Microbial communities as indicators of anthropogenic impacts in Antarctic lakes

Thèse

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Résumé

La péninsule Fildes est l'une des régions antarctiques présentant la plus forte intensité d'activité humaine, et elle est également fortement influencée par le réchauffement climatique. La zone libre de glace de la péninsule abrite plusieurs lacs, dont certains servent de sources d'eau pour les stations antarctiques. J'ai d'étudié une série de lacs de la péninsule Fildes en utilisant une approche combinée limnologique et paléolimnologique afin d'évaluer et de reconstituer la structure des communautés microbiennes avant et après l'arrivée humaine en Antarctique et l'intensification des activités. Cette thèse se compose de trois chapitres:

Dans le premier chapitre, j'ai étudié sept lacs autour de la péninsule Fildes en analysant les communautés de phytoplancton et de bactérioplancton lorsque les lacs étaient couverts de glace (printemps) et à la fin de l'été, lorsque les lacs étaient libres de glace. Nous avons constaté que les différences saisonnières étaient les principales causes structurant la diversité microbienne. Ces résultats suggèrent que des périodes plus longues sans glace, telles que prévues, ainsi que la présence de contaminants liés à l'augmentation de l'impact humain dans la région, pourraient affecter les communautés microbiennes et, par conséquent, l'ensemble des systèmes.

Le deuxième chapitre évalue l'effet anthropique dans la péninsule Fildes en examinant les sédiments pré- et post-anthropiques des mêmes lacs, en estimant l'enrichissement en métaux et les changements dans les communautés bactériennes entre les deux périodes. Deux lacs très proches des routes et des infrastructures aéroportuaires ont montré une augmentation des métaux ainsi que de taxons bactériens associés à des environnements pollués. Cette étude confirme que l'ADN bactérien est bien préservé dans les sédiments antarctiques et constitue un excellent proxy sédimentaire pour inférer les changements liés aux impacts anthropiques.

Le troisième chapitre a évalué des profils sédimentaires détaillés des concentrations de métaux, de l'ADN bactérien, de la diversité des diatomées et des tératologies dans deux des lacs proches des infrastructures, ainsi que dans un lac plus éloigné des stations. Les changements observés dans le lac plus distant se sont avérés êtreliés au changement climatique, tandis que nous confirmons l'impact humain dans les autres lacs par l'augmentation des concentrations de métaux, de la diversité des diatomées et des tératologies liées à l'enrichissement en métaux. Cette étude contribue à une meilleure compréhension de l'effet des activités humaines et du changement climatique sur le paysage local de la péninsule Fildes et sur la manière dont les écosystèmes de la région répondront aux changements futurs.

Cette thèse démontre que les communautés microbiennes sont des sentinelles de la réponse adaptative aux changements environnementaux liés à la fois au changement climatique et à l'impact humain local. Elle constitue également une contribution à la prise de conscience de l'impact de l'homme sur l'Antarctique.

Abstract

Fildes Peninsula is one of the Antarctic regions having the highest intensity of human activity, and the region is also greatly influenced by global warming. The ice-free area of the peninsula is the site of numerous lakes, some of which are used as water sources for Antarctic stations. I studied a series of lakes of the Fildes Peninsula using a combined limnological-paleolimnological approach in order to assess and reconstruct microbial community structure before and after the onset of human presence and activity in Antarctica. This thesis consists of three chapters:

In the first chapter, I studied seven lakes around Fildes Peninsula through the analysis of phytoplankton and bacterioplankton communities when the lakes were ice-cover (spring) and before the summer, when the lakes were ice-free. We found that seasonal differences were the main causes structuring microbial diversity. These results suggest that longer ice-free periods such as those predicted for the future, and the presence of contaminants due to the increase in human impact in the area, may affect microbial communities and thus overall system functioning.

The second chapter asses the anthropogenic effect in Fildes Peninsula evaluating the pre- and post-anthropic sediments of the same lakes, by estimating metal enrichment and changes in bacterial communities before and after the establishment of year-round human presence in the area. Two lakes very close to roads and airport infrastructure showed increases of metals as well as bacteria taxa associated with polluted environments. This study confirms that bacterial DNA is well preserved in Antarctic lake sediments and is a good sedimentary proxy for inferring changes related to anthropogenic impacts.

