances of Pseudanabaenales OTUs (Figure 16). At this taxonomic resolution, our samples were divided in two major clusters. The cluster I consists of samples from the maritime and Sub-Antarctic regions. In these samples, the OTUs richness and abundances were higher than in samples from cluster II. The cluster II is composed by samples from the continent. In these samples, the Pseudanabaenales community is often dominated by only one OTU, as shown by the subclustering of cluster II.

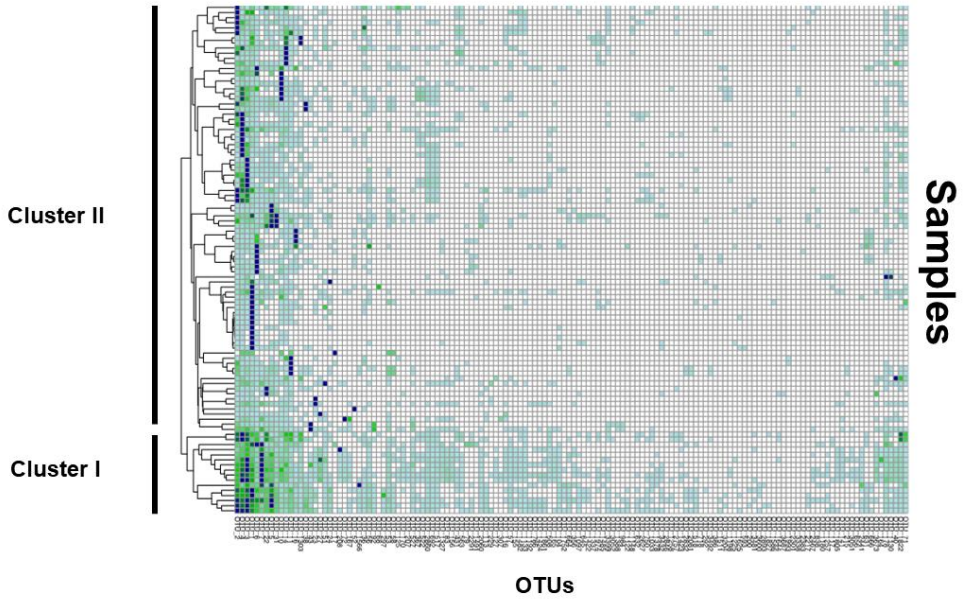

Figure 16, Heatmap showing a sample by sample matrix of Pseudanabaenales OTUs relative abundances using relatice CDM in R. Clustering was performed using Euclidean distances.

In the present study, a significant fraction of the phylotypes found were associated with cyanobacterial lineages currently restricted to Antarctica ("Endemic" OTUs, 17.9% for the pyrosequencing study, 10.5% for the Illumina study) and phylotypes which had no related (≥99% similarity) sequences in GenBank ("Novel" OTUs, 37.5% for the pyrosequencing study, 51.8% for the Illumina study). Similar results have been reported for cyanobacterial communities in other Antarctic lakes (Taton et al., 2006a, 2006b), as well as for diatom and green algal communities (De Wever et al., 2009; Sabbe et al., 2004; Vyverman et al., 2010, this report). The high level of endemism observed within Antarctic cyanobacterial communities provides additional evidence for an ancient, pre-Holocene origin for a meaningful portion of the contemporary Antarctic cyanobacterial biodiversity.

On the other hand, another fraction (33.0% for the pyrosequencing run, 34% for the Illumina run) of the cyanobacterial communities consisted of phylotypes with a current cosmopolitan distribution, suggesting that cyanobacterial propagules are dispersed globally, including to and from Antarctica. These may be transported to Antarctica by the wind or migratory birds (Pearce et al., 2009) and likely portrait contemporary colonization events. An interesting trend observed here was that highly abundant and frequent OTUs were usually cosmopolitan, while rare OTUs were usually novel or endemic. This could be explained by differences in dispersal ability between cyanobacterial taxa, with cosmopolitan taxa being better adapted to transportation and dispersal (Taton et al., 2006a; Vyverman et al., 2010). In addition, populations of rare

taxa would have a lower probability to successfully disperse to a different environment in comparison to abundant ones (Fierer et al., 2010).

3.5. Phylogeographic studies of focal taxa

3.5.1. Isolation and characterization of selected bacterial strains

Focus was on bacteria of the genera *Deinococcus* and *Flavobacterium* as these groups had previously (BELSPO AMBIO project) been found to be well represented with many new, potentially endemic species. Three soil samples from Utsteinen, KP2, KP15 and KP43, were used. One gram of sample was used to make a dilution series that was plated on R2A and 1/10R2A media. Plates were incubated at 15 and 20°C and red (possible *Deinococcus*) and yellow (possible *Flavobacterium*) colonies were purified. Isolates were grouped by MALDI-TOF MS and representatives were identified by partial 16S rRNA gene sequence analysis.

A total of 199 colored isolates were characterized and assigned to the following genera: *Hymenobacter* (24%), *Arthrobacter* (21%), *Sphingomonas* (18%), *Deinococcus* (16%), *Roseomonas* (5%), *Spirosoma* (4%), *Pedobacter* (4%), *Modestobacter* (3%), *Novosphingobium* (1%), *Noviherbaspirillum* (1%), *Brevundimonas* (1%), Sandarkinorhabdus (1%), *Rhodococcus* (1%), *Nakamurella* (1%) and *Adhaeribacter* (1%). Five isolates (3%) could not be identified. Thirty-two isolates were assigned to *Deinococcus* and, surprisingly, none to *Flavobacterium*.

For the molecular phylogenies of housekeeping genes, we focused on *Deinococcus* because no new Flavobacteria isolates were recovered. Previous results (AMBIO project) had indicated that many of the Deinococci isolated are potentially restricted to Antarctica and thus adapted to cold temperatures. Using sequence data retrieved from genomes available in public databases, amplification primers for the housekeeping genes *rpoB*, *gyrB*, *purA*, *dnaK*, *tdh* and *recA* were designed and used to amplify and sequence these genes in newly obtained isolates. Maximum likelihood phylogenetic analysis was performed using MEGA software.

Based on the phylogenetic relationships of the partial 16S rRNA gene (~350 bp) of Antarctic *Deinococcus* isolates with those of reference strains of known *Deinococcus* species, we had previously established that the Antarctic strains seemed to represent at least 10 potentially novel species as well as the previously described species *D. saxicola* and *D. marmoris* (Peeters et al., 2011). To improve the confidence in these groupings, we completed the 16S rRNA gene sequences (~1350 bp) and this confirmed the previous lineages and indicated a few more potential new species. Because 16S rRNA is a conserved marker, we compared with the phylogenies of the selected more variable housekeeping genes to assess the novel groups. Primers for *rpoB* and *gyrB* performed best, yielding PCR products for all strains tested, while for *purA*, *dnaK*, *tdh* and *recA,* a

portion of the strains failed to yield an amplicon. The reason may be the large number of ambiguous positions in some of the primers. The phylogenetic analysis of all the genes confirmed the existence of the same individual groups. However, the available reference data for housekeeping genes is limited and therefore the distance to existing species could not be confirmed clearly. As an example, the *rpo*B phylogeny is shown in Figure 17.

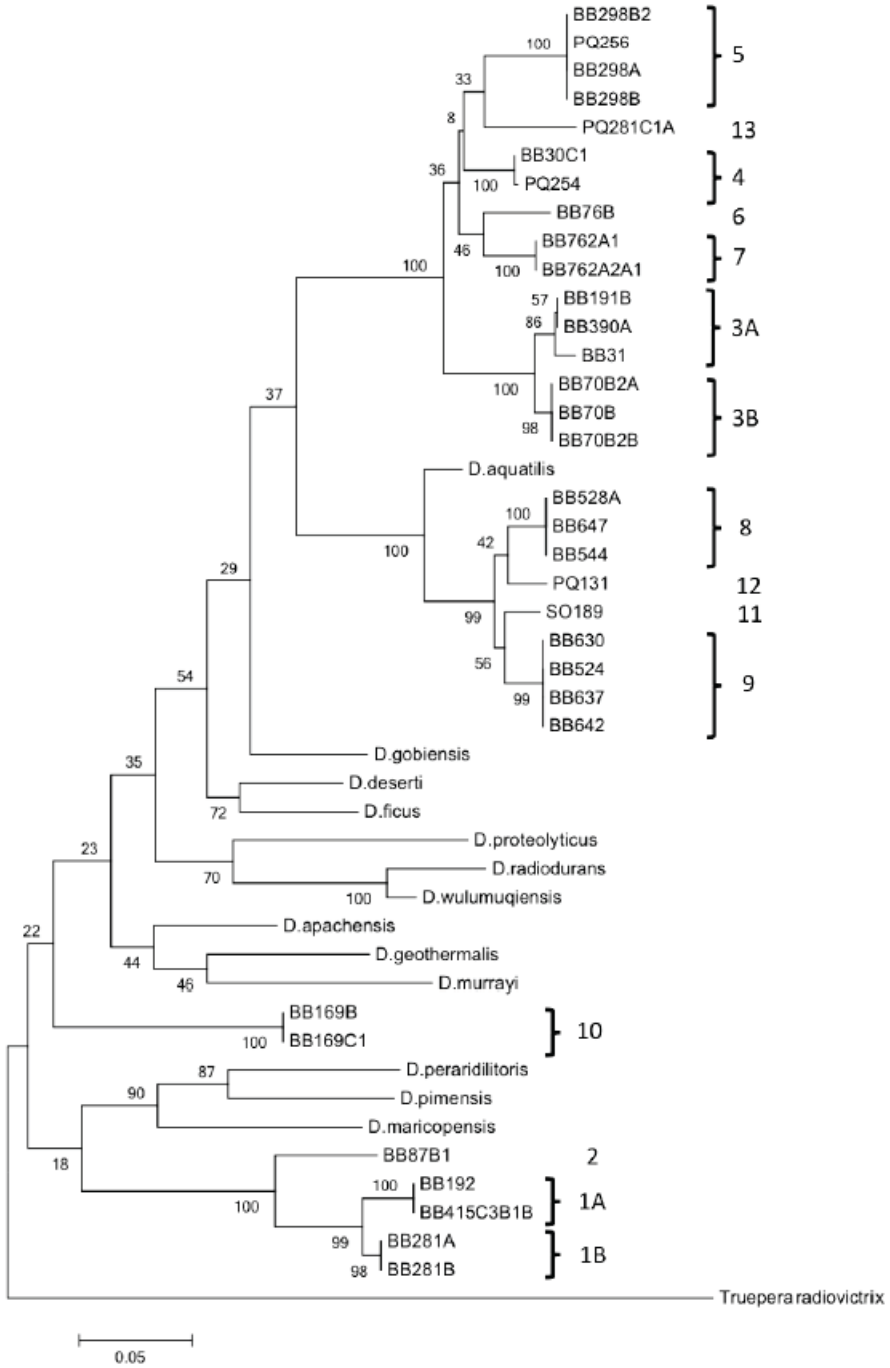

Figure 17. Maximum likelihood tree based on *rpo*B sequences (705 bp) of *Deinococcus* strains. The Tamura-Nei substitution model was used in MEGA. Bootstrap values at branching points are based on 500 replications. Cluster numbers refer to our groups of Antarctic isolates.

With the increasing availability of whole genome sequences, and the declining cost in sequencing whole genomes, it can be anticipated that more data for comparison will become available or can be obtained in follow-up research. Availability of the whole genome sequences of our own isolates will allow description of new species. For this purpose, we have already determined fatty acids profiles and some phenotypic characterization of representative strains of the new groups.

3.5.2. Isolation and characterization of cyanobacterial strains

The selected cyanobacterial strains clustered in 4 main lineages according to the *rpoC1* phylogeny (Figure 18).

Figure 18. *rpoC1* phylogeny (501 bp) of Antarctic cyanobacterial strains. The ML tree was constructed using RAXML with GTR G+I model. Bootstrap values at branching points are based on 1000 replications. Cluster numbers refer to our groups of Antarctic isolates.

The first cluster (I) is composed by nine strains previously described as *Phormidesmis priestleyi* (Taton et al., 2006b) from 3 different regions (Dronning Maud Land, East Antarctica, and Transantarctic Mountains). This group of strains also included *Phormidium* D1 (from a cave in Greece) and *Phormidesmis* sp. WJT36-NPBG28 (Czech Republic) that had a 16S rRNA similarity ranging from 97.1% to 100% with our

Antarctic strains and also correspond to the genus *Phormidesmis* (Komárek et al., 2009). According to the *rpoC1* phylogeny, the nine Antarctic strains were subdivided into three groups plus two isolated strains but there was no relation with the geographic origin (Figure 18). The second cluster (II) included 6 *Leptolyngbya antarctica* strains (ULC017, ULC031, ULC32, ULC036, ULC037, ULC043) that had identical *rpoC1* sequences but shared only 78.8% *rpoC1* and 91-91.2% 16S rRNA similarity with the most closely related strain, *Leptolyngbya antarctica* ULC023. The third clade (III) was composed by strains assigned to different morphotypes. However, the Antarctic strains in this cluster shared between 96.3% and 100% 16S rRNA similarity and 83.4% to 100% *rpoC1* similarity, and thus, probably belong to the same genus. Finally, the fourth clade (IV) is composed of 5 Antarctic strains previously named as *L. antarctica* but that should be renamed as *Nodosilinea* sp. because they share 16S rRNA similarities > 96.1% with several non-Antarctic strains that belong to *Nodosilinea* sp. (Perkerson et al., 2011). Besides, they formed a well-supported clade according to the *rpoC1* phylogeny, including a well-supported sub-cluster of four Antarctic strains (ULC041, ULC047, ULC073, and ULC090) from the Larsemann Hills, Transantarctic Mountains and McMurdo Ice Shelf. Genetic analyses using *rpoC1* and 16S rRNA sequences allowed an improvement in the characterization of strains from the problematic polyphyletic genera *Leptolyngbya* and *Phormidium*, which represent the two dominant OTUs observed in our HTS environmental surveys (task 2.4.). However, no clear sub-clustering was observed according to the strain's bioregion origin.

The ITS phylogenies allowed to reconstruct the sub-clustering of strains within each of the studied lineages. However, this clustering was similar to the one observed with the *rpoC1* phylogeny.

The occurrence of *nifH/D* genes was observed in the cluster of *Leptolyngbya antarctica* (clade II) leading to the hypothesis that strains from this clade are potential nitrogen fixers. This is important as, till now, *Nostoc* was considered to be the only nitrogen-fixer in Antarctic biotopes.

For the genome of the Antarctic axenic strain ULC007 (*Phormidesmis priestleyi*), the first Illumina run generated 4,404,753 reads, and the second run generated 8,628,205 reads. Final assembly using the CISA3 assembler led to 45 contigs ranging from 1530 to 495420 bp length, for a total length of 5,262,658 bp (48.8 %GC). The genome was annotated with RAST (Aziz et al., 2008). This leads to the prediction of 5975 coding sequences grouped in 393 subsystems (Lara et al. 2017). Three subsystems have been studied in more detail because they are often associated with organisms growing in cold biotopes (Fatty Acid Biosynthesis FASII, Oxidative stress, Protection from Reactive Oxygen Species (ROS)). The number of protein-encoding genes (pegs) for each subsystem is distributed as followed: 27 in Fatty Acid Biosynthesis FASII, 31 in Oxidative stress, 4 in Protection from Reactive Oxygen Species. For the subsystems

'Fatty Acid Biosynthesis' and 'Protection from ROS', the strain ULC007 does not show a particular pattern in comparison to the genomes in other lineages in the public databases.

However, for the 'Oxidative stress' subsystem, a high number of pegs is observed for the order Oscillatoriales and is particularly high for the *Phormidiaceae* family (Figure 17). Indeed, 3 pegs coded for the HPIIb (Catalase) and 6 pegs coded for Crp transcriptional regulator, in the Crp/Fnr family. The catalase is an enzyme involved in the degradation of hydrogen peroxide whereas the transcriptional regulator of type Crp/Fn regulates various metabolic pathways in bacteria and typically functions in response to environmental stresses (oxidative, osmotic, etc.) (Zhou et al., 2012). Besides HPIIB and Crp for the Phormidiaceae, 2 other pegs are involved in 3 different roles: Fr (Ferroxidase), IBP (Iron-binding ferritin-like antioxidant protein) and Dps (Non-specific DNA-binding protein). Dps protects cells from oxidative stress by binding directly to the DNA (Martinez et al., 1997). Another interesting parameter is the relative importance of the oxidative stress response genes within the genome's features. It can be approximately evaluated by computing the ratio between the number of pegs for the oxidative stress subsystem and the total number of pegs within the genome (ratio_OX). ULC007 shows a relatively high ratio_OX within cyanobacteria and Oscillatoriales and the highest ratio_OX of the *Phormidiaceae* family. Strains which have the smallest ratio_OX grow in the marine environment where conditions are stable. However, the strains with the highest ratio_OX have been isolated from freshwater habitats where environmental conditions are variable (lakes, rivers and streams).

Finally, 13 clusters potentially encoding for the biosynthesis of unknown secondary metabolites were identified. It included NRPS, PKS, NRPS/PKS, and bacteriocin clusters. This type of clusters is probably responsible for the antibacterial and antifungal activities observed by Taton et al. (2006b) in strain ULC007.

The lack of enough genomes from Antarctic representatives and the paucity of calibration priors is complicating the attempts to achieve molecular clocks using multiple loci. A 16S rRNA molecular clock analysis was perfomed with a selected strain dataset which includes the Antarctic *P. priestleyi* ULC007 and its Arctic relative BC1401. This analysis suggested that divergence between the two polar strains occurred at least 700 Ma before the division of the Gondwana (1.6 – 0.8 Ga) (Figure 19). However, large deviations in node ages' estimates are observed.