The third chapter evaluated detailed sedimentary profiles of metal concentrations, bacterial DNA and diatom diversity and teratologies in two of the impacted lakes close to logistics and scientific infrastructure, and in one lake more distant to the stations. Diatom and bacteria changes observed in the lake more distant from infrastructure were shown to be related to climate change, while we confirmed human impacts in the other lakes from the observed increase in metal concentrations, changes in diatom taxa and diatom teratologies related to metal enrichment. This study helps to better understand how human activities and climate change have affected the local landscape around Fildes Peninsula and how the region's ecosystems will respond to future changes.

This thesis demonstrates that microbial communities are sentinels of adaptive response to environmental change related both to climate change as well as to local human impacts. Moreover, it contributes to the growing awareness of the impact that human beings are having on sensitive Antarctic ecosystems.

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AP: Antarctic Peninsula
ASV: Amplicon sequence variant
BP: Before the present
CTAB: Hexadecyltrimethylammonium bromide
Chl-*a*: Chlorophyll *a*
Chl-*b*: Chlorophyll *b*
chl-c_1: Chlorophyll c_1
chl-c_2: Chorophyll c_2
CE: Common Era
CRS: Constant rate of supply
db-RDA: Distance-based redundancy analysis
DIC: Differential interference contrast
EDTA: Ethylenediaminetetraacetic acid
HPLC: High performance liquid chromatography
H_2O_2: Hydrogen peroxide
HNO_3: Nitric acid
HCl: Hydrochloric acid
ICP-MS: Inductively coupled plasma-mass spectrometry
LCBD: Local contribution to beta-diversity
MgDVP: Mg-3,8-divinyl-pheoporphyrin a5 monomethyl ester
NGS: Next- generation sequencing
OM: Organic matter
PCA: Principal component analysis
PCR: polymerase chain reaction
PDA: Photo-diode array
PERMANOVA: Permutational multivariate analysis of variance
SEM: Scanning electron microscope
SIMPER: Similarity percentage analysis
TBE: Tris-Borate-EDTA buffer
TBI: Temporal beta diversity index

μ-XRF: Micro-X-ray fluorescence

^{210}Pb: Lead 210

^{209}Po: Polonium 209

^{214}Bi: Bismuth 214

As: Arsenic

Cd: Cadmium

Cr: Chromium

Co: Cobalt

Cu: Copper

Fe: Iron

Mn: Manganese

Ni: Nickel

Pb: Lead

Se: Selenium

Ti: Titanium

Zn: Zinc

Era clara, era vida, de mis manos
Se escurría
Me besaba, me envolvía
Pero siempre agua seguía
Amanecer, desnuda en tu ritual
Y así te encuentro, serena siempre
Era clara, era vida, de mis manos
Se escurría

Extraído de la canción Agua de los Piojos

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Preface

This thesis includes the work carried out as part of my PhD under the supervision of Professor Dermot Antoniades. Three chapters are presented in the form of scientific articles for which I am the first author. The first two chapters have been published in international scientific journals, while the third chapter has been submitted for publication. All three chapters are written in English.

Chapter 1- Seasonal shifts in microbial diversity in the lakes of the Fildes Peninsula, King George Island, Maritime Antarctica

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I participated in the design of this project, sampled the sites in 2017, analyzed the samples and data, and created the figures. I wrote the article as the first author. To include this article in the thesis, the reference format and figure numbering have been modified.

Chapter 2- Sedimentary indicators of anthropogenic impact in Fildes Peninsula lakes (King George Island, Maritime Antarctica).

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I participated in the design of this project, analyzed the samples and data, and created the figures. I wrote the article as the first author. To include this article in the thesis, the figure numbering has been modified.

Chapter 3- Reconstructing the effects of anthropogenic activities and climate change in three lakes of the Fildes Peninsula, Maritime Antarctica

Authors: Florencia Bertoglio, Samuel Yergeau, Claudia Piccini, Santiago Giralt, Roberto Urrutia, Dermot Antoniades

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I participated in the design of this project, analyzed the samples and data, and created the figures. I wrote the chapter in scientific article format as the first author.

Introduction

The limnology of Maritime Antarctica

Most of the Antarctic continent is permanently covered by ice or snow, but there are also ice-free areas located in the Maritime Antarctic and sub-Antarctic regions where many lakes, ponds, and streams characterize the landscape. The Maritime Antarctic region includes the west coast of the Antarctic Peninsula and extends from the South Sandwich Islands through the South Orkney and South Shetland Islands to approximately 72°S along the Antarctic Peninsula (Camacho, 2006; Fig. 1). Freshwater lakes and ponds in Maritime Antarctica include epishelf lakes, deep and shallow inland lakes and ponds lying on rock substrates, as well as coastal lagoons influenced by sea sprays and marine animals (Smith et al., 2006; Camacho, 2006, Toro et al., 2007).

These regions are characterized by higher mean temperatures and precipitation than continental Antarctica, even though average annual temperature is below 0°C, summer temperatures are commonly over the freezing point (Camacho, 2012). This means that the snow and ice cover on lakes melts each summer, when lakes become ice-free until the following winter (Izaguirre et al., 2021). These cycles of freeze-melting along with the variation in solar irradiance caused by the high latitudes, result in large annual variations in levels of photosynthetically available radiation (Vincent et al., 2008). During the winter, the ice cover prevents mixing resulting in thermal isolation of the lake and light penetration (Hawes and Schwarz, 2001), while during the summer increases the input of nutrients from the catchment with consequences for planktonic and benthic microbial populations (Quayle, 2002). These lakes are thus subjected to harsh environmental conditions during most of the year, not only due to variations in light availability and low temperatures but also due to the oligotrophic conditions that characterize most Antarctic lakes. There are exceptions, as the Maritime Antarctic region also hosts meso-eutrophic shallow lakes and hypertrophic ponds influenced mainly by their size and proximity to marine animal colonies (e.g., Izaguirre et al., 1998, 2003).

Antarctic freshwater lakes are characterized by short food webs dominated by the microbial loop (Vincent et al., 2008). However, the lakes of the Maritime Antarctic have slightly more complex food webs often including crustacean zooplankton (Camacho, 2006). There are several studies analyzing the composition of freshwater biological communities from South Georgia, the South Orkney Islands, the northern part of the Antarctic Peninsula (Hope Bay)

Figure 1. Map of the Maritime Antarctic region showing the King George Island (South Shetland Islands). Source: National Geographic.

and the South Shetland Islands (Livingston Island) (Hansson and Tranvik, 1996; Almada et al., 2004; Butler et al., 2005, Camacho et al., 2012). However, few studies have included the study of bacterial communities from these Antarctic aquatic systems (e.g. Picazo et al., 2019; Zhang et al., 2024) although they are essential components of the polar aquatic food web, playing critical roles in nutrient cycling and energy flow.

Lakes from Maritime Antarctica have been affected by some of the most rapid warming on Earth since 1969 (READER, 2025). Warming impacted several aspects, such as extending ice-free periods and increasing nutrient levels and primary production (Quayle, 2002). This exemplifies the sensitive nature of lakes to environmental changes, as they register events related to biological and geochemical processes and human perturbations occurring in their catchments and in the water bodies themselves. Because of this, lakes are considered sentinels of environmental changes and among high-latitude ecosystems, lakes are particularly sensitive to climate change and human perturbation (Mueller et al., 2009; Antoniades et al., 2011).