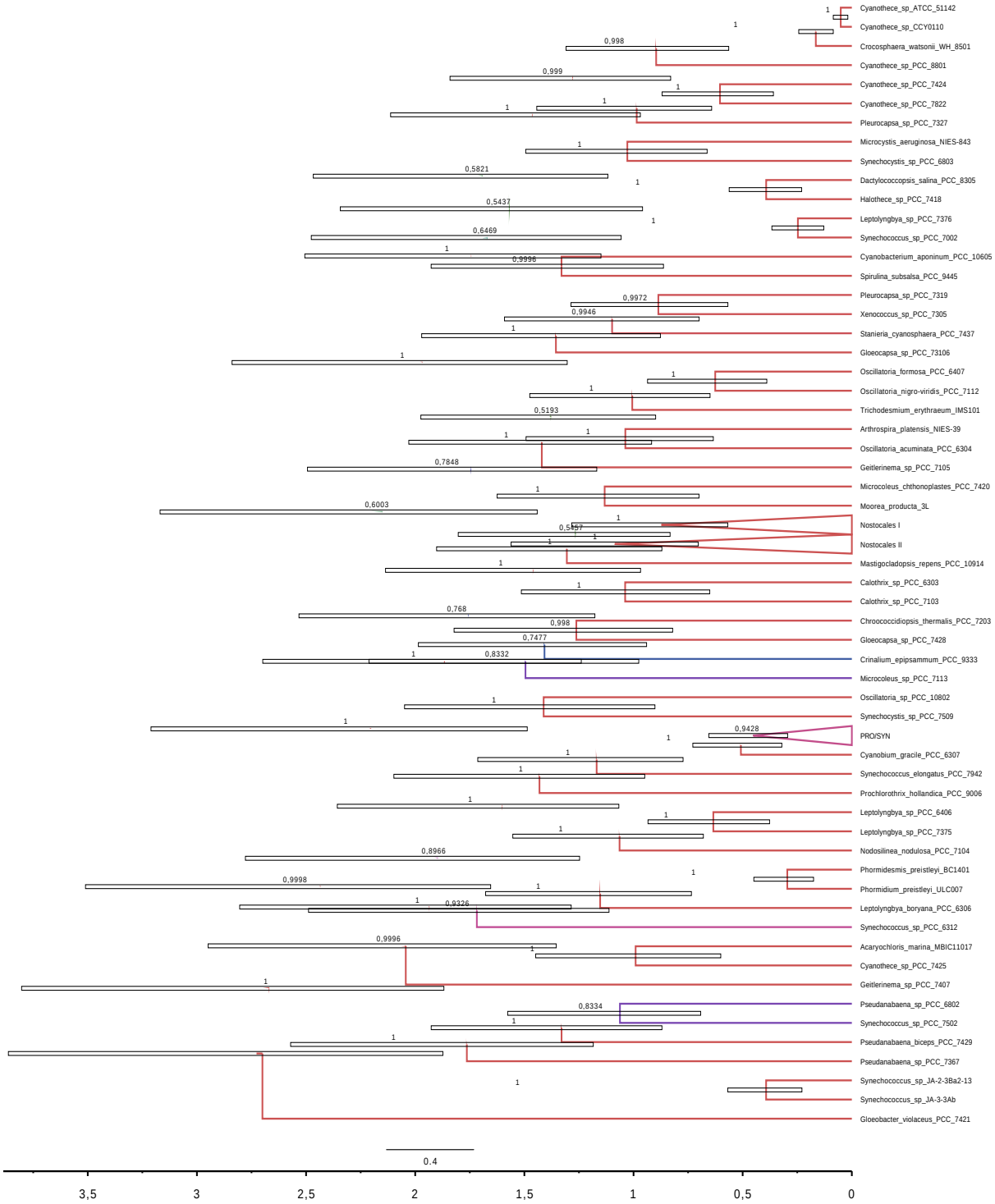

Figure 19. Relaxed molecular clock using partial 16S rRNA, root prior was set at 3.8 Ga. Akinetes were used as calibration prior (2.1 Ga) to support the Nostocales clades.

3.5.3. Phylogeography of diatoms

About 300 monoclonal diatom cultures of *Pinnularia borealis*, a cosmopolitan species complex, were established from various Maritime Antarctic, Arctic and Alpine regions, including Svalbard, James Ross Island and the South Shetland Islands, and added to the existing dataset (Souffreau et al., 2013)(Figure 20). Currently, 22 different lineages of *P.*

borealis are distinguished in the phylogenetic tree, with the Maritime Antarctic region exhibiting a relatively high regional diversity being home at 10 different lineages of which several are new to science and in need of formal species descriptions. Whereas some lineages seem to have a rather restricted distribution, others have been found on several continents and might be truly cosmopolitan. Future planned expansions of the phylogeny including strains from other (polar) regions should allow gaining more insight in the diversity and biogeography of this species complex.

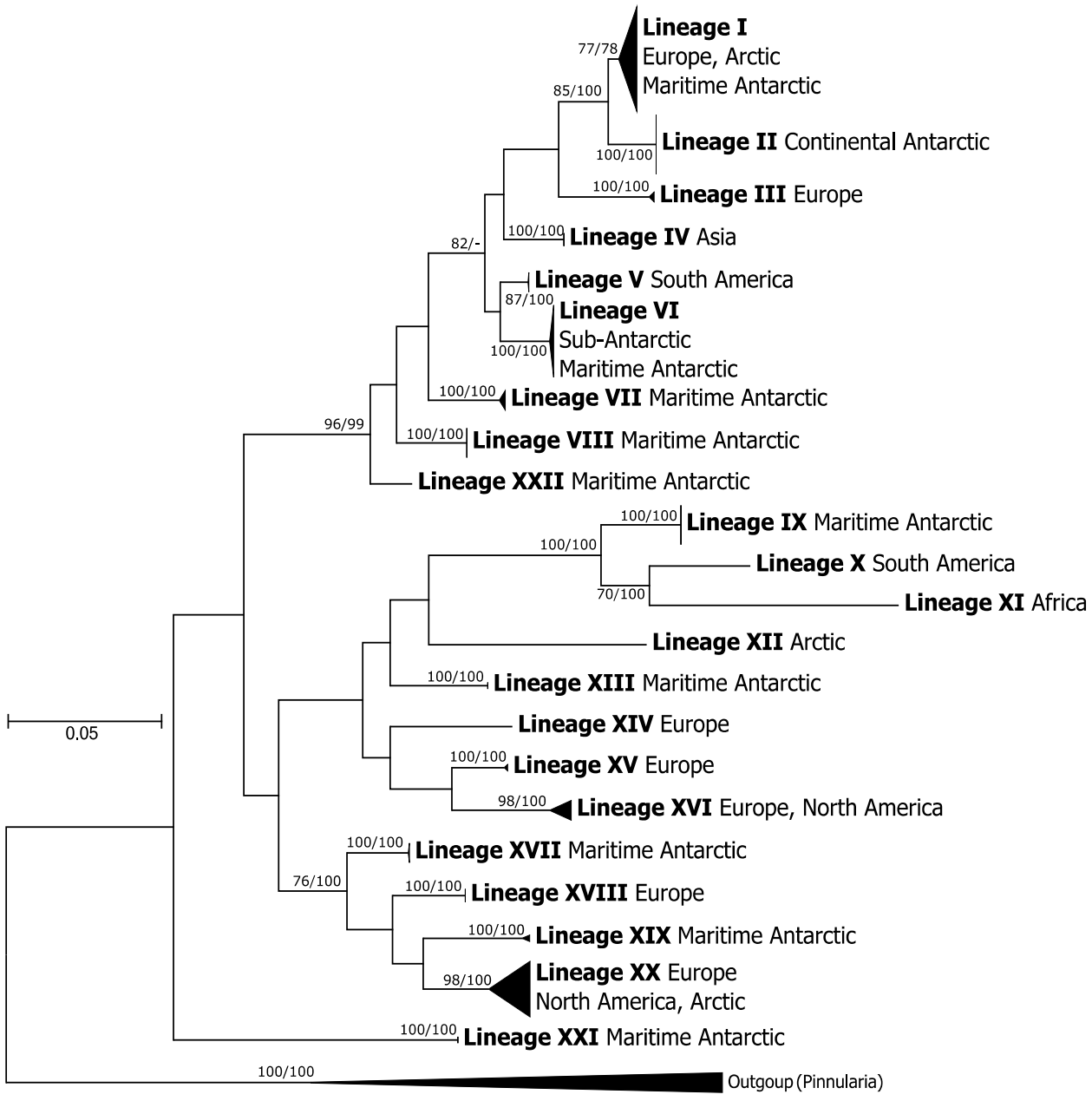

Figure 20**:** Maximum likelihood molecular phylogeny based on D1-D3 LSU rDNA with indication of bootstrap values (≥70) and posterior probabilities, showing the major lineages of *P. borealis* (consensus from three different automatic species delimitation methods: GMYC, statistical parsimony network analysis and bayesian PTP) .

3.5.4. Phylogeography of green algae

Strains from the 18S rRNA type EO2-14, II-11, VPL6-4, B6-6, WO1L-3 were isolated from lakes in Maritime and Continental Antarctica (De Wever et al., 2009) and coupled to the dataset of Dr. K. Sciuto, Dr. I Moro, and Dr. N. La Rocca (University of Padova, Italy) which contained the 18S rRNA sequences of the Gondwana strain stored in the International Nucleotide Sequence Database (INSD) with the accession number AM419228.

A new Scenedesmacean species from Antarctica was described, *Chodatodesmus australis* Sciuto, Verleyen, Moro & La Rocca, in collaboration with the team from the University of Padova and based on molecular and phylogenetic analyses of the ITS2 spacer, *rbc*L gene, and *tuf*A gene. Morphological (light microscopy and scanning electron microscopy) and ultrastructural observations carried out both on the holotype of *C. australis* and on the generitype of the genus *Chodatodesmus* Hegewald, Bock & Krienitz allowed us to emend the original description of this genus (Sciuto et al., 2015).

3.5.5. Comparison of evolution of microbial and multicellular organisms

HTS of Arctic and Antarctic lakes, as well as morphology based inventories of the diatom community structure in lakes from the Antarctic Realm revealed a number of striking similarities with patterns found in multicellular organisms. First, HTS of bacteria and eukaryotes revealed clear bipolar differences in microbial community structure between the Arctic, Antarctica and the sub-Antarctic Islands (Figure 8).

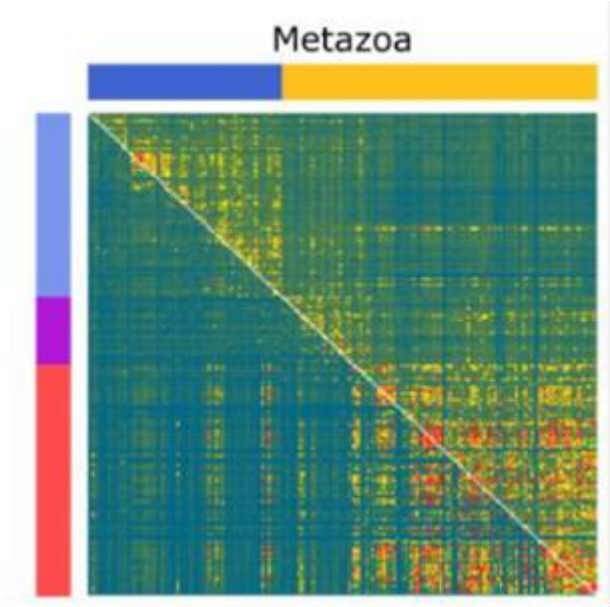

Figure 21. Heatmap showing a sample by sample matrix of metazoan sequences, ordered following their geographical location and proximity, while intersecting cells were colour-coded according to the ecological dissimilarities between individual lakes (Bray-Curtis, based on presence-absence data for the upper triangle, and abundances for the lower). See Figure 8 for colour coding.

When only the metazoan sequences from the HTS database are analysed separately (Figure 21), the patterns are highly congruent.

Second, the percentage of diatom species endemic to Antarctica (74 ± 16% in Continental Antarctica, 61 ± 9% in Maritime Antarctica, and 46 ± 13% in the sub-Antarctic Islands) is highly similar to numbers found in multicellular organisms. More in particular, approximately 30 to 50% of the lichens are endemic (Peat et al., 2007) and up to 58% of the free-living fauna is considered to be endemic to Antarctica (Pugh et al., 2008). In Tardigrades the proportion of endemic species is more than 80% (Guidetti et al., 2017) and for rotifers this is even thought to exceed 95% (Iakovenko et al., 2015).

Third, the biogeographic zoning in freshwater diatoms of the AR is highly congruent with that in multicellular terrestrial taxa (Chown et al., 2007 ; Figures 1, 5). Diatom floras in Continental Antarctica, Maritime Antarctica and the sub-Antarctic Islands are each characterised by specific species and have only few species in common. In addition, pronounced differences in diatom community structure between the different oceanic provinces and ACBRs (Terauds & Lee, 2016; Van der Putten et al., 2010) within Sub-Antarctica, and Maritime and Continental Antarctica, respectively.

Combined, these data point to a common evolutionary history of microorganisms and macroscopic taxa. Hence, the strong biogeographic structuring in our datasets is likely a reflection of the high degree of geographic isolation and polar climatic conditions, as well as the timing and rate of deglaciation of the different ice-free regions (Convey et al., 2008; Fraser et al., 2014). The importance of isolation and hence dispersal limitation was indeed confirmed by the variation partitioning analysis in the diatom dataset, which revealed that historical and spatial factors independently explained a significant (and the largest) portion of the variation in diatom community structure between the lakes. In addition, multiple regression analysis revealed that the number of endemic diatom species can be significantly explained by differences in temperature and geographic isolation, and hence dispersal limitation. This, together with the strong bioregionalisation patterns observed, has a number of important implications for conservation planning (Chown et al., 2015; Fraser et al., 2014). First, management plans for terrestrial ice-free regions and their lakes should include measures to prevent the introduction of non-native microbes into the AR, as exotic taxa might potentially affect local communities and competitively exclude endemic and sometimes rare species (Cowan et al., 2011). Second, because our data revealed that different ACBRs and oceanic provinces are characterized by highly dissimilar diatom communities and few species are shared between regions, also the unintentional transportation of microorganisms from one region to another should be avoided in order to protect regions against increased homogenization of their diatom floras. This evidently requires more stringent measures than those currently taken by national scientific program managers and tourist operators (Hughes et al., 2018). Considering the steady increase in

tourism and scientific activities in the AR (Coetzee et al., 2017), as well as forecasted climate and environmental changes (Lee et al., 2017) render this issue a high priority on the international conservation agenda.

3.6. Functional genetic and biochemical capacities

3.6.1. Metagenomics

In total, we obtained 118.5 million 2x 125bp raw reads (on average 39.5 +-2.9 M reads per sample). An inhouse pipeline was tested to process and analyze these data. Preliminary analyses showed that bacteria accounted for the majority of the diversity (Figure 22). At the phylum level, the groups Proteobacteria, Bacteroidetes and Actinobacteria, which are typically found in lakes (Newton et al., 2011), accounted for most reads. In the littoral sample naga1, Cyanobacteria (*Nostoc*) were also relatively abundant, while naga5 and naga6, from respectively 5 and 10 m deep contained more Acidobacteria and DNA viruses. These two latter samples showed a higher similarity in bacterial community composition compared to the most shallow sample. Interestingly, Archaea were, however, found only in the naga5 sample. Clear differences in functional potential were noticeable between the different depths. The shallow sample was dominated by genes involved in lipid, amino acid, fatty acids transport and metabolism. The middle depth was dominated by genes involved in carbohydrate metabolism related to carbon fixation, DNA metabolism and photosynthesis pathways. The deepest sample was characterized by genes involved in cell wall biogenesis and signal transduction mechanisms.

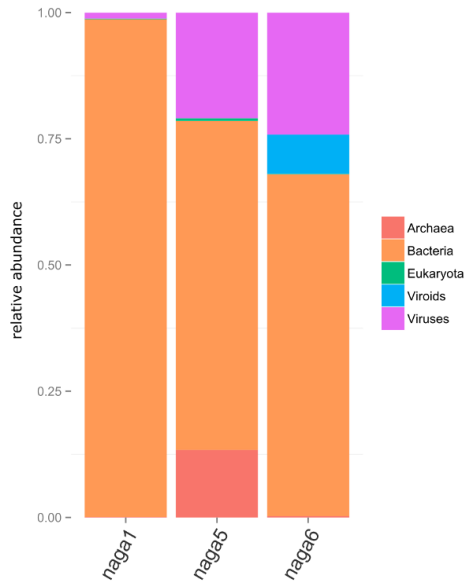

Figure 22. Relative abundance of Archaea, Eukarya, Bacteria and Viruses/viroids, based on the analysis with Metaphlan.

Because Antarctic ecosystems are notoriously oligotrophic and nitrogen availability is potentially limited, we focussed on the nitrogen pathways of these samples. Nitrogen fixation genes were most abundant at depths 1 and 3, although the actual genes present differed with *nifH* and *nifD* being abundant at depth 3, while *nifK* and *nifN* were dominant in the shallow sample. Genes involved in denitrification (*norB* and *nirK*) were also more present in this shallow sample. Dissimilatory nitrate reduction to ammonium (DNRA), an anaerobic pathway, was clearly more present in the deeper samples (Figure 23), and especially in the deepest sample. This might indicate that oxygen availability is limited or even absent in this part of the lake, and likely the result of the accumulation of dead organic matter. A further analysis of pathways involved in remineralization or anaerobic processes are needed to corroborate this hypothesis.