Human presence in Antarctica

Human activities in Antarctica have progressively increased since its discovery, beginning with hunting from the early 19th until the mid-20th century, when limited numbers of explorers, sealers and whalers arrived seasonally to the Antarctic (Schiffer, 2013). These hunters rapidly depleted certain species and dramatically altered the terrestrial and marine environments adjacent to their stations (e.g., Whaler’s Bay, Deception Island; Deception Island Management Group, 2006). The Antarctic Treaty was signed in 1959, which designated the continent as a region for peaceful and scientific exploration. Due to logistic reasons there is a high concentration of scientific activity in Maritime Antarctica, where the many stations present resulting in an area with amongst the highest anthropogenic influences of the continent. More recently, the intensification of tourism has surpassed scientific presence as the largest annual contributor of visitors to Antarctica (Liggett et al., 2011), a growing industry that was probably not contemplated as such in the treaty.

Although the obligations of countries engaging in Antarctic research are outlined in the Protocol on Environmental Protection to the Antarctic Treaty, which came into effect in 1998, the increase in scientific, tourism and fishing activities in Antarctica inevitably contributes to the introduction of alien species and localized environmental contamination through combustion of fuels, accidental oil spills, wastewater and waste production (e.g., Tin et al., 2009; Fryirs et al., 2015). The anthropogenic impact has been high in areas adjacent to stations because in the past wastes were simply disposed of in landfills, burned in the open air or dumped into the sea (Bargagli and Rota, 2024). As a result, the diversity of biotic communities and the functioning of ecosystems has been affected in some coastal areas (Kennicutt II et al., 2010; Aronson et al., 2011; Fryirs et al., 2015). More recently, evidence of pollutants such as microplastics (Reed et al., 2018), organic pollutants including polycyclic aromatic hydrocarbons (Szopińska et al., 2019), pharmaceutical, recreational and psychotropic drug residues (González-Alonso et al., 2017) and metals (Webb et al., 2020) occur in points close to stations from local deposition as well as from long distance transport.

Despite the existence of abundant evidence for widespread human impacts in terrestrial and marine ecosystems, the focus of research to date has been to examine samples from near Antarctic stations and compare them to non-impacted sites, instead of considering a temporal baseline prior to the intensification of local human activities.

Paleolimnology for the reconstruction of past conditions

Physical, chemical and biological information from a lake's catchment and its water column is chronologically deposited and recorded in sediments of inland waters. These sediments thus archive a record of environmental change as it occurs over time. Paleolimnology is the discipline that uses the information stored in lake sediments to reconstruct the paleoenvironmental conditions of an aquatic system and its catchment by analyzing these indicators in the sediment (Smol, 2008).

Studies focused on the natural development of environments address issues such as global climate change, providing an understanding of natural cycles and information on baseline conditions. These data are needed to discern effects caused by human activity from those that are natural. Establishment of a reliable and accurate chronology is key for all temporal interpretations, and there are a number of dating techniques at their disposal to help resolve depth with respect to time. These techniques are based on the quantification of radioisotopes in the vertical sedimentary profile. Due to the natural disintegration, deepest sediment layers contain lower proportions of a radioisotope. Each date technique is appropriate for a certain age range and sediment type. For events that have occurred within the past 150 years, ^{210}Pb and ^{137}Cs radioactive isotopes are commonly used (Appleby, 2001). Another radioactive isotope, ^{14}C, has been used to date lake sediments up to roughly 50,000 years of age (Björck and Wohlfarth, 2001).

Different variables can be analyzed for environmental reconstructions including physicochemical indicators (e.g., organic matter, granulometry, geochemistry, stable isotopes), and biological indicators (Smol, 2008). The biological indicators need to be readily preserved in sediments and ecologically constrained to specific environmental conditions. Problems certainly do occur with both physical (e.g., breakage) and chemical (e.g., dissolution) degradation under certain conditions, as well as other taphonomic processes. These problems, however, can often be assessed and the quality of the record evaluated. In fact, if the processes responsible for these problems are well understood, paleoenvironmental information can, in some cases, be surmised from the preservation status of indicators (Smol, 2008).

Although some organic compounds are not preserved in the paleolimnological record due to diagenetic processes, there are a large number of biological indicators represented as morphological fossils, such as the hard structures composed of chitin (e.g., insects), biogenic silica (e.g., diatoms and chrysophytes), calcium carbonate (e.g., mollusks), cellulose (e.g., plant macrofossils), or other biopolymers such as sporopollenin (e.g., pollen grains) (Smol, 2008). Biological indicators also include “chemical fossils” derived from the “soft” parts of organisms such as pigments and biomolecular signatures, derived for example from alkenones and DNA fragments preserved in sediment (Smol, 2008).