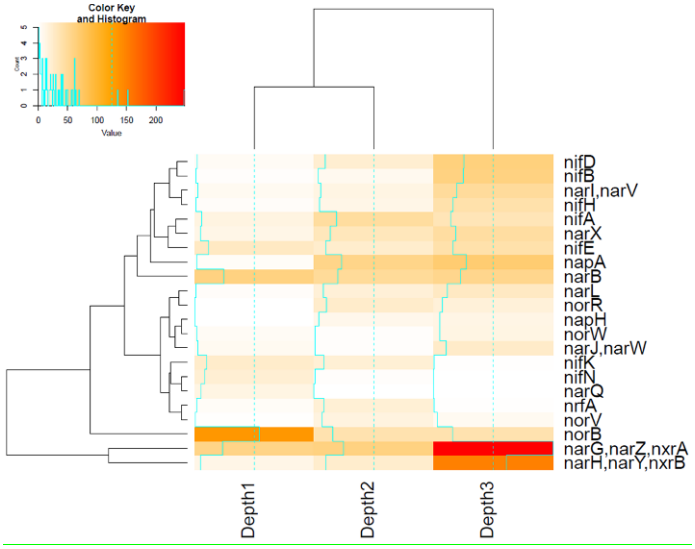

Figure 23: Relative abundances of genes involved in the nitrogen cycle

3.6.2. Metatranscriptomics

Quality control of co-extracted RNA (see 2.4.1) on a Bioanalyzer revealed that the quality of the RNA was insufficient to continue with cDNA synthesis and sequencing. This was probably caused by temperature fluctuation induced RNA degradation during the prolonged storage of the samples when these were transported from Antarctica to Japan by boat. Therefore, we decided not to sequence the RNA, as sufficient quality of the resulting data could not be guaranteed.

3.7. Microorganisms as early warning indicators

This work package was aimed at assessing the potential to use microorganisms as early indicators for climate and environmental changes. We focused on the most complete datasets resulting from the CCAMBIO project, namely those (i) with the Illumina sequences of the 18S and 16S rRNA genes in the Arctic and Antarctic lakes, and (ii) the

dataset containing diatom species distributions in the Antarctic realm. Because modern day species distribution and diversity patterns are the result of both past processes (e.g. deglaciation history) as well as dispersal limitation (spatial processes) and present-day environmental properties of habitats, we used two approaches to assess the relative importance of each of these sets of variables. First, we modelled the effect of environmental, climatic and spatial/historical factors on OTU and species richness using multiple regressions. The response variable were the number of OTUs standardized to an equal number of sequences (n = 5000) and the local diatom richness. The predictor variables included the climatic variables (see 2.2.3.1), the minimum age that lakes exist in the region, the distance of the region to the closest southern hemisphere landmass (excluding Antarctica), and the pH and specific conductance. Second, we applied redundancy analysis and variation partitioning (Borcard et al., 1992) to assess the importance of local environmental and climate variables versus spatial and hirstorical variables in explain patterns in microbial community structure in both datatsets (see also 3.2 and 3.3). The multiple coefficients of determination were adjusted (R^2_{adj}) (Peres-Neto et al., 2006) was used to correct for differences in the number of samples and the number of independent variables in both groups of predictors. For the Illumina dataset we only included pH and specific conductance and the climatic variables (see above). The limnological dataset for which we have diatom counts was more complete so we could also include data on the concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, NH_4^+ and PO_4^{3+} in 213 lakes. The geographic data consisted of the eigenvectors corresponding to the positive eigenvalues resulting from a principal coordinate analysis of a matrix of geographic distances between the sampling sites (Borcard et al., 2002). Using Moran's I (Dray et al., 2006) calculated in R package PCNM v2.1, only the significant spatially autocorrelated PCNM vectors were selected. For the latter, latitude and longitude were modelled by distance based Moran Eigenvector Maps (dbMEM) (Borcard et al., 2002; Dray et al., 2006; Legendre et al., 2012), which are created by orthogonal projections of the variation within a matrix of Euclidian distances between the sampling sites (Legendre et al., 2012). We retained only those dbMEM vectors that were strongly and positively spatially structured based on Moran's I (Dray et al., 2006) using the PCNM package in R (version 2.1; Legendre et al., 2013). The historical variable is the minimum age that lakes became ice-free, which is based on ^{14}C, optically stimulated luminescence and/or $^{234}U/^{230}$ dates of lacustrine sediments and algal limestones (Hendy, 2000; Hodgson et al., 2004b; Mackintosh et al., 2014; Verleyen et al., 2012). When age constraints from lacustrine sediments were not available, we assumed that lakes originated after the region became ice-free. Initial deglaciation was in these cases inferred from cosmogenic isotope dating of landforms, and/or ^{14}C dating of (i) marine fossils in raised beaches on land (Bentley et al., 2014; Mackintosh et al., 2014; Ocofaigh et al., 2014) or (ii) organic material in peat cores for the sub-Antarctic

islands (Hodgson et al., 2004b). All statistical analyses were performed in R with the packages vegan (Oksanen et al., 2015).

3.7.1. Multiple regression analysis

Multiple generalized linear regression (GLM) showed the significant interaction term between mean annual temperature and the hemisphere variable in the eukaryote GLM further suggested that mean annual temperature positively correlated to OTU-richness in the Southern Hemisphere, while this relation was considerably weaker in the Arctic. Similarly as in eukaryotes, bacterial OTU-richness in the Southern Hemisphere increased with mean annual temperature, while in the Northern Hemisphere, this relation was marginally negative, as indicated by the interaction term between annual average temperature and the hemisphere variable.

Stepwise multiple linear regressions of the diatom dataset revealed that the trends in local species richness between the lake districts can be significantly explained by seasonal variation in temperature, geographic isolation (i.e. distance of each region to the nearest Southern Hemisphere continent), and pH (R^2_{adj} = 0.81; $P < 0.001$). The geographic factor uniquely explains 28.2% of variation in species richness between the lake districts and the limnological and climatic factors combined account for 25.3% of variation. This relation was confirmed by the analysis of the entire dataset (i.e., at the individual lake level), which revealed a significant correlation between richness and isolation (Spearman's rho = -0.60, $P<0.001$), variation in temperature (Spearman's rho =-0.47, $P<0.001$).

3.7.2. Ordination and variation partitioning analysis

The importance of the climatic factors in explaining microbial community structure became also evident in the variation partitioining analysis of the three datasets. In both the eukaryotes and bacteria (Illumina datasets), temperature related variables explained c. 3% of the variation in community structure, independent of the other explanatory variables (Figure 24). However, the spatial variables accounted for c. 6 and 5% of the variation on the eukaryotes and bacteria, respectively.

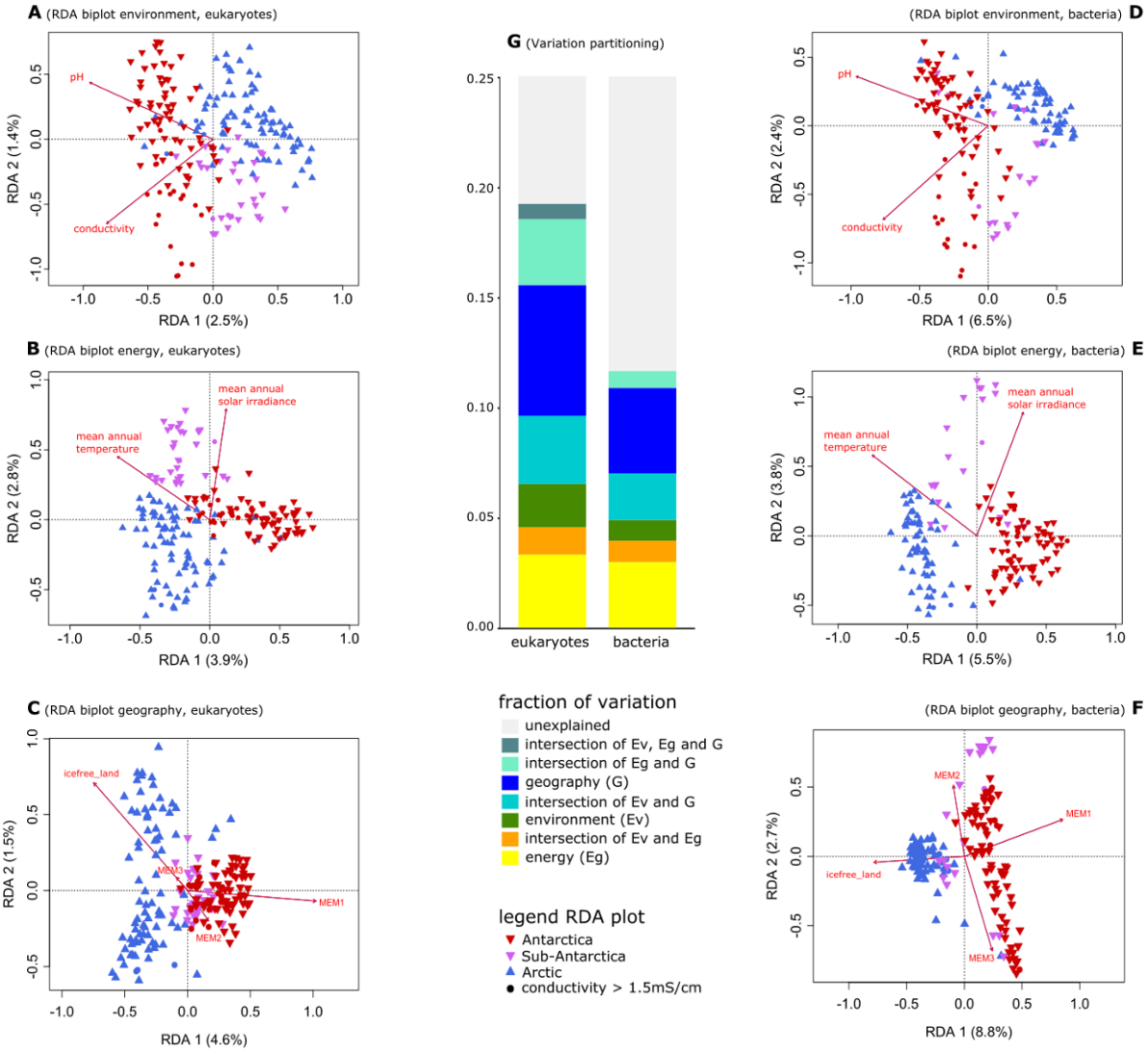

Figure.24: Biplots of redundancy analysis (RDA, plots A-F) and variation partitioning (G), showing the relationship between community composition and environmental, contemporary climate/energy and geographic data for eukaryotes (A-C, respectively) and bacteria (D-F, respectively). The total explained variation in the community data was partitioned in the proportions explained by environmental, energy, geographic variables and their shared proportions (plot G), showing geographic variables still explain the largest proportion of explained variation in both eukaryotes and bacteria, after correcting for spatial autocorrelation by measured environmental and climate/energy related variables. As expected by the large number of response variables (OTUs), a significant part of the observed variation remained unaccounted for.

Redundancy analysis and variation partitioning of the diatom dataset, revealed that variation in local diatom community structure is significantly ($P < 0.05$) explained by both (i) environmental, as well as (ii) historical and geographic factors (Figure 25). Combined, geographic and historical factors explain 44.4% of the total variation in local

diatom community structure. The significant local and climatic variables are the difference between mean summer and winter temperature, pH, specific conductance, and the concentrations of Cl^-, Na^+, Ca^{2+}, PO_4^{2+}, K^+, NO_3^-, Mg^{2+} and NH_4^+. These factors jointly explain 33.6% of the variation in diatom turnover between the lakes. As expected, the overlap between both sets of predictors is relatively large (24.8%), given that much of the variation in environmental conditions is spatially structured at such a large scale. Additionally, however, geographic and historical variables uniquely explain 10.8% of the total variation in diatom data, while the unique contribution of local environmental and climatic variables amounts to only 8.8%.

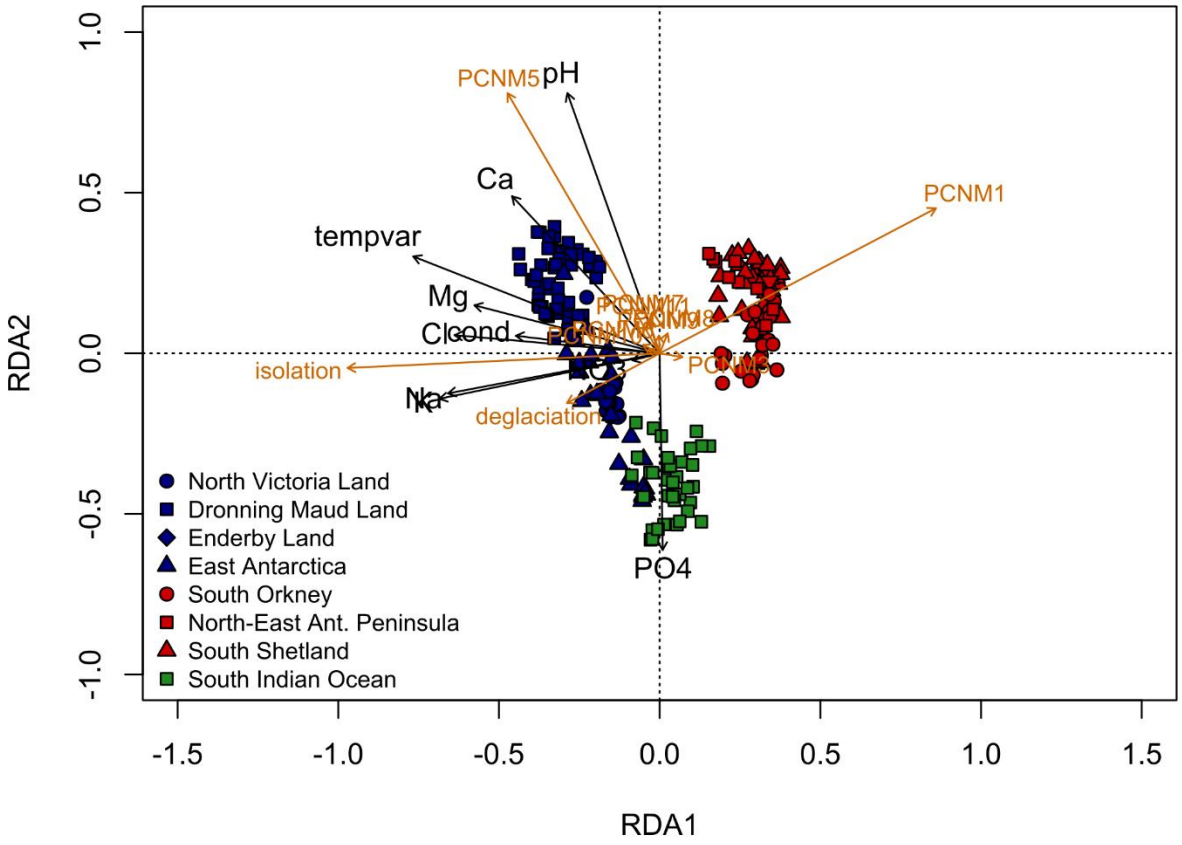

Figure 25: Redundancy analysis of 213 lakes showing those variables significantly explaining the variation in diatom community structure

We conclude that although temperature and limnological variables significantly explained patterns in richness and community structure in the three datasets, geographic and historical factors explain a significant, unique and generally the largest amount of the variation in biotic data. This suggests that lacustrine microbial communities in Antarctica will likely respond to future climate change and variations in limnological properties of the lakes, and that their present-day distribution is an imprint of hirstorical events (e.g. glacial-interglacial cycles and differences in the deglaciation history between regions), as well as past and present dispersal limitation. The latter calls for stringent

measures to be taken to prevent the introduction of non-native microbial species and the homogenization of floras between regions of the AR.

3.8. Publication of datasets in open access systems

The metadata of the eukaryote and bacterial HTS amplicon sequencing datasets of LM and PAE have been documented on the microbial Antarctic Resource System (mARS) portal at mars.biodiversity.aq. MARS is an SCAR initiative developed at RBINS, and aims to make microbial data from Antarctica more visible for the wider scientific community, as such ensuring optimal access to the obtained data.

By using internationally supported standardized terminology of the Genomics Standards Consortium (GSC), the data (metadata, nucleotide sequence data and environmental data) can later also be integrated with other projects. In addition, the metadata of these datasets have also been registered on the Global Biodiversity Information Facility (GBIF, available at http://ipt.biodiversity.aq/resource?r=microbial_bacteria_fungi_and_eukaryotes_in_arctic_antarctic_and_subantarctic_lacustrine_biofilms). After publication of the manuscripts that are currently in preparation, the sequencing and environmental data will be formatted using the GSC terminology, and will be made public through the European Nucleotide Archive (ENA, part of the International Nucleotide Sequence Database Consortium, INSDC) in collaboration with RBINS. Analogously, after publication of the results of the high throughput shotgun sequencing experiment, the data will be documented on mARS and GBIF, and will be made publicly available through ENA. The Cyanobacteria 454 pyrosequencing dataset of CIP that was discussed in Pessi et al. (2016 and 2018) has also been documented on mARS and GBIF (available at http://ipt.biodiversity.aq/resource?r=lacustrine_cyanobacteria_antarctica), while the sequence data has been submitted to the NCBI's Short Read Archive (SRA, part of INSDC) under the Bioproject number PRJNA512453. These data will be made public on 31-12-2019, after publication of a third manuscript. An assembly of the contigs of the genome of ULC007 was deposited in the genome database of NCBI. Finally, the diatom occurrence dataset of PAE has been formatted as a Darwin Core archive, and will be publicly available through the Biofresh metadata portal (http://data.freshwaterbiodiversity.eu) and GBIF after publication of the manuscript.

4. POLICY SUPPORT

In relation to the stated priorities of the 'Science for a Sustainable Development' (SSD) program, CCAMBIO addressed major research questions of high importance for the policy issues regarding the environmental protection and conservation of Antarctic biodiversity:

- Extent and uniqueness of microbial diversity in the Antarctic Realm
- Vulnerability of microbial diversity to climate change
- A demonstration of the usefulness of HTS methodologies as a tool for biodiversity assessments, which can be used in future monitoring programs and projects aimed at assessing the response of microbial communities to climate and environmental changes
- Recommendations for sustainable management of lacustrine and terrestrial Antarctic ecosystems and design of ASPA based on microbial diversity data

4.1. Environmental protection and conservation

The rather high ratio of endemic and novel microbial phylotypes observed on the Antarctic continent shows that a significant fraction of the microbial diversity may have evolved *in situ* over larger temporal scales. Combined, the strong bioregionalization and macroecological patterns point to past and present dispersal limitations, evolution in isolation and persistence of Eukarya and Bacteria on the continent in glacial refugia during ice ages. This is largely in agreement with patterns for macroscopic organisms, and calls for stringent measures to avoid the introduction of alien microbial species into the Antarctic Biogeographic Realm, and to prevent the homogenisation of microbial communities between terrestrial ice-free regions. The loss of this Antarctic microbial diversity and its replacement by cosmopolitan invasive taxa would impair the scientific understanding of the functioning of these native communities and the study of their evolutionary history, specific adaptations and properties.

The proposed conservation and management measures would include the following:

1) Management plans for terrestrial ice-free regions and their lakes should include measures to prevent the introduction of non-native microbes into the AR, as exotic taxa might potentially affect local communities and competitively exclude endemic and sometimes rare species (Cowan et al., 2011).

2) The unintentional transportation of microorganisms from one region to another should be avoided in order to protect regions against increased homogenization of their microbial floras. This evidently requires more stringent measures than those currently

taken by national scientific program managers and tourist operators. Moreover, the awareness of scientists of other disciplines working in Antarctica would also need to be raised, as they also might disperse microorganisms to and from different local and regional sources during field work. This is particularly important for areas of Antarctica that are still pristine. Considering the steady increase in tourism and scientific activities in the AR (Coetzee et al., 2017) as well as forecasted climate and environmental changes render this issue a high priority on the international conservation agenda. A special attention to this point could be integrated into the Environmental Impact Assessments that are mandatory to carry out activities in Antarctica following the Protocol on Environmental Protection of the Antarctic Treaty. In the sub-Antarctic Islands, the national authorities are responsible for the environmental management.

3) Antarctic Specially Protected Areas (ASPA) should be designated in areas to protect unique microbial diversity, that is currently undervalued and rarely considered as being worth protection. Of the 72 ASPAs that existed in 2015, 19 and 7 were mentioning algae or cyanobacteria, respectively. These ASPA would also include 'inviolate areas' that would be closed to human presence for long periods and serve as 'reference areas' for future studies with methods that will be even more sensitive and sophisticated that those available today (Hughes et al., 2015).

Though it is not possible to protect microbial habitats from the impact of climate change *per se*, and there will be climatic modifications resulting in larger deglaciated areas (Lee et al., 2017), we advocate to work with the SCAR and CEP to avoid that anthropogenic dissemination and homogenization would destroy the legacy of a unique and fascinating evolutionary history (Chown et al., 2015). A new SRP called 'Integrated Science to Support Antarctic and Southern Ocean Conservation (Ant-ICON)' is presently being prepared to be submitted to SCAR in 2020 and if accepted, would aim to address fundamental scientific questions related to the conservation and management of Antarctica, and aim to enable science-based international decision-making and development of new policies.

4.2. Preliminary steps to the use of HTS methods for the assessment of Polar Biodiversity

Confronted with the technological progresses allowed by HTS but also the risks that this technology could generate artefactual data to an unknown extent and could result in an artificial inflation of the observed diversity, a workshop was organised with international experts on 21/11/2012 as described in 5.2. This allowed to take into account many advices and recommendations that were helpful for CCAMBIO, e.g. the use of artificial communities to validate the bioinformatic pipelines used for analysis.

For the cyanobacteria, a pilot study was performed to test the choice of primers and DNA extraction methods, but also to test and calibrate different bioinformatic pipelines by using two artificial communities (based on cyanobacterial strains). This showed that PCR- and sequencing-derived artefacts inflated richness up to five times, even after a very stringent quality control. The relative abundances were also skewed, as some taxa seem to be preferentially amplified over others, so that the method can only be considered as qualitative (Pessi et al., 2016).

Also for microbial eukaryotes, the analysis of artificial communities provided new insights in errors associated with NGS data. These errors included large insertions and deletions in sequences that otherwise have a good quality score and easily get through the most stringent quality controls. We found that by adding an alignment step after OTU clustering this issue can be partially resolved. Although the insertions and deletions are not difficult to spot, we also found some sequences with a considerable amount of errors. We expect that they have arisen during PCR amplification. Although this problem merits further investigation, a possible solution is to introduce a cut-off value that removes all OTUs with a small number of sequences. We found that OTUs containing a large number of sequences robustly represented the species present in the artificial community. In some rare cases, however, spurious OTUs could reach a significant size, while true species would fall under any sensible cut-off level. This difficulty is of less importance when we aim to investigate large-scale patterns in community composition of the dominant species.

4.3. Activities and expertise of CCAMBIO partners for policy support

At the international level, CCAMBIO partners translated their expertise and results obtained during CCAMBIO in working groups or international organizations that are working on the environmental protection of Antarctic biotopes, their long-term monitoring, and the conservation of the microbial diversity.

The CCAMBIO partners have co-organized various sessions concerning the Antarctic biogeography of microorganisms and the conservation of microbial communities during SCAR meetings (e.g. Session S22 at the XXXIII SCAR Biennal Meetings and Open Science Conference 2014, "Scientific Advice for Policymakers and Evidence-based Conservation (AntEco)", August 28 2014, Auckland, New-Zealand or Session S10 'Understanding physiology (including 'omics' approaches), July 10-13 2018, Leuven, Belgium, as well as during meetings concerning Arctic and Antarctic microbial diversity (i.e. the Arctic summit meeting in Prague, 6-7/04/2017).

Presently, E. Verleyen is member of the Board of the ANTOS Action group of SCAR ANTOS (Antarctic near-shore and terrestrial observing system) and was invited to a SCAR workshop 'Interactions between biological and climate processes in the Antarctic'

(16-18/09/2015, Barcelona Spain). He was also invited to a workshop initiated by the SCAR SRP "Antarctic Thresholds - Ecosystem Resilience and Adaptation", which focused on challenges in identifying and applying cross-disciplinary approaches in the Antarctic and resulted in a publication (Gutt et al., 2018) where eight themes were highlighted. These ranged from scale problems, through risk maps, and organism/ecosystem responses to multiple environmental changes and evolutionary processes.

A. Wilmotte is member of the Board of the SCAR SRP 'Ant-Eco' and member of the Belgian delegation to the Committee for Environmental Protection (CEP) of the Antarctic Treaty Meeting, and is also member of a group of CEP delegates who organized a workshop on education and dissemination in the frame of the ATCM38, just before the latter in Sofia in May 2015. She was co-organisor of mini-symposium MS 3. "Linking Antarctic science with environmental protection: Celebrating the 25th anniversary of the Madrid Protocol", Kuala Lumpur, Malaysia, 23 August 2016, that resulted in a publication (Hughes et al., 2018). She was invited to the "Antarctic Environments Portal Climate Change Content Development Workshop" to advise on the priorities and content of subjects to put on the Antarctic Environments Portal (17-18/03/2015, Cambridge, UK) and the "Priority Threat Management for Antarctic Biodiversity Workshop" to identify the management strategies available to mitigate threats to Antarctic biodiversity and quantify the cost, feasibility and benefit of each action (8-9/07/2017, Leuven , Belgium). She was invited by EU-POLARNET to be the lead author of the White Paper on Polar Biology touching upon the most pressing issues in the Polar Regions (https://www.eu-polarnet.eu/fileadmin/user_upload/www.eu-polarnet.eu/Events/White_Paper_Broschuere-Korr-nach-Druck.pdf).

5. DISSEMINATION AND VALORISATION

Results from CCAMBIO have been disseminated in a number of ways. To the scientific community, an output of 42 peer-reviewed papers, 40 oral conference contributions and 15 posters at international meetings were presented. Students in the partners' laboratories also benefited from CCAMBIO knowledge and methodologies. To the general public, the project was presented via the website and a large number of outreach activities as detailed below.

Valorization can also be judged from the success of CCAMBIO partners to obtain new projects based on the expertise gained during CCAMBIO. In 2013, the partners were selected by the INTERACT network to sample microbial biofilms in 80 lakes in Greenland (Sabbe et al., 2015) that were included in the CCAMBIO study by PAE. In 2016, the CCAMBIO partners have obtained the BRAIN-BE project MICROBIAN to study the microbiome diversity and function in the Sør Rondane Mountains, East Antarctica. Since 2017, PAE is coordinating the Biodiversa project CLIMARCTIC - Climate change impacts on Arctic soil and lake microbiomes. In UL-CIP, after working for 1 year on CCAMBIO, Igor S. Pessi has obtained in 2013 a FRIA fellowship to characterize the colonization by cyanobacterial communities of the forefields of retreating glaciers in the Poles. UL-CIP also collaborated on the polyphasic taxonomic study of Oscillatoriales from Maritime Antarctica, with Prof. Kovacik of the Comenius University of Bratislava (Slovakia). The sequencing of genomes was also the occasion of a collaboration with Prof. Baurain and EJ Javaux (ULiège) and resulted in 3 publications and the PhD thesis of Luc Cornet. At LM, an FWO-funded research project (2012-2015) allowed samples from the PEA to be studied in great detail for the presence of photoautotrophic and photoheterotrophic bacteria. This included enrichment and purification cultures as well as amplicon sequencing (Illumina) of functional genes and lead to synergies with CCAMBIO regarding cultures and data analysis, additional peer-reviewed papers and a PhD thesis by Guillaume Tahon.

During the taxonomic revision of diatoms of Maritime Antarctica, B. Van de Vijver gave names to newly described species that were inspired by the CCAMBIO project: *Nitzschia annewillemsiana, N. kleinteichiana, N. velazqueziana, N. willmotteana, N. stelmachpessiana, N. vancauwenberghiana; N. vandeputteana* (Hamsher et al. 2015) and *Mayamaea sweetloveana, M. tytgatiana* (Ziderova et al. 2016).

5.1. Website

A project website was designed to increase the visibility of the project and is available at http://www.ccambio.ulg.ac.be. It includes a description of the project, news, activities, publications, links and contact information.

5.2. Workshop on the use of HTS methods for Polar Biodiversity

The "Next Generation Sequencing at the Poles" workshop was organized by the CCAMBIO project partners at the University of Liège (Liège, Belgium) between 21 and 23 November 2012. The first day was dedicated to lectures, poster presentations and discussions centered around proposing guidelines for inter-laboratory comparisons and was attended by 53 participants. The book of abstracts can be downloaded from the project website.

The speakers were David Pearce (British Antarctic Survey, Cambridge, UK), Antonio Quesada, (Autonomous University Madrid, Spain), Alison Murray (Desert Research Institute, NV, USA), Jean-François Ghiglione (Laboratoire d'Océanographie Microbienne, France), George Kowalchuk* (Netherlands Institute of Ecology, Netherlands), Thomas Pommier (Université de Lyon 1, France), Craig Herbold (University of Waikato, New Zealand), Étienne Yergeau* (National Research Council of Canada, Montréal). The speakers indicated with * gave a talk by videoconference, decreasing the carbon footprint of the workshop.

During the second and third days, a training course on bioinformatic analyses was given by Alison Murray, Thomas Pommier, Craig Herbold and Bjorn Tytgat (LM) and was followed by 24 young researchers.

5.3. Biological resources

Bacterial isolates obtained at LM have been stored in the research collection of the laboratory. Their characterization is still ongoing. Once their identity is fully established, a subset of representative strains is selected by the BCCM/LMG public collection curator for deposit in the public collection. This has already been done for a strain, LMG 29911^{T}, which we named *Abitibacterium utsteinensis* that represents the novel phylum Abditibacteriota (Tahon et al. 2018).

Nineteen cyanobacterial strains isolated by CCAMBIO members (Dagmar Obbels for PAE, Guillaume Tahon for LMG and Y. Lara for ULg) were deposited in the public collection BCCM/ULC.

5.4. Collection of samples

The samples collected and combined within the CCAMBIO project resulted so far in two new initiatives.

First, 16 samples were selected from Arctic, Antarctic and sub-Antarctic lakes for a metagenomics analysis on a Illumina HiSeq platform. The samples were processed as described in 3.4.1. This resulted in nearly 500 million paired end reads. Bacteria made

up the majority of the reads. Preliminary analysis showed that Archaea were present in at least 4 samples. Eukaryotic reads are mainly metazoan from the southern Greenland sample, while most other eukaryotic reads are assigned to the Bacillariophyta. The sample from the highly saline Forlidas Pond (Transantarctic Mountains) showed a very low diversity and different functional genes from the other samples (DNRA being the most important nitrogen-related process) and a high number of genes related to stress response. Future analysis of this unique dataset will especially focus on carbon and nitrogen metabolism related processes, which we expect to differ significantly between (and within) the different biogeographical regions.
In a second study, we selected 87 benthic microbial mats of Arctic and (sub)Antarctic lakes from the CCAMBIO dataset for studying the biodiversity of aquatic fungi using high-throughput amplicon sequencing (Illumina, MiSeq) of the 5.8S-ITS2 segment of the nuclear ribosomal ITS region. This dataset was combined with the 16S and 18S rRNA datasets obtained within CCAMBIO for bacteria and microbial eukaryotes, respectively (see 3.3). This analysis revealed that these benthic mats harbour a diverse pool of fungi, dominated by Cryptomycota and Chytridiomycota, as well as many yeast-like forms of Ascomycota and Basidiomycota.

5.5. Outreach and education

5.5.1. Articles in newspapers, media activities

- Website of the University of Liège: https://www.uliege.be/cms/c_3065991/fr/annick-wilmotte-deleguee-belge-au-comite-pour-la-protection-de-l-environnement-lors-de-la-reunion-consultative-du-traite-sur-l-antarctique
- 2013/05/01-2013/05/12 Interviews of Dr A. Wilmotte on WebTV sur le Traité Antarctique (http://www.ulg.ac.be/cms/c_3155716/en/est-on-en-trainde-detruire-l-antarctique) and during the public TV broadcast 'Le jardin extraordinaire' (https://www.rtbf.be/tv/emission/detail_le-jardin-extraordinaire/actualites/article_la-protection-de-l-antarctique-la-preservation-de-la-biodiversite-des-eaux-douces?id=7988975&emissionId=30)
- Interview of E. Verleyen for the kids krant about scientific expeditions. 21/02/2018.
- Durieu B.: Regular posts on the website of APECS Belgium (Association of Polar Early Career Scientists: popular Science, data tutorial, etc. in 2017 (https://apecsbelgium.wordpress.com/category/data-tutorials/)

5.5.2. Valorisation for a wider audience

- Verleyen E., D. Obbels, A. De Wever, C. Souffreau, P. Vanormelingen, K. Sabbe, W. Vyverman, K. Peeters, B. Tytgat, A. Willems, M.-J. Mano, P. De Carvalho Maalouf, R. Fernandez-Carazo, Z. Namsaraev, A. Wilmotte, D. Ertz. 2013. 'Out of sight, out of mind, Antarctic microbial diversity as an additional criterion for conservation purposes'// Uit het oog uit het hart? Antarctische microbiële diversiteit als criterium voor naruurbehoud. // Loin des yeux, loin du coeur? Et si on prenait en compte la diversité microbienne dans les stratégies de conservation en Antarctique? Science Connection (BelSPO) 41:44-47. Special edition for the organisation of the 36th Antarctic Treaty Consultative Meeting (ATCM2013) with translation in English, dutch and french, May 2013, Brussels, Belgium. (www.belspo.be/belspo/organisation/Publ/pub_ostc/sciencecon/41sci_en.pdf)
- LM, PAE and CIP contributed to the exhibition "Inside the Station Exhibition"of the International Polar Foundation, by providing bacterial cultures for fotosessions, photographs and information material for the Biology part. December 2012 to May 2013, Tour & Taxis, Brussels.
- Lecture given by Dr A. Wilmotte during the Science Fair of the Association for Polar Early Career Scientists (APECS) at the Royal Academy: "The forgotten heroes of Antarctica (microbial life)", 26 May 2013, Brussels, Belgium.
- A. Wimotte organised the course Collège Belgique at the Royal Academy 'Le traité sur l'Antarctique: une gouvernance originale pour un continent exceptionnel', Le système du traité sur l'Antarctique : un mécanisme de coopération unique. Le Comité pour la protection de l'environnement : un outil mis en place par le protocole de Madrid pour protéger l'Antarctique. 28 May 2013, Brussels, Belgium.
- A. Wilmotte gave an outreach lecture "Les héros oubliés de l'Antarctique » on the subject of microbial diversity» for the association 'Connaissance et vie' in Mons (22 January 2015) and Courtrai (1 October 2015).
- A. Wilmotte gave two outreach lectures during 'Cours Espace Universitaire' in Liège ≪ Le traite sur l'Antarctique, une gouvernance originale pour un continent exceptionnel ≫, 18 February 2016, and ≪ S'adapter pour survivre : la biodiversité terrestre antarctique≫, 3 March 2016, Liège, Belgium.
- B. Durieu, K. Beets & A. Wilmotte, 'Hands on workshop on the pigments of Antarctic cyanobacteria' for a secondary school Saint-Roch de Theux,Belgique, 31 March 2017, University of Liège, Belgium.
- E. Verleyen: Presentation "Antarctische kustmeren: biodiversiteitshotspots voor micro-organismen". Rotary club Dendermonde, 2 May 2017, Dendermonde, Belgium.