Since polar lakes are particularly very sensitive to environmental change, their sediments provide detailed records of climate change and its influence on the biosphere, hydrosphere and cryosphere (Vincent et al., 2008). The rapid response of living communities and sedimentology from lake sediments make it possible to infer local and/or regional changes in temperature, precipitation, evaporation, light, ice cover, deglaciation and ice shelf decay (Hodgson et al., 2004). For example, by studying diatom assemblages it is possible to reconstruct climatic variations due to their sensitivity to environmental changes and because they are well preserved in sediments thanks to their silica cell wall (Smol and Stoermer, 2010). Paleolimnological proxies also reveal rates of isostasy and relative sea-level changes, which makes them an unequivocal proof of how changes in the ice sheet mass balance have influenced sea level (Hodgson et al., 2004).

Sedimentary pigments

There are many algal groups that do not leave reliable morphological fossils and so their populations can only be reconstructed using biogeochemical markers such as fossil pigments (Smol, 2008). Fossil pigments preserve a record of past photosynthetic organisms in aquatic ecosystems. They serve as an archive of changes in algal and bacterial biomass, as well as changes in community composition (Leavitt and Hodgson, 2001). Since fossil pigment concentrations are proportional to algae concentrations in the water column at the time of deposition, they can be used as paleoproductivity proxies (Leavitt, 1993), especially the broadly distributed pigments (e.g. chlorophyll *a*, β-carotene, pheophytin *a*). Other pigments that are more taxonomically diagnostic (e.g., different carotenoids: alloxanthin, lutein, echinenone, fucoxanthin, peridinin) can be used to examine changes in the abundance of specific phytoplankton groups (Leavitt and Hodgson, 2001).

Pigments degradation is conditioned by several factors that can act more rapidly in the water column compared with the sediment, such as the presence of oxygen and light (Mcgowan, 2007). However, preservation of pigments in the sediment depends on conditions at the sediment-water interface and once incorporated into the sediment, degradation via oxidation and digestion may be significant (Mcgowan, 2007). Therefore, it is important to consider the importance of such diagenetic process for interpreting record results.

Sedimentary DNA

The use of sedimentary ancient DNA enables reconstructions of the past diversity of ecosystems by indicating the abundances of species from microbes to mammals (Crump, 2021). Given the high abundance of aquatic and sedimentary bacteria, their rapid responses to environmental changes and their variety of ecological requirements for variables such as light, nutrients and oxygen levels, past changes in their populations can be used to infer important limnological conditions that give information about the history of a lake (Smol, 2008; Martínez de la Escalera et al., 2014). This approach is extremely effective, as even low concentrations of DNA can be successfully amplified by primers and polymerase chain reaction (PCR) to facilitate sequencing of a region or regions of a gene, allowing sequencing of diverse populations including taxa present in low proportions (Fouhy et al., 2016). A sequence most employed to identify environmental bacteria is the marker gene encoding for 16S rRNA, which possesses nine hypervariable regions flanked by conservative stretches that enable its amplification (Stackebrandt and Goebel, 1994). The sequencing and analysis of 16S rRNA gene has become the method of choice for nucleic acid-based detection and identification of microorganisms by comparing the sequence data with a reference database to make taxonomic assignments, which enables inferences about organism existing at the time the sediment was deposited as well bacteria currently living in the sediment.

Diatoms

Diatoms are unicellular algae from the class Bacillariophyceae surrounded by cell walls made of silica (known as a frustule). They are widely used in paleolimnology since they are well-preserved in sediments and they possess excellent bioindicator properties (Reid et al., 1995; Smol and Stoermer, 2010). Amongst these properties are that their siliceous cell walls generally fossilize, they have a well-established taxonomic classification and their identification by microscopy often reaches species level. Diatoms are sensitive to changes in water chemistry and have well-defined ecological niches. They have rapid life cycles and rapidly colonize new habitats, and changes in diatom communities thus generally reflect changes in water chemistry.