- E. Verleyen: Presentation "Antarctische kustmeren: microbiële biodiversiteitshotspots en archieven voor de reconstructie van vroegere veranderingen in de ijskapdynamiek". Vrienden van de Wetenschap' (Faculteit Wetenschappen), 4 May 2017, Ghent, Belgium.
- E. Verleyen: Outreach presentation for the Ny Ålesund science forum, 19 July 2017, Svalbard.
- A. Wilmotte: Presentation of research projects in Antarctica at the « Closing event » of an exhibition of pictures of Antarctica "Sentinels of the extreme", Atrium Covent Garden, 7 September 2017, Brussels, Belgium.
- B. Durieu & V. Savaglia: organization of a projection of the movie "Antarctica, sur les traces de l'Empereur", plus the interview of the film-maker and debate at the University of Liège, 27 September 2017. (https://www.facebook.com/events/480535182315165/), Liège, Belgium.
- E. Verleyen: Presentation for 'Iedereen UGent' - UGent 200 jaar: Wat Antarctische meersedimenten ons leren over vroegere klimaatveranderingen (en voorspellingen nauwkeuriger maken). 08 October 2017, Ghent, Belgium.
- A. Wilmotte & V. Savaglia gave an outreach lecture ≪Qui vit dans les conditions extrêmes de l'Antarctique? ≫ Université du troisième âge, 14 November 2017 Liége, Belgium.
- B. Durieu & V. Savaglia : for the "Antarctica day", creation and publication of the "Maps of Belgian polar research and expedition in 2017/2018": https://apecsbelgium.wordpress.com/maps-of-belgian-polar-research/belgian-polar-expeditions/, 26 November 2017.
- Durieu B & V. Savaglia: organization of an APECS-BELSPO contest of Antarctic stories for 5th and 6th primary school and 1st secondary school, 2017-2018: (http://rejouisciences.uliege.be/activites/concours/concours-antarctique/). Visit of the primary school of Wegnez Centre (Pepinster) who participated to the Story book contest : https://apecsbelgium.wordpress.com/2018/07/21/back-to-school/, 21 June 2018, Pepinster, Belgium.
- B. Durieu & V. Savaglia: for "Antarctica Day", visits of 3 schools (British International School of Bruxelles, primary school Sainte-Louise of Schaerbeek, Collège Saint-Pierre of Uccle) to propose different activities about Antarctic research (presentations, experiments, games, etc.), 23, 28, 30 November 2018. https://apecsbelgium.wordpress.com/2018/12/01/school-visits-for-antarctica-day/
- B. Durieu & V. Savaglia: presentation of CCAMBIO/MICROBIAN expeditions and laboratory analyses to the public during the "Printemps des Sciences 2018" at University of Liège : http://rejouisciences.uliege.be/2018/ll37/, 24-25 March 2018, Liège, Belgium.

5.5.3. Training and education

- A. Willems includes the research outcomes in classes on Antarctic prokaryotic diversity in the course of Molecular microbial ecology to students of the MSc Biotechnology program at Ghent University.
- W. Vyverman, E. Verleyen and B. Tytgat use the CCAMBIO data as case studies in several courses within the bachelor and master programs in Biology at Ghent University. E. Verleyen also gives guest lectures at the University of Lille (France) and the Free University of Brussels in which the CCAMBIO data are integrated.
- The LM team hosted two master students (4th year Biochemistry and Biotechnology) who received 4 weeks of laboratory training. One bachelor thesis (2013-2014) and three master theses (Sam Lambrechts, MSc Biology 2012-2013 and Hanneloor Heyndrickx and Karen Van Raemdonck, both MSc Biology and Biotechnology 2013-2014) were done in the framework of CCAMBIO.
- The PAE laboratory trained three bachelor students (3th year biology) for one month within the CCAMBIO framework (bioinformatics and statistical analysis techniques of NGS amplicon data, design of group-specific primers with high taxonomic resolution, and) HPLC-pigment analysis techniquesà.
- At LM and PAE, Bjorn Tytgat prepared and successfully defended a PhD thesis in the framework of CCAMBIO (28/04/2016, title: Distribution and characterization of bacterial communities in diverse Antarctic ecosystems by high-throughput sequencing). In the PAE lab, a PhD thesis in the framework of CCAMBIO was also written and defended by Maxime Sweetlove (30/08/2018, title: Biogeography, macro-ecology and biodiversity of lacustrine microbiomes).
- In CIP, a PhD thesis was written and defended (05/09/2017, title: The cyanobacterial biota of Polar Regions) by Igor S. Pessi that included two chapters on the project CCAMBIO.

5.5.4. Final workshop

No final workshop was organized as three oral talks on CCAMBIO data were given during the SCAR Biology Symposium in Leuven in July 2017.

6. PUBLICATIONS

6.1 Articles in peer-reviewed international journals

1. Tytgat B., E. Verleyen, D. Obbels, K. Peeters, A. De Wever, S. D'hondt, T. De Meyer, W. Van Criekinge, W. Vyverman and A. Willems. 2014. Bacterial diversity assessment in Antarctic terrestrial and aquatic microbial mats: a comparison between bidirectional pyrosequencing and cultivation. PLoS One. 9: e97564.
2. Taylor JC, Cocquyt C, Karthick B & Van de Vijver B (2014) Analysis of the type of *Achnanthes exigua* Grunow (Bacillariophyta) with the description of a new Antarctic species. Fottea 14: 43-51.
3. Kopalová K, Ochyra R, Nedbalová L & Van de Vijver B (2014) Moss-inhabiting diatoms from two islands in the Maritime Antarctic Region. Plant Ecology & Evolution 147: 67–84.
4. Van de Vijver B & Crawford RM (2014) Orthoseira *limnopolarensis sp. nov.* (Bacillariophyta), a new *d*iatom species from Livingston Island (South Shetland Islands, Antarctica). Cryptogamie, Algologie 35: 245-257.
5. Van de Vijver B & Kopalová K (2014) Four *Achnanthidium species* (Bacillariophyta) formerly identified as *Achnanthes minutissima* from the Antarctic Region. European Journal of Taxonomy 79: 1–19.
6. Van de Vijver B, Zidarova R & Kopalová K (2014) New species in the genus *Muelleria* (Bacillariophyta) from the Maritime Antarctic Region. Fottea 14: 77–90.
7. Van de Vijver B, Morales EA & Kopalová K (2014) Three new araphid diatoms (Bacillario*phyta) f*rom the Maritime Antarctic Region. *Ph*ytotaxa 167: 256–266.
8. Van de Vijver B, de Haan M & Lange-Bertalot H (2014) Revision of the genus *Eunotia* (Bacillariophyta) in the Antarctic Region. Plant Ecology & Evolution 147: 256–284.
9. Van de Vijver B, Kopalová K, Zidarova R & Levkov Z (2014) Revision of the g*enus Halamphora* (Bacillariophyta) in the Antarctic Region. Plant Ecology & Evolution 147: 374–391. doi: 10.5091/plecevo.2014.979
10. Zidarova R, Kopalová K & Van de Vijver B (2014) The genus *Stauroneis* (Bacillariophyta) from the South Shetland Islands and James Ross Island (Antarctica). Fottea 14: 201–207.
11. Pinseel E., Kopalová K. & Van de Vijver B. (2014) *Gomphonema svalbardense* sp. nov., a new freshwater diatom species (Bacillariophyta) from the Arctic Region. Phytotaxa 170: 250–258.

12. Chattová B., Lebouvier M. & Van de Vijver B. (2014) Freshwater diatom communities from Ile Amsterdam (TAAF, southern Indian Ocean). Fottea 14: 101–119.
13. Van de Vijver B.(2014) *Brachysira sandrae*, a new raphid diatom (Bacillariophyceae) from the Iles Kerguelen (TAAF, sub-Antarctica, southern Indian Ocean) with an analysis of the type material of *B. brebissonii* R.Ross. Phytotaxa 184: 139–147.
14. Van de Vijver, B., Kopalova, K., Kociolek, J.P. & Ector L. (2015) *Denticula jamesrossensis*, a new freshwater diatom (Bacillariophyta) species from the Maritime Antarctic Region. Fottea 15: 105–111.
15. Kohler T.J., Kopalová K., Van de Vijver B. & Kociolek P.J. (2015) The genus *Luticola* D.G. Mann (Bacillariophyta) from the McMurdo Sound Region, Antarctica, with the description of 4 new species. Phytotaxa 208: 103–134.
16. Van de Vi*jver B.,* Kopalová K. & Zidarova R. (2015) Three new *Craticula* spec*ies* (Bacillariophyta) from the Maritime Antarctic Region. Phytotaxa 213: 45–55.
17. Kopalová K., Kociolek J.P., Lowe R.L., Zidarova R. & Van de Vijver B. (2015) Five new species of the genus *Humidophila* (Bacillariophyta) from the Maritime Antarctic Region. Diatom Research 30: 117–131.
18. Van de Vijver B. & Cox E.J. (2015) *Fallacia emmae* sp. nov., a new soil-inhabiting diatom species from the sub-Antarctic Region. Cryptogamie, Algologie 36: 245-254.
19. Hughes, K., Cowan, D., & Wilmotte, A. (2015). Protection of Antarctic microbial communities – 'out of sight, out of mind'. Frontiers in Microbiology, 6(151), 1-6.
20. Obbels D., E. Verleyen, M.-J. Mano, Z. Namsaraev, M. Sweetlove, B. Tytgat, R. Fernandez-Carazo, A. De Wever, S. D'hondt, D. Ertz, J. Elster, K. Sabbe, A. Willems, A. Wilmotte, and W. Vyverman (2016). Bacterial and eukaryotic biodiversity patterns in terrestrial and aquatic habitats of the Sør Rondane Mountains, Dronning Maud Land, East Antarctica. FEMS Microbiol. Ecol. 92:fiw041.
21. Tytgat Bjorn, Elie Verleyen, Maxime Sweetlove, Sofie D'hondt, Pia Clercx, Eric Van Ranst, Karolien Peeters, Stephen Roberts, Zorigto Namsarev, Annick Wilmotte, Wim Vyverman, Anne Willems (2016). Bacterial community composition in relation to bedrock type and macrobiota in soils from the Sør Rondane Mountains, East Antarctica. FEMS Microbiol. Ecol. 92:fiw126
22. Jancusova, M., Kovacik, L., Pereira, A. B., Dusinsky, R., & Wilmotte, A. (2016). Polyphasic characterization of 10 selected ecologically relevant filamentous

cyanobacterial strains from the South Shetland Islands, Maritime Antarctica. FEMSMicrobiology Ecology, 92(7), 100.

23. Tahon G, Tytgat B, Stragier P & Willems A (2016) Analysis of *cbbL*, *nifH*, and *pufLM* in soils from the Sør Rondane Mountains, Antarctica, reveals a large diversity of autotrophic and phototrophic bacteria. Microbial Ecology 71: 131-149.
24. Tahon G, Tytgat B & Willems A (2016) Diversity of phototrophic genes suggests multiple bacteria may be able to exploit sunlight in exposed soils from the Sør Rondane Mountains, East Antarctica. Frontiers in Microbiology 7: Article 2026.
25. Stelmach Pessi, I., De Carvalho Maalouf, P., Laughinghouse, H. D., Baurain, D., & Wilmotte, A. (2016). On the use of high-throughput sequencing for the study of cyanobacterial diversity in Antarctic aquatic mats. Journal of Phycology, 52, 356–368.
26. Kopalová K., Zidarova R. & Van de Vijver B. (2016) Four new monoraphid diatom species (Bacillariophyta, Achnanthaceae) from the Maritime Antarctic Region. European Journal of Taxonomy 217: 1–19.
27. Hamsher S., Kopalová K., Kociolek J.P., Zidarova R. & Van de VIjver B. (2016) Revision of the genus *Nitzschia* in the Maritime Antarctic Region. Fottea 16: 79–102.
28. Van de Vijver B., Kopalová K. & Zidarova R. (2016) Revision of the *Psammothidium germainii* complex (Bacillariophyta) in the Maritime Antarctic Region Fottea 16: 145–156.
29. Van de Vijver B., Kopalová K., Zidarova R. & Kociolek J.P. (2016) Two new *Gomphonema* species (Bacillariophyta) from the Maritime Antarctic Region. Phytotaxa 269: 209–220.
30. Kochman-Kędziora N., Noga T., Zidarova R., Kopalová K. & Van de Vijver B. (2016) *Humidophila komarekiana* sp. nov. (Bacillariophyta), a new limnoterrestrial diatom species from King George Island (Maritime Antarctica). Phytotaxa 272: 184–190.
31. Zidarova R., Kopalová K. & Van de Vijver B. (2016) Ten new Bacillariophyta species from James Ross Island and the South Shetland Islands (Maritime Antarctic Region). Phytotaxa 272: 037–062.
32. Lara, Y., Durieu, B., Cornet, L., Verlaine, O., Rippka, R., Stelmach Pessi, I., Misztak, A., Joris, B., Javaux, E., Baurain, D., & Wilmotte, A. (2017). Draft genome sequence of the axenic strain *Phormidesmis priestleyi* ULC007, a cyanobacterium isolated from Lake Bruehwiler (Larsemann Hills, Antarctica). Genome Announcements , 01546-16.

33. Tahon G & Willems A (2017) Isolation and characterization of aerobic anoxygenic phototrophs from exposed soils from the Sør Rondane Mountains, East Antarctica. Systematic and Applied Microbiology 40: 357-369.
34. Pinseel E, Hejdukova E, Vanormelingen P, Kopalová K, Vyverman W, Van De Vijver B (2017) *Pinnularia catenaborealis sp. nov*. (Bacillariophyceae), a unique chain-forming diatom species from James Ross Island and Vega Island (Maritime Antarctica). Phycologia 56: 94-107.
35. Van de Vijver B., Lange-Bertalot, H., Wetzel C.E. & Ector L. (2017) *Michelcostea*, a new diatom genus (Bacillariophyta) from the sub-Antarctic Region. Nova Hedwigia Beihefte 146: 125–136.
36. Pessi, I. S., Lara, Y., Durieu, B., Maalouf, P., Verleyen, E., & Wilmotte, A. (2018). Community structure and distribution of benthic cyanobacteria in Antarctic lacustrine microbial mats. FEMS Microbiology Ecology, 94 (5), 042.
37. 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.
38. Cornet, L., Meunier, L., Van Vlierberghe, M., Léonard, R., Durieu, B., Lara, Y., Misztak, A., Sirjacobs, D., Javaux, E., Philippe, H., Wilmotte, A., & Baurain, D. (2018). Consensus assessment of the contamination level of publicly available cyanobacterial genomes. PLoS ONE, 13(7), 0200323.
39. Cornet, L., Bertrand, A., Hanikenne, M., Javaux, E., Wilmotte, A., & Baurain, D. (2018). Metagenomic assembly of new (sub)polar Cyanobacteria and their associated microbiome from non-axenic cultures. Microbial Genomics 4(9), 1-15.
40. Cornet, L., Wilmotte, A., Javaux, E., & Baurain, D. (2018). A constrained SSU-rRNA phylogeny reveals the unsequenced diversity of photosynthetic Cyanobacteria (Oxyphotobacteria). BMC Research Notes, 11(1), 435.

6.2. Articles in preparation for refereed international journals

1. Sweetlove Maxime, Tytgat Bjorn, Verleyen Elie, Van den Berge Koen, members of the Polar lake Sampling Consortium, Hodgson A. Dominic, Clement Lieven, Sabbe Koen, Wilmotte Annick, Willems Anne and Vyverman Wim (in prep.) Divergent evolutionary trajectories of Arctic and Antarctic lake microbiomes.
2. Tytgat Bjorn, Sweetlove Maxime, Verleyen Elie, Van den Berge Koen, members of the Polar Sampling Consortium, Hodgson Dominic, Clement Lieven, Sabbe Koen, Wilmotte Annick, Willems Anne and Vyverman Wim (in prep.) An

interhemispheric asymmetry in the latitude-diversity relation is common among microorganisms
3. Tytgat Bjorn, Verleyen Elie, Pargana, Aikaterini, Sweetlove Maxime, D'hondt Sofie, Tsujimoto Megumu, Imura, Satoshi, Wilmotte Annick, Willems Anne, Vyverman Wim, (in prep.) Shotgun metagenomic profile of an east-Antarctic lake along a depth gradient.
4. Tytgat Bjorn, Verleyen Elie, Sweetlove Maxime, D'hondt Sofie, Wilmotte Annick, Willems Anne and Vyverman Wim (in prep.) Interhemispheric biogeographical patterns in the functional potential of polar lake microbiomes.
5. Elie Verleyen, Bart Van de Vijver, Bjorn Tytgat, Dominic A. Hodgson, Eveline Pinseel, Kate Kopalova, Steven L. Chown, Eric Van Ranst, ANTDIAT consortium, Koen Sabbe, Wim Vyverman. (in prep.) bioregionalisation and biogeographic provincialism in Antarctic freshwater diatom communities
6. Durieu B, Lara Y, Pessi I S, Lambion A, Verleyen E, Wilmotte A (In prep) A multi-scale biogeographical analysis of Antarctic mat-forming cyanobacteria.
7. Lara Y, Durieu B, Rippka, R, Javaux EJ, Wilmotte A (In prep.) The black-pigmented cyanobacterium *Phormidesmis priestleyi* ULC007: a bacterial survival toolkit from Antarctica.

6.3. Articles in non-refereed journals

1. Deng, L., Sweetlove, M., Blank, S., Obbels, D., Verleyen, E., Vyverman, W. & Kurmayer R. (2017). Monitoring of Toxigenic Cyanobacteria Using Next Generation Sequencing Techniques. In R., Kurmayer, K., Sivonen, A., Wilmotte, & N., Salmaso (eds.) Molecular Tools for the Detection and Quantification of Toxigenic Cyanobacteria, pp. 277-282. Hoboken, NJ: John Wiley and sons LTD.
2. Sweetlove M, Obbels D, Verleyen E, Pessi I.S., Wilmotte A & Vyveman W (2017). Bioinformatic processing of amplicon sequencing datasets. In R., Kurmayer, K., Sivonen, A., Wilmotte, & N., Salmaso (eds.) Molecular Tools for the Detection and Quantification of Toxigenic Cyanobacteria, pp. 283-287. Hoboken, NJ: John Wiley and sons LTD.

6.4. Oral presentations at scientific meetings

1. Mano, M.-J., De Carvalho, P., Verleyen, E., Obbels, D., De Wever, A., Namsaraev, Z., Willems, A., Vyverman, W., & Wilmotte, A. Out of sight, out of mind? Diversity of microscopic organisms as an overlooked criterion for conservation purposes. Paper presented at XXXII SCAR OPEN SCIENCE

CONFERENCE "Antarctic Science and Policy Advice in a Changing World", July 2012, Portland, OR, USA.

2. Wilmotte A. Antarctic cyanobacterial diversity: how important are the geographical and ecological factors? Paper presented at the XXI Congresso Latinoamericano de Microbiology – ALAM 2012, 31 October 2012, Santos, Brazil.(Invited Speaker)
3. Verleyen E, Van de Vijver B, Hodgson DA, Sabbe K, Souffreau C, Nedbalová L, Tavernier I, Sterken M, Jones VJ, Vanormelingen P, Antoniades D, Van Nieuwenhuyze W, Satoshi I, Kudoh S & Vyverman W. Poles apart: Interhemispheric contrasts in polar diatom diversity driven by differences in tectonics and glacial history. 30th Polar Biology Symposium, 24 November-01 December 2012, Tokyo, Japan.
4. Verleyen E, Van de Vijver B, Souffreau C, Sabbe K, Hodgson DA, Nedbalová L, Antoniades D, Vanormelingen P, Convey P & Vyverman W. Diatom distributions in space and time - a case study from the polar regions. 1st Polar Ecology Symposium, 1-3 October 2012. České Budějovice, Czech Republic.
5. Vyverman W, Verleyen E, Obbels D, Tytygat B, Wilmotte A, Willems A, Van Nieuwenhuyze W, Tavernier I, Hodgson DA & Sabbe K. The imprint of glacial history on the biogeography of Antarctic lake-dwelling micro-organisms. 5th International Conference on Polar and Alpine Microbiology, 8-12 September 2012, Big Sky, Montana, USA.
6. Van de Vijver B, Wetzel CA & Ector L. Revision of the genus *Planothidium*: the importance of type material in a better identiftication of some widespread taxa. NORBAF meeting, 18-22 November 2013, Erken, Sweden.
7. Kopalová K, Nedbalová L, Verleyen E & Van de Vijver B. James Ross Island: diatom gate to two biogeographical zones. 7th Central European Diatom Meeting, 16-20 September 2013, Thonon-les-Bains, France.
8. Van de Vijver B, Kopalová K, Zidarova R & Verleyen E. Freshwater diatoms from the Maritime Antarctic Region: biodiversity hotspot or taxonomical artefact? 7th Central European Diatom Meeting, 16-20 September 2013, Thonon-les-Bains, France.
9. Van de Vijver B, Kopalová K, Zidarova R, Wetzel CE & Ector L. Le genre *Planothidium* dans la région antarctique. 32ème Colloque de l'Association des Diatomistes de Langue Française, 16-20 September 2013, Thonon-les-Bains, France.
10. Kopalová K, Nedbalová L & Van de Vijver B. James Ross Island: diatom gate to two biogeographical zones. 22nd North American Diatom Symposium, 13-17 August 2013, Bar Harbor, Maine, USA.

11. Van de Vijver B, Kopalová K & Zidarova R. Freshwater diatoms from the Maritime Antarctic Region: biodiversity hotspot or taxonomical artefact? 22nd North American Diatom Symposium, 13-17 August 2013, Bar Harbor, Maine, USA.
12. Hughes K., E. Verleyen, W. Vyverman, D. Obbels, A. Willems, I. Stelmach Pessi, H. D. Laughinghouse, A. Wilmotte. Human impacts on Antarctic ecosystems : do not forget the microorganisms! XIth SCAR Biology Symposium, 15-19 July 2013, Barcelona, Spain.
13. Vyverman W., E. Verleyen, D. Obbels, B. Tytgat, A. Wilmotte, A. Willems, W. Van Nieuwenhuyze, I. Tavernier, D.A. Hodgson, K. Sabbe. The imprint of glacial history on the biogeography of Antarctic lake-dwelling micro-organisms. 5th International Conference on Polar and Alpine Microbiology, 8-13 September 2013, Big Sky, Montana, USA.
14. Van de Vijver B, Kopalová K, Zidarova R & Verleyen E. Freshwater diatoms from the Maritime Antarctic Region: biodiversity hotspot or taxonomical artefact? 23rd International Diatom Symposium, 6-11 September 2014, Nanjing, China.
15. Verleyen E, Tavernier I, Van Nieuwenhuyze W, Hodgson DA, Souffreau C, Sabbe K, Van de Vijver B & Vyverman W. Biogeografisch provincialisme in Oost-Antarctische diatomeeëngemeenschappen: lokale extinctie versus selectieve overleving in glaciale refugia. NVKD studiedagen, 15-17 May 2014, Leiden, Netherlands.
16. Van de Vijver B., Kopalová K., Zidarova R. & Verleyen E. Freshwater diatoms from the Maritime Antarctic Region: biodiversity hotspot or taxonomical artefact? 23rd International Diatom Symposium, 06-11 September 2014, Nanjing, China.
17. Stelmach Pessi, I**.,** de Carvalho Maalouf, P., Laughinghouse IV, H. D., & Wilmotte, A. Unveiling Antarctic cyanobacterial diversity by 454 pyrosequencing. Paper presented at DFG Workshop on Antarctic Research, May 2015, Göttingen, Germany.
18. Wilmotte A., I. Stelmach-Pessi, M. Sweetlove, D. Obbels, P. Vanormelingen, B. Tytgat, A. Willems, E. Verleyen, W. Vyverman, B. Van De Vijver. Molecular diversity of microorganisms in Antarctic lacustrine microbial mats. Aquatic Sciences Meeting: Global And Regional Perspectives — North Meets South. 22-27 February 2015, Granada, Spain.
19. Tahon G., B. Tytgat, A. Willems. Diversity of *cbb*L, *nif*H and *puf*LM genes in soils around the Princess Elisabeth Station, Sør Rondane Mountains, Antarctica. 6th International Conference on Polar and Alpine Microbiology, České

Budějovice, Czech Republic, 6-10 September 2015. Oral presentation by G. Tahon. Second prize for a lecture by a young researcher.

20. Vyverman W, Verleyen E, Pinseel E, Kopalová K, Antoniades D, Sterken M, Nedbalová L, Jones VJ, Tavernier I, Tytgat B, Souffreau C, Imura S, Kudoh S, Convey P, Hodgson DA, Sabbe K, Van de Vijver B. Post-Miocene divergence of polar diatom biomes. 6th International Conference on Polar and Alpine Microbiology, 6-10 September 2015, České Budějovice, Czech Republic (invited keynote lecture).
21. Sweetlove M., E. Verleyen, B. Tytgat, D. Obbels, S. D'hondt, A. Willems, W. Vyverman. Biogeographic zoning of aquatic microeukaryotes in the Antarctic realm. 6th International Conference on Polar and Alpine Microbiology, 6-10 September 2015, České Budějovice, Czech Republic.
22. Tytgat B., D. Obbels, E. Verleyen, M. Sweetlove, Z. Namsaraev, M.J. Mano, R. Fernandez-Carazo, K. Peeters, S. D'hondt, P. Clercx, A. De Wever, D. Ertz, J. Elster, E. Van Ranst, S. Roberts, K. Sabbe, A. Wilmotte, W. Vyverman, A. Willems. Bacterial and eukaryotic biodiversity patterns in the Sør Rondane Mountains, Dronning Maud Land, East Antarctica. BNCAR symposium. Unlocking a continent: scientific research at the Belgian Princess Elisabeth Station, Antarctica 2008-2016. 29 April 2016, Brussels, Belgium.
23. Tahon G., B. Tytgat, K. Peeters, A. Willems. Shining a light on exposed high-altitude Antarctic ecosystems provides a clearer view on the diversity of phototrophic bacteria. BNCAR symposium. Unlocking a continent: scientific research at the Belgian Princess Elisabeth Station, Antarctica 2008-2016. 29 April 2016, Brussels, Belgium.
24. Durieu B., Y. Lara, D. Obbels, E. Pinseel, I. Stelmach Pessi, M. Sweetlove, B. Tytgat, E. Verleyen, W. Vyverman, A. Van De Putte, B. Van De Vijver, A. Willems, A. Wilmotte. Diversity and distribution of microorganisms in microbial mats of Antarctic lakes. XXXIV Scientific Committee on Antarctic Research Open Science Conference , 20-30 August 2016, Kuala Lumpur, Malaysia.
25. Wilmotte A., A. Willems, E. Verleyen, W. Vyverman, D. Velazquez, A. Quesada, D. H. Laughinghouse, J. Kleinteich, D. A Pearce, J. Elster, K. Hughes. Inviolate areas to protect reference sites for future microbiology research in Antarctica. XXXIV Scientific Committee on Antarctic Research Open Science Conference, 20-30 August 2016, Kuala Lumpur, Malaysia.
26. Stelmach Pessi, I., Lara, Y., Durieu, B., Wilmotte, A. Cyanobacterial Diversity in Antarctic Aquatic Microbial Mats, Third annual Belgian Interdisciplinary Biofilm Research meeting, September 2016, Liège, Belgium.

27. Van de Vijver B., Kopalová K. & Zidarova R. The *Psammothidium germainii*-complex. 10th Central European Diatom Meeting, 20-24 April 2016, Boedapest, Hungary.
28. Van de Vijver B., Kociolek J.P., Kopalová K., Hamsher S.E., Kohler, T.J., Convey P. & McKnight D.M. Freshwater diatom biogeography and the genus *Luticola:* An extreme case of endemism in Antarctica. 24th International Diatom Symposium, 20-26 August 2016, Quebec, Canada.
29. Verleyen E. The origin of the polar lacustrine diatom biome: evidence from paleolimnology, macroecology and high-throughput sequencing. The Micropalaeontological Society Annual Conference, 17-18 November 2016, Lille,France. Invited keynote presentation
30. Verleyen E., Vyverman W., Van de Vijver B., Sweetlove M., Pinseel E., Tytgat B., Sabbe K, and the CCAMBIO consortium. The polar lacustrine microbiome: centres of endemism under changing climates. Flanders Annual Meeting on Ecology, 19 December 2016, Gent, Belgium.
31. Verleyen E. The origin of the polar lacustrine diatom biome: evidence from macroecology and paleolimnology. 11th Central European Diatom meeting, 22-25 March 2017, Prague, Czech Republic. Invited keynote
32. Wilmotte, A. Antarctic cyanobacteria: from diversity to genomics. Paper presented at 'Marine microorganisms and their contribution to global biogeochemical cycles' Symposium in honour of Professor Lucas Stal, 30 June 2017, Amsterdam, Netherlands. http://hdl.handle.net/2268/212648
33. Sweetlove M., B. Tytgat, E. Verleyen, K. Van den Berge, S. D'hondt, D. Obbels, E. Pinseel, D. A. Hodgson, K. Sabbe, A. Wilmotte, L. Clement, A. Willems and W. Vyverman.Biogeography and macroevolution in the Arctic and Antarctic lacustrine microbiomes. XIIth SCAR Biology Symposium, 10-14 July 2017, Leuven, Belgium.
34. Durieu B, Pessi IS, Lara Y, de Carvalho Maalouf P, Lambion A & Wilmotte A. Biogeographic patterns and genomic adaptation of benthic cyanobacteria in Antarctic lakes. XIIth SCAR Biology Symposium, 10-14 July 2017, Leuven, Belgium.
35. Verleyen E., Pinseel E., Van de Vijver B., Hodgson D.A., Harper M., Wolfe A.P., Lewis A.R., Dickinson W., Ashworth A.C., the ANTDIAT consortium, Sabbe K., Vyverman W. The imprint of Neogene and Quaternary climate filtering on contemporary biogeographic patterns in the Antarctic lacustrine diatom biome. SCAR PAIS symposium, 10-15 September 2017, Trieste, Italy.
36. Tytgat, B., Obbels, D., Sweetlove, M., Namsaraev, Z., Mano, M-J., Fernandez-Carazo, R., Peeters, K., D'hondt, S., Clercx, P., De Wever, A., Ertz, D., Elster, J., Van Ranst, E., Roberts, S., Sabbe, K., Wilmotte, A., Willems, A., Vyverman,

W. and Verleyen, E. Bacterial and eukaryotic biodiversity patterns in the Sør Rondane Mountains. Ecology Across Borders, 11-15 December 2017, Ghent, Belgium.

37. Verleyen E., Van de Vijver B., Pinseel E., Hodgson D.A., Tytgat B., POLDIAT consortium, Sabbe K., Vyverman W. The origin of the polar lacustrine diatom biome: evidence from macroecology and paleolimnology. German Polar Conference, 25-29 March 2018, Rostock, Germany.
38. Lara, Y., Durieu, B., Pessi, I., Cornet, L., Baurain, D., Javaux, E., & Wilmotte, A. The survival toolkit of the Antarctic cyanobacterium *Phormidesmis priestleyi* ULC007. COST Life Origin Final Workshop, 20 March 2018, Bertinoro, Italie.
39. Vincent W and Wilmotte A. Conservation issues in the High Arctic and Pole-to-Pole comparisons. Polar 2018, Where the Poles come together, Open Science Conference, 21 June 2018, Davos, Switzerland.
40. Durieu, B., Lara, Y., Stelmach Pessi, I., Lambion, A., Verleyen, E., Baurain, D., & Wilmotte, A. Biogeography of Cyanobacteria in Antarctic Mats and Implication for Conservation. Polar 2018, Where the Poles come together, Open Science Conference, 19 June 2018, Davos, Switzerland.

6.5. Poster presentations at scientific meetings

1. Obbels D., P. De Carvalho Maalouf, A. Lambion, A. De Wever, K. Peeters, A. Willems, E. Verleyen, W. Vyverman, A. Wilmotte. Antarctic Microbial BIOdiversity : the importance of geographical versus ecological factors. SCAR 5th Open Science Conference, , 16-19 July 2012, Portland, Oregon, USA.
2. Convey P., B. Danis, H. D. Laughinghouse, D. Obbels, D. Pearce, I. Stelmach Pessi, B. Tytgat, B. Van de Vijver, E. Verleyen, W. Vyverman, A. Willems, A. Wilmotte. The CCAMBIO project: responses of the aquatic microbial mats to Climate Change. SCAR-EBA Workshop on "Next-Generation Sequencing at the Poles". 21-23 November 2012, Liège, Belgium.
3. Obbels D., E. Verleyen, B. Tytgat, J. Elster, O. Strunecky, A. Wilmotte, A. Willems, K. Sabbe, W. Vyverman. The diversity and tolerance to osmotic stress of East Antarctic filamentous Cyanobacteria. XIth SCAR Biology Symposium, 15-19 July 2013, Barcelona, Spain.
4. Kopalová K., D. Obbels, I. Stelmach Pessi, M. Sweetlove, B. Tytgat, B. Van de Vijver, P. Vanormelingen, E. Verleyen, W. Vyverman, A. Willems, A. Wilmotte. CCAMBIO: Climate Change and Antarctic Microbial Biodiversity. SCAR-Open Science Conference, 25-28 August 2014, Auckland, New Zealand.
5. Stelmach Pessi, I., De Carvalho, P., Laughinghouse, H. D., & Wilmotte, A. Use of 454 pyrosequencing protocol for the assessment of cyanobacterial diversity.

Poster session presented at Empowering Biodiversity Research, May 2015, Brussels, Belgium.

6. Lara, Y., Verlaine, O., Kleinteich, J., Stelmach Pessi, I., Rippka, R., Renard, M., Cornet, L., Baurain, D., & Wilmotte, A. Genome sequencing of an endemic filamentous Antarctic cyanobacterium. Poster session presented at 15th International Symposium on Phototrophic Prokaryotes, 03 August 2015, Tübingen, Allemagne.
7. Tytgat B., E. Verleyen, S. D'hondt, P. Clercx, K. Peeters, E. Van Ranst, W. Vyverman, A. Willems. Exploring diversity patterns in the Sør Rondane Mountains (East-Antarctica) using Next Generation Sequencing and ARISA. 6th FEMS Congress of European Microbiologists, 7-11 June 2015, Maastricht, The Netherlands.
8. Tytgat B., E. Verleyen, M. Sweetlove, D. Obbels, S. D'hondt, A. Wilmotte, W. Vyverman, A. Willems. Biogeographic patterns in Antarctic lacustrine prokaryotes. 6th International Conference on Polar and Alpine Microbiology, 6-10 September 2015, České Budějovice, Czech Republic. (1st prize of the poster competition).
9. Tytgat B., E. Verleyen, S. D'hondt, P. Clercx, E. Van Ranst, S. J. Roberts, A. Wilmotte, W. Vyverman, A. Willems. Bedrock and biotic influence on community composition in soils from the Sør Rondane Mountains, East Antarctica. 6th International Conference on Polar and Alpine Microbiology, 6-10 September 2015, České Budějovice, Czech Republic.
10. Wilmotte A., A. Willems, E. Verleyen, W. Vyerman, D. Velázquez, H. Dail Laughinghouse IV, J. Elster, K. Hughes. A plea for the creation of inviolate areas to protect reference areas for future microbiology research in Antarctica. 6th International Conference on Polar and Alpine Microbiology, 6-10 September 2015, České Budějovice, Czech Republic.,
11. Lara, Y., Durieu, B., Borderie, F., Stelmach Pessi, I., Crahay,C., Deblander, V., Geelen, N., Iovino, M., Defise, A., Laughinghouse, H. D., Wilmotte, A. Characterization of *Leptolyngbya* and *Phormidium* diversity in Antarctic biotopes, XXXIV SCAR Biennal Meetings and Open Science Conference, August 2016, Kuala Lumpur, Malaysia.
12. Tytgat B., E. Verleyen, M. Sweetlove, B. Van de Vijver, K. Van den Berge, S. D'hondt, D. Obbels, E. Pinseel, D. Hodgson, S. Imura, K. Sabbe, A. Wilmotte, L. Clement, A. Willems, the ANTDIAT consortium and W. Vyverman. Multi-domain evidence for fine-scale bioregionalisation patterns in the Antarctic lacustrine microbiome. XIIth SCAR Biology Symposium, 10-14 July 2017, Leuven, Belgium.

13. Wilmotte A., B. Durieu, Y. Lara, D. Obbels, I. S. Pessi, E. Pinseel, M. Sweetlove, B. Tytgat, A. Van De Putte, B. Van De Vijver, E. Verleyen, W. Vyverman and A. Willems. Diversity and biogeography of microorganisms in microbial mats of Antarctic lakes. XIIth SCAR Biology Symposium, 10-14 July 2017, Leuven, Belgium.
14. Wilmotte A., A. Willems, E. Verleyen, W. Vyverman, D. Velazquez, A. Quesada, D. H. Laughinghouse, J. Kleinteich, D. A Pearce, J. Elster, K. Hughes. A strategy to protect reference sites for future microbiology research in Antarctica. XIIth SCAR Biology Symposium, 10-14 July 2017, Leuven, Belgium.
15. Durieu, B., Baurain, D., Wilmotte, A., & Lara, Y. Cold Adaptation Strategy of the Antarctic Cyanobacterium *P. priestleyi* ULC007. Polar 2018, Where the Poles come together, Open Science Conference, 19 June 2018, Davos, Switzerland.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

Adams, B., Wall, D., Virginia, R., Broos, E., & Knox, M. (2014). Ecological Biogeography of the Terrestrial Nematodes of Victoria Land, Antarctica. *ZooKeys*, *419*(419), 29–71.

Alneberg, J., Bjarnason, B. S., De Bruijn, I., Schirmer, M., Quick, J., Ijaz, U. Z., Lahti, L., Loman, N. J., Andersson, A. F., & Quince, C. (2014). Binning metagenomic contigs by coverage and composition. *Nature Methods*, *11*(11), 1144–1146.

Arctic Council. (2004). ACIA - Arctic Climate Impact Assessment.

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G. D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., & Zagnitko, O. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. *BMC Genomics*, *9*(1), 75.

Baselga, A. (2010): Partitioning the turnover and nestedness components of beta diversity. *Global Ecology & Biogeography 19,* 134-143.

Bentley, M. J., Ocofaigh, C., Anderson, J. B., Conway, H., Davies, B., Graham, A. G. C., Hillenbrand, C. D., Hodgson, D. A., Jamieson, S. S. R., Larter, R. D., Mackintosh, A., Smith, J. A., Verleyen, E., Ackert, R. P., Bart, P. J., Berg, S., Brunstein, D., Canals, M., Colhoun, E. A., Crosta, X., Dickens, W. A., Domack, E., Dowdeswell, J. A., Dunbar, R., Ehrmann, W., Evans, J., Favier, V., Fink, D., Fogwill, C. J., Glasser, N. F., Gohl, K., Golledge, N. R., Goodwin, I., Gore, D. B., Greenwood, S. L., Hall, B. L., Hall, K., Hedding, D. W., Hein, A. S., Hocking, E. P., Jakobsson, M., Johnson, J. S., Jomelli, V., Jones, R. S., Klages, J. P., Kristoffersen, Y., Kuhn, G., Leventer, A., Licht, K., Lilly, K., Lindow, J., Livingstone, S. J., Massé, G., McGlone, M. S., McKay, R. M., Melles, M., Miura, H., Mulvaney, R., Nel, W., Nitsche, F. O., O'Brien, P. E., Post, A. L., Roberts, S. J., Saunders, K. M., Selkirk, P. M., Simms, A. R., Spiegel, C., Stolldorf, T. D., Sugden, D. E., van der Putten, N., van Ommen, T., Verfaillie, D., Vyverman, W., Wagner, B., White, D. A., Witus, A. E., & Zwartz, D. (2014). A community-based geological reconstruction of Antarctic Ice Sheet deglaciation since the Last Glacial Maximum. *Quaternary Science Reviews*, *100*, 1–9.

Bik, H. M., Lambshead, P. J. D., Thomas, W. K., & Lunt, D. H. (2010). Moving towards a complete molecular framework of the Nematoda: a focus on the Enoplida and early-branching clades. *BMC Evolutionary Biology*, *10*(1), 353.

Borcard, D., & Legendre, P. (2002). All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*, *153*(1–2), 51–68.

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology*, *73*(3), 1045–1055.

Buchfink, B., Xie, C., & Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, *12*(1), 59–60.

Cavicchioli, R. (2015). Microbial ecology of Antarctic aquatic systems. *Nature Reviews Microbiology*, *13*(11), 691–706.

Chown, S. L., Clarke, A., Fraser, C. I., Cary, S. C., Moon, K. L., & McGeoch, M. A. (2015). The changing form of Antarctic biodiversity. *Nature*, *522*(7557), 431–438.

Chown, S. L., & Convey, P. (2007). Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1488), 2307–2331.

Coetzee, B. W. T., Convey, P., & Chown, S. L. (2017). Expanding the Protected Area Network in Antarctica is Urgent and Readily Achievable. *Conservation Letters*, *10*(6), 670–680.

Convey, P. (2011). Antarctic terrestrial biodiversity in a changing world. *Polar Biology*, *34*(11), 1629–1641.

Convey, P., Chown, S. L., Clarke, A., Barnes, D. K. A., Bokhorst, S., Cummings, V., Ducklow, H. W., Frati, F., Green, T. G. A., Gordon, S., Griffiths, H. J., Howard-Williams, C., Huiskes, A. H. L., Laybourn-Parry, J., Lyons, W. B., McMinn, A., Morley, S. A., Peck, L. S., Quesada,

A., Robinson, S. A., Schiaparelli, S., & Wall, D. H. (2014). The spatial structure of antarctic biodiversity. *Ecological Monographs*, *84*(2), 203–244.

Convey, P., Gibson, J. A. E., Hillenbrand, C. D., Hodgson, D. A., Pugh, P. J. A., Smellie, J. L., & Stevens, M. I. (2008). Antarctic terrestrial life - Challenging the history of the frozen continent? *Biological Reviews*, 82(*2*), 103-117.

Convey, P., & Stevens, M. I. (2007). Ecology: Antarctic Biodiversity. *Science*, *317*(5846), 1877–1878.

Corinaldesi, C., Danovaro, R., & Dell'Anno, A. (2005). Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. *Applied and Environmental Microbiology*, *71*(1), 46–50.

Cornet, L., Bertrand, A. R., Hanikenne, M., Javaux, E. J., Wilmotte, A., & Baurain, D. (2018a). Metagenomic assembly of new (sub)polar Cyanobacteria and their associated microbiome from non-axenic cultures. *Microbial Genomics*, *4*(9), 1–15.

Cornet, L., Meunier, L., Van Vlierberghe, M., Léonard, R. R., Durieu, B., Lara, Y., Misztak, A., Sirjacobs, D., Javaux, E. J., Philippe, H., Wilmotte, A., & Baurain, D. (2018b). Consensus assessment of the contamination level of publicly available cyanobacterial genomes. *PLoS ONE*, *13*(7), e0200323.

Cowan, D. A., Chown, S. L., Convey, P., Tuffin, M., Hughes, K., Pointing, S., & Vincent, W. F. (2011). Non-indigenous microorganisms in the Antarctic: assessing the risks. *Trends in Microbiology*, *19*(11), 540–548.

Cox, F., Newsham, K. K., Bol, R., Dungait, J. A. J., & Robinson, C. H. (2016). Not poles apart: Antarctic soil fungal communities show similarities to those of the distant Arctic. *Ecology Letters*, *19*(5), 528–536.

De Wever, A., Leliaert, F., Verleyen, E., Vanormelingen, P., Van der Gucht, K., Hodgson, D. A., Sabbe, K., & Vyverman, W. (2009). Hidden levels of phylodiversity in Antarctic green algae: further evidence for the existence of glacial refugia. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1673), 3591–3599.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a Chimera-Checked 16S rRNA Gene Database and Workbench Compatible with ARB. *Applied and Environmental Microbiology*, *72*(7), 5069–5072.

Dray, S., Legendre, P., & Peres-Neto, P. R. (2006). Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). *Ecological Modelling*, *196*(3–4), 483–493.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods*, *10*(10), 996–998.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*, *27*(16), 2194–2200.

Esposito, R. M. M., Horn, S. L., McKnight, D. M., Cox, M. J., Grant, M. C., Spaulding, S. A., Doran, P. T., & Cozzetto, K. D. (2006). Antarctic climate cooling and response of diatoms in glacial meltwater streams. *Geophysical Research Letters*, *33*(7), L07406.

Fernandez-Carazo, R., Hodgson, D. A., Convey, P., & Wilmotte, A. (2011). Low cyanobacterial diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80-82°S), Antarctica. *FEMS Microbiology Ecology*, *77*(3), 503–517.

Fierer, N., Nemergut, D., Knight, R., & Craine, J. M. (2010). Changes through time: Integrating microorganisms into the study of succession. *Research in Microbiology*, , 161(8), 635-642.

Finlay, B. J. (2002). Global Dispersal of Free-Living Microbial Eukaryote Species. *Science*, *296*(5570), 1061–1063.

Foissner, W. (2008). Protist diversity and distribution: Some basic considerations. *Biodiversity and Conservation*, *17*(2), 235–242.

Fraser, C. I., Nikula, R., Ruzzante, D. E., & Waters, J. M. (2012). Poleward bound: biological

impacts of Southern Hemisphere glaciation. *Trends in Ecology & Evolution*, *27*(8), 462–471.

Fraser, C. I., Terauds, A., Smellie, J., Convey, P., & Chown, S. L. (2014). Geothermal activity helps life survive glacial cycles. *Proceedings of the National Academy of Sciences*, *111*(15), 5634–5639.

Frenot, Y., Chown, S. L., Whinam, J., Selkirk, P. M., Convey, P., Skotnicki, M., & Bergstrom, D. M. (2005). Biological invasions in the Antarctic: Extent, impacts and implications. *Biological Reviews of the Cambridge Philosophical Society*, 80(1), 45-72.

Green, J., & Bohannan, B. J. M. (2006). Spatial scaling of microbial biodiversity. *Trends in Ecology & Evolution*, *21*(9), 501–507.

Guidetti, R., McInnes, S. J., Cesari, M., Rebecchi, L., & Rota-Stabelli, O. (2017). Evolutionary scenarios for the origin of an Antarctic tardigrade species based on molecular clock analyses and biogeographic data. *Contributions to Zoology*, *86*(2), 97–110.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., De Vargas, C., Decelle, J., Del Campo, J., Dolan, J. R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W. H. C. F., Lara, E., Le Bescot, N., Logares, R., Mahé, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A. L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P., & Christen, R. (2013). The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Research*, *41*(D1), 597–604.

Gutt, J., Isla, E., Bertler, A. N., Bodeker, G. E., Bracegirdle, T. J., Cavanagh, R. D., Comiso, J. C., Convey, P., Cummings, V., De Conto, R., De Master, D., di Prisco, G., d'Ovidio, F., Griffiths, H. J., Khan, A. L., López-Martínez, J., Murray, A. E., Nielsen, U. N., Ott, S., Post, A., Ropert-Coudert, Y., Saucède, T., Scherer, R., Schiaparelli, S., Schloss, I. R., Smith, C. R., Stefels, J., Stevens, C., Strugnell, J. M., Trimborn, S., Verde, C., Verleyen, E., Wall, D. H., Wilson, N. G., & Xavier, J. C. (2018). Cross-disciplinarity in the advance of Antarctic ecosystem research. *Marine Genomics*, *37*, 1–17.

Hendy, C. H. (2000). Late quaternary lakes in the mcmurdo sound region of antarctica. *Geografiska Annaler: Series A, Physical Geography*, *82*(2–3), 411–432.

Hodgson, D. A., Verleyen, E., Squier, A. H., Sabbe, K., Keely, B. J., Saunders, K. M., & Vyverman, W. (2006). Interglacial environments of coastal east Antarctica: Comparison of MIS 1 (Holocene) and MIS 5e (Last Interglacial) lake-sediment records. *Quaternary Science Reviews*, *25*(1–2), 179–197.

Hodgson, D. A., Vyverman, W., Verleyen, E., Sabbe, K., Leavitt, P. R., Taton, A., Squier, A. H., & Keely, B. J. (2004a). Environmental factors influencing the pigment composition of in situ benthic microbial communities in east Antarctic lakes. *Aquatic Microbial Ecology*, *37*(3), 247–263.

Hodgson, D. A., Vyverman, W., Verleyen, E., Sabbe, K., Leavitt, P. R., Taton, A., Squier, A. H., & Keely, B. J. (2004b). Environmental factors influencing the pigment composition of in situ benthic microbial communities in east Antarctic lakes. *Aquatic Microbial Ecology*, *37*(3), 247–263.

Hughes, K. A., Constable, A., Frenot, Y., López-Martínez, 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.

Hughes, K. A., Cowan, D. A., & Wilmotte, A. (2015). Protection of Antarctic microbial communities - "out of sight, out of mind." *Frontiers in Microbiology*, *6*(FEB), 151.

Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, *17*(3), 377–386.

Iakovenko, N. S., Smykla, J., Convey, P., Kašparová, E., Kozeretska, I. A., Trokhymets, V., Dykyy, I., Plewka, M., Devetter, M., Duriš, Z., & Janko, K. (2015). Antarctic bdelloid rotifers: diversity, endemism and evolution. *Hydrobiologia*, *761*(1), 5–43.

Jeffrey, S. W., Mantoura, R. F. C., & Wright, S. W. (1997). Phytoplankton pigments in

oceanography: guidelines to modern oceanography. *UNESCO Publishing*.

Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A. F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S. Y., Lopez, R., & Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, *30*(9), 1236–1240.

Jones, P. D., Lister, D. H., Osborn, T. J., Harpham, C., Salmon, M., & Morice, C. P. (2012). Hemispheric and large-scale land-surface air temperature variations: An extensive revision and an update to 2010. *Journal of Geophysical Research Atmospheres*, *117*(D05127).

Jungblut, A. D., Hawes, I., Mountfort, D., Hitzfeld, B., Dietrich, D. R., Burns, B. P., & Neilan, B. A. (2005). Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. *Environmental Microbiology*, *7*(4), 519–529.

Jungblut, A. D., Lovejoy, C., & Vincent, W. F. (2010). Global distribution of cyanobacterial ecotypes in the cold biosphere. *ISME Journal*, *4*(2), 191–202.

Kleinteich, J., Hildebrand, F., Bahram, M., Voigt, A. Y., Wood, S. A., Jungblut, A. D., Küpper, F. C., Quesada, A., Camacho, A., Pearce, D. A., Convey, P., Vincent, W. F., Zarfl, C., Bork, P., & Dietrich, D. R. (2017). Pole-to-Pole Connections: Similarities between Arctic and Antarctic Microbiomes and Their Vulnerability to Environmental Change. *Frontiers in Ecology and Evolution*, *5*, 137.

Komárek, J., Kaštovský, J., Ventura, S., Turicchia, S., & Šmarda, J. (2009). The cyanobacterial genus <I>Phormidesmis</I>. *Algological Studies*, *129*(1), 41–59.

Kopylova, E., Noé, L., & Touzet, H. (2012). SortMeRNA: Fast and accurate filtering of ribosomal RNAs in metatranscriptomic data. *Bioinformatics*, *28*(24), 3211–3217.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.

Lanzén, A., Jørgensen, S. L., Huson, D. H., Gorfer, M., Grindhaug, S. H., Jonassen, I., Øvreås, L., & Urich, T. (2012). CREST – Classification Resources for Environmental Sequence Tags. *PLoS ONE*, *7*(11), e49334.

Laybourn-Parry, J., & Pearce, D. A. (2007). The biodiversity and ecology of Antarctic lakes: models for evolution. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *362*(May), 2273–2289.

Lee, J. R., Raymond, B., Bracegirdle, T. J., Chadès, I., Fuller, R. A., Shaw, J. D., & Terauds, A. (2017). Climate change drives expansion of Antarctic ice-free habitat. *Nature*, *547*(7661), 49–54.

Legendre, P., Borcard, D., Blanchet, F., & Dray, S. (2013). PCNM: MEM spatial eigenfunction and principal coordinate analyses.

Legendre, P., & Legendre, L. (2012). Multiscale analysis: Spatial eigenfunctions. In *Developments in Environmental Modelling* (Vol. 24, pp. 859–906).

Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics*, *31*(10), 1674–1676.

Mackintosh, A. N., Verleyen, E., O'Brien, P. E., White, D. A., Jones, R. S., McKay, R., Dunbar, R., Gore, D. B., Fink, D., Post, A. L., Miura, H., Leventer, A., Goodwin, I., Hodgson, D. A., Lilly, K., Crosta, X., Golledge, N. R., Wagner, B., Berg, S., van Ommen, T., Zwartz, D., Roberts, S. J., Vyverman, W., & Masse, G. (2014). Retreat history of the East Antarctic Ice Sheet since the Last Glacial Maximum. *Quaternary Science Reviews*, *100*, 10–30.

Martinez, A., & Kolter, R. (1997). Protection of DNA during oxidative stress by the nonspecific DNA- binding protein Dps. *Journal of Bacteriology*, *179*(16), 5188–5194.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., Horner-Devine, M. C., Kane, M., Krumins, J. A., Kuske, C. R., Morin, P. J., Naeem, S., Øvreås, L., Reysenbach, A.-L., Smith, V. H., & Staley, J. T. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology*, *4*(2), 102–112.

McArdle, B. H., & Anderson, M. J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, *82*(1), 290–297.

McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., Desantis, T. Z., Probst, A., Andersen, G. L., Knight, R., & Hugenholtz, P. (2012). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal*, *6*(3), 610–618.

Mittelbach, G. G., & Schemske, D. W. (2015). Ecological and evolutionary perspectives on community assembly. *Trends in Ecology and Evolution*, *30*(5), 241–247.

Mortimer, E., van Vuuren, J. B., Lee, J. E., Marshall, D. J., Convey, P., & Chown, S. L. (2011). Mite dispersal among the southern ocean islands and antarctica before the last glacial maximum. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1709), 1247–1255.

Nemergut, D. R., Costello, E. K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S. K., Fierer, N., Townsend, A. R., Cleveland, C. C., Stanish, L., & Knight, R. (2011). Global patterns in the biogeography of bacterial taxa. *Environmental Microbiology*, *13*(1), 135–144.

Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D., & Bertilsson, S. (2011). A Guide to the Natural History of Freshwater Lake Bacteria. *Microbiology and Molecular Biology Reviews*, *75*(1), 14–49.

Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. A. (2017). MetaSPAdes: A new versatile metagenomic assembler. *Genome Research*, *27*(5), 824–834.

Ocofaigh, C. Ó., Davies, B. J., Livingstone, S. J., Smith, J. A., Johnson, J. S., Hocking, E. P., Hodgson, D. A., Anderson, J. B., Bentley, M. J., Canals, M., Domack, E., Dowdeswell, J. A., Evans, J., Glasser, N. F., Hillenbrand, C. D., Larter, R. D., Roberts, S. J., & Simms, A. R. (2014). Reconstruction of ice-sheet changes in the Antarctic Peninsula since the Last Glacial Maximum. *Quaternary Science Reviews*, *100*, 87–110.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2015). vegan: Community Ecology Package. R package version 2.3-0. *Http://CRAN.R-Project.Org/Package=vegan*.

Olson, J. B., Steppe, T. F., Litaker, R. W., & Paerl, H. W. (1998). N 2 -Fixing Microbial Consortia Associated with the Ice Cover of Lake Bonney, Antarctica. *Microbial Ecology*, *36*(3), 231–238.

Osborn, T. J., & Jones, P. D. (2014). The CRUTEM4 land-surface air temperature data set: Construction, previous versions and dissemination via Google earth. *Earth System Science Data*, *6*(1), 61–68.

Pearce, D. A., Bridge, P. D., Hughes, K. A., Sattler, B., Psenner, R., & Russell, N. J. (2009). Microorganisms in the atmosphere over Antarctica. *FEMS Microbiology Ecology*, *69*(2), 143–157.

Peat, H. J., Clarke, A., & Convey, P. (2007). Diversity and biogeography of the Antarctic flora. *Journal of Biogeography*, *34*(1), 132–146.

Peeters, K., Ertz, D., & Willems, A. (2011). Culturable bacterial diversity at the Princess Elisabeth Station (Utsteinen, Sør Rondane Mountains, East Antarctica) harbours many new taxa. *Systematic and Applied Microbiology*, *34*(5), 360–367.

Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of species data matrices: Estimation and comparison of fractions. *Ecology*, *87*(10), 2614–2625.

Perkerson, R. B., Johansen, J. R., Kovácik, L., Brand, J., Kaštovský, J., & Casamatta, D. A. (2011). A unique pseudanabaenalean (cyanobacteria) genus Nodosilinea gen. nov. based on morphological and molecular data. *Journal of Phycology*, *47*(6), 1397–1412.

Pessi, I. S., Maalouf, P. D. C., Laughinghouse, H. D., Baurain, D., & Wilmotte, A. (2016). On the use of high-throughput sequencing for the study of cyanobacterial diversity in Antarctic aquatic mats. *Journal of Phycology*, *52*(3), 356–368.

Pointing, S. B., Büdel, B., Convey, P., Gillman, L. N., Körner, C., Leuzinger, S., & Vincent, W. F. (2015). Biogeography of photoautotrophs in the high polar biome. *Frontiers in Plant Science*, *6*, 692.

Priscu, J. C., Fritsen, C. H., Adams, E. E., Giovannoni, S. J., Paerl, H. W., McKay, C. P., Doran, P. T., Gordon, D. A., Lanoil, B. D., & Pinckney, J. L. (1998). Perennial antarctic lake ice: An oasis for life in a polar desert. *Science*, *280*(5372), 2095–2098.

Pugh, P. J. a, & Convey, P. (2008). Surviving out in the cold: Antarctic endemic invertebrates and their refugia. *Journal of Biogeography*, *35*(12), 2176–2186.

Quayle, W. C., Peck, L. S., Peat, H., Ellis-Evans, J. C., & Harrigan, P. R. (2002). Extreme responses to climate change in Antarctic lakes. *Science*, *295*(5555), 645.

Quesada, A., Fernández-Valiente, E., Hawes, I., & Howard-Williams, C. (2009). Benthic primary production in polar lakes and rivers. In *Polar Lakes and Rivers: Limnology of Arctic and Antarctic Aquatic Ecosystems* (pp. 179–196). Oxford University Press.

Quesada, A., & Vincent, W. F. (2012). Cyanobacteria in the cryosphere: Snow, ice and extreme cold. In *Ecology of Cyanobacteria II: Their Diversity in Space and Time* (Vol. 9789400738, pp. 387–399).

Roeselers, G., Norris, T. B., Castenholz, R. W., Rysgaard, S., Glud, R. N., Kühl, M., & Muyzer, G. (2007). Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland). *Environmental Microbiology*, *9*(1), 26–38.

Sabbe, K., Hodgson, D. A., Verleyen, E., Taton, A., Wilmotte, A., Vanhoutte, K., & Vyverman, W. (2004). Salinity, depth and the structure and composition of microbial mats in continental Antarctic lakes. *Freshwater Biology*, *49*(3), 296–319.

Sabbe, K., Obbels, D., Vanormelingen, P., Strunecky, O., Vijver, B. Van De, Elster, J., Wilmotte, A., Verleyen, E., & Vyverman, W. (2015). Microbial biodiversity in polar lake ecosystems: why is it different at the North and South Pole? In *INTERACT : stories of arctic science*. (Danish Cen, pp. 146–147). Aarhus University.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., & Weber, C. F. (2009). Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541.

Sciuto, K., Lewis, L. A., Verleyen, E., Moro, I., & La Rocca, N. (2015). Chodatodesmus australis sp. nov. (Scenedesmaceae, Chlorophyta) from Antarctica, with the emended description of the genus Chodatodesmus, and circumscription of Flechtneria rotunda gen. et sp. nov. *Journal of Phycology*, *51*(6), 1172–1188.

Smol, J. P., & Douglas, M. S. V. (2007a). From controversy to consensus: Making the case for recent climate change in the Arctic using lake sediments. *Frontiers in Ecology and the Environment*, *5*(9), 466–474.

Smol, J. P., & Douglas, M. S. V. (2007b). Crossing the final ecological threshold in high Arctic ponds. *Proceedings of the National Academy of Sciences*, *104*(30), 12395–12397.

Souffreau, C., Vanormelingen, P., Van de Vijver, B., Isheva, T., Verleyen, E., Sabbe, K., & Vyverman, W. (2013). Molecular Evidence for Distinct Antarctic Lineages in the Cosmopolitan Terrestrial Diatoms Pinnularia borealis and Hantzschia amphioxys. *Protist*, *164*(1), 101–115.

Stackhouse, P. W., & Kusterer, J. M. (2008). *Surface meteorology and Solar Energy (SSE) release 6.0. NASA SSE 6.0, Earth Science Enterprise Program*.

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, *22*(21), 2688–2690.

Stanier, R. Y., Deruelles, J., Rippka, R., Herdman, M., & Waterbury, J. B. (1979). Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. *Microbiology*, *111*(1), 1–61.

Stevens, M. I., Greenslade, P., Hogg, I. D., & Sunnucks, P. (2006). Southern hemisphere springtails: Could any have survived glaciation of antarctica? *Molecular Biology and Evolution*, *23*(5), 874–882.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M. D. M., Breiner, H. W., & Richards, T. A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly

complex eukaryotic community in marine anoxic water. *Molecular Ecology*, *19*(SUPPL. 1), 21–31.

Strunecký, O., Elster, J., & Komárek, J. (2012). Molecular clock evidence for survival of Antarctic cyanobacteria (Oscillatoriales, Phormidium autumnale) from Paleozoic times. *FEMS Microbiology Ecology*, *82*(2), 482–490.

Taton, A., Grubisic, S., Balthasart, P., Hodgson, D. A., Laybourn-Parry, J., & Wilmotte, A. (2006a). Biogeographical distribution and ecological ranges of benthic cyanobacteria in East Antarctic lakes. *FEMS Microbiology Ecology*, *57*(2), 272–289.

Taton, A., Grubisic, S., Brambilla, E., De Wit, R., & Wilmotte, A. (2003). Cyanobacterial Diversity in Natural and Artificial Microbial Mats of Lake Fryxell (McMurdo Dry Valleys , Antarctica): a Morphological and Molecular Approach. *Applied and Environmental Microbiology*, *69*(9), 5157–5169.

Taton, A., Grubisic, S., Ertz, D., Hodgson, D. A., Piccardi, R., Biondi, N., Tredici, M. R., Mainini, M., Losi, D., Marinelli, F., & Wilmotte, A. (2006b). Polyphasic study of antarctic cyanobacterial strains. *Journal of Phycology*, *42*(6), 1257–1270.

Tavernier, I., Verleyen, E., Hodgson, D. A., Heirman, K., Roberts, S. J., Imura, S., Kudoh, S., Sabbe, K., De Batist, M., & Vyverman, W. (2014). Absence of a medieval climate anomaly, little ice age and twentieth century warming in skarvsnes, lützow holm bay, east antarctica. *Antarctic Science*, *26*(5), 585–598.

Terauds, A., Chown, S. L., Morgan, F., Peat, H. J., Watts, D. J., Keys, H., Convey, P., & Bergstrom, D. M. (2012). Conservation biogeography of the Antarctic. *Diversity and Distributions*, *18*(7), 726–741.

Terauds, A., & Lee, J. R. (2016). Antarctic biogeography revisited: updating the Antarctic Conservation Biogeographic Regions. *Diversity and Distributions*, *22*(8), 836–840.

Theodorakopoulos, N., Bachar, D., Christen, R., Alain, K., & Chapon, V. (2013). Exploration of Deinococcus-Thermus molecular diversity by novel group-specific PCR primers. *MicrobiologyOpen*, *2*(5), 862–872.

Turner, J., & Overland, J. (2009). Contrasting climate change in the two polar regions. *Polar Research*, *28*(2), 146–164.

Tytgat, B., Verleyen, E., Sweetlove, M., D'hondt, S., Clercx, P., Van Ranst, E., Peeters, K., Roberts, S., Namsaraev, Z., Wilmotte, A., Vyverman, W., & Willems, A. (2016). Bacterial community composition in relation to bedrock type and macrobiota in soils from the Sør Rondane Mountains, East Antarctica. *FEMS Microbiology Ecology*, *92*(9), fiw126.

Van der Putten, N., Verbruggen, C., Ochyra, R., Verleyen, E., & Frenot, Y. (2010). Subantarctic flowering plants: pre-glacial survivors or post-glacial immigrants? *Journal of Biogeography*, *37*(3), 582–592.

Verleyen, E., Hodgson, D. A., Gibson, J., Imura, S., Kaup, E., Kudoh, S., De Wever, A., Hoshino, T., McMinn, A., Obbels, D., Roberts, D., Roberts, S., Sabbe, K., Souffreau, C., Tavernier, I., Van Nieuwenhuyze, W., Van Ranst, E., Vindevogel, N., & Vyverman, W. (2012). Chemical limnology in coastal East Antarctic lakes: Monitoring future climate change in centres of endemism and biodiversity. *Antarctic Science*, *24*(1), 23–33.

Verleyen, E., Hodgson, D. A., Sabbe, K., Cremer, H., Emslie, S. D., Gibson, J., Hall, B., Imura, S., Kudoh, S., Marshall, G. J., McMinn, A., Melles, M., Newman, L., Roberts, D., Roberts, S. J., Singh, S. M., Sterken, M., Tavernier, I., Verkulich, S., de Vyver, E. Van, Van Nieuwenhuyze, W., Wagner, B., & Vyverman, W. (2011). Post-glacial regional climate variability along the East Antarctic coastal margin-Evidence from shallow marine and coastal terrestrial records. *Earth-Science Reviews*, *104*(4), 199–212.

Verleyen, E., Sabbe, K., Hodgson, D. A., Grubisic, S., Taton, A., Cousin, S., Wilmotte, A., De Wever, A., Van Der Gucht, K., & Vyverman, W. (2010). Structuring effects of climate-related environmental factors on Antarctic microbial mat communities. *Aquatic Microbial Ecology*, *59*(1), 11–24.

Verleyen, E., Vyverman, W., Sterken, M., Hodgson, D. A., De Wever, A., Juggins, S., Van De Vijver, B., Jones, V. J., Vanormelingen, P., Roberts, D., Flower, R., Kilroy, C., Souffreau, C.,

& Sabbe, K. (2009). The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. *Oikos*, *118*(8), 1239–1249.

Vincent, W. F. (2000). Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarctic Science*, *12*(03), 374–385.

Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D. A., Mann, D. G., Juggins, S., Van De Vijver, B., Jones, V., Flower, R., Roberts, D., Chepurnov, V. A., Kilroy, C., Vanormelingen, P., & De Wever, A. (2007). Historical processes constrain patterns in global diatom diversity. *Ecology*, *88*(8), 1924–1931.

Vyverman, W., Verleyen, E., Wilmotte, A., Hodgson, D. A., Willems, A., Peeters, K., Van de Vijver, B., De Wever, A., Leliaert, F., & Sabbe, K. (2010). Evidence for widespread endemism among Antarctic micro-organisms. *Polar Science*, *4*(2), 103–113.

Walther, G.-R., Post, E., Convey, P., Menzel, A., Parmesank, C., Beebee, T. J. C., Fromentin, J.-M., Hoegh-Guldberg, O., & Bairlein, F. (2002). Ecological responses to recent climate change. *Nature*, *416*(28), 389–395.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*(16), 5261–5267.

Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence classification using exact alignments. *Genome Biology*, 15(3), R46.

Xu, Y., Vick-Majors, T., Morgan-Kiss, R., Priscu, J. C., & Amaral-Zettler, L. (2014). Ciliate diversity, community structure, and Novel Taxa in lakes of the McMurdo Dry Valleys, Antarctica. *Biological Bulletin*, *227*(2), 175–190.

Yergeau, E., Bokhorst, S., Kang, S., Zhou, J., Greer, C. W., Aerts, R., & Kowalchuk, G. a. (2012). Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments. *The ISME Journal*, *6*(3), 692–702.

Zeglin, L. H. (2015). Stream microbial diversity in response to environmental changes: Review and synthesis of existing research. *Frontiers in Microbiology*, *6*, 454.

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614–620.

Zhou, A., Chen, Y. I., Zane, G. M., He, Z., Hemme, C. L., Joachimiak, M. P., Baumohl, J. K., He, Q., Fields, M. W., Arkin, A. P., Wall, J. D., Hazen, T. C., & Zhou, J. (2012). Functional characterization of Crp/Fnr-type global transcriptional regulators in Desulfovibrio vulgaris hildenborough. *Applied and Environmental Microbiology*, *78*(4), 1168–1177.

Zidarova,Ralitsa., Kopalová,Kateřina., Van der Vijver, B. (2016). Diatoms from the Antarctic Region: Maritime Antarctica. In *Iconographia Diatomologica* (Vol. 24, pp. 1–509).

Zwart, G., Huismans, R., Van Agterveld, M. P., Van de Peer, Y., De Rijk, P., Eenhoorn, H., Muyzer, G., Van Hannen, E. J., Gons, H. J., & Laanbroek, H. J. (1998). Divergent members of the bacterial division Verrucomicrobiales in a temperate freshwater lake. *FEMS Microbiology Ecology*, *25*(2), 159–169.

ANNEX 1: COPY OF THE PUBLICATIONS
ANNEX 2: FOLLOW-UP COMMITTEE MEETINGS

The annexes are available on our website:
http://www.belspo.be/belspo/SSD/science/pr_biodiversity_en.stm#CCAMBIO