Diatoms sometimes develop teratological forms responding to environmental stress caused by various conditions. Teratological forms can manifest in different ways, for example deformations can be observed at the level of the striae, the raphe or the contour (Falasco et al., 2009; Lavoie et al., 2017). Causes of teratologies can include organic pollution, heavy metal pollution, lack of light or lack of nutrients (Morin et al., 2014; Pandey et al., 2014; Lavoie et al., 2017; Falasco et al., 2021).

Mineralogy and trace metals

Inorganic elements and metals are important indicators, since they provide insight into the genesis and geological history of sediments. Using high resolution sediment chemistry datasets, it is possible to reconstruct processes at scales from regional to in-lake, such as erosion, primary productivity and volcanism. Trace metals, such as copper and zinc, are micronutrients that are required by biota in small quantities, but which become toxic at higher concentrations. Other metals, including cadmium, chromium, lead, and mercury are toxic even at low concentrations. The natural concentrations of trace metals in aquatic ecosystems derived from rock weathering may vary widely between sites because they are linked to catchment geology and hydrological regimes (Boyle, 2001). Trace metals produced by anthropogenic activities (e.g., mining, traffic, agriculture, industries, wastewater treatment plants, waste landfills) are often transported to lakes through atmospheric deposition, increasing the concentrations of metals in the sediments above the background levels (Hernández-Crespo and Martín, 2015). Determining background metal levels permits the quantification of the anthropogenic contribution to sedimentary metal concentrations through the calculation of enrichment factors (Hilton et al., 1985).

A reliable, fast and non-destructive technique for the measurement of the chemical composition of sediments is x-ray fluorescence (XRF) measured with a core scanner (Croudace et al., 2006; Richter et al., 2006). This technique can simultaneously produce measurements of a suite of elemental activities at extremely high resolution. Additionally, metal concentrations in sediment can be reliably quantified by inductively coupled plasma mass spectrometry (ICP-MS). This is a very sensitive technique which poses detection limits that are lower than typical sediment abundances level and renders the ability to perform isotopic analysis (Kuznetsova et al., 2019).

Studies in polar aquatic ecosystems have demonstrated the effectiveness of paleolimnological methods for the reconstruction of local human impacts by both recent (Douglas and Smol, 2000; Antoniades et al., 2011; Michelutti et al., 2007) and pre-industrial societies (Douglas et al., 2004; Michelutti et al., 2013). In addition, landscape evolution in Maritime Antarctica has been reconstructed by diatom and mineralogical analysis indicating proglacial lake formation during the Holocene (Schmidt et al., 1990; Lee et al., 2009), as well as the reconstruction of climate fluctuations studying bacteria diversity by ancient DNA (García-Rodríguez et al., 2021; Piccini et al., 2024).

Specific objectives of the thesis chapters

The general objective of this thesis was to assess and reconstruct the microbial community structure in lakes from Fildes Peninsula before and after human presence in the region, and thereby to infer any environmental changes that may have resulted from human activity. The general hypothesis was that the local anthropogenic impact in Fildes Peninsula will be preserved in the sedimentary record of the lakes and the degree of contamination due to the stations can therefore be reconstructed using paleolimnological techniques. The thesis is divided into three chapters written in the format of scientific articles.

Chapter 1: “Seasonal shifts in microbial diversity in the lakes of the Fildes Peninsula, King George Island, Maritime Antarctica”. The general objective was to examine phytoplankton and bacterioplankton communities of seven lakes from the Fildes Peninsula and explore compositional differences both amongst lakes and between seasons (spring and autumn). In this study water samples were taken from seven lakes in Fildes Peninsula and their microbial diversity was compared between the ice-covered season and the period after the summer when lakes were ice free.

Chapter 2: “Sedimentary indicators of anthropogenic impact in Fildes Peninsula lakes (King George Island, Maritime Antarctica)”. The general objective was to determine the extent of anthropogenic impact on aquatic ecosystems through the assessment of microbial community structure and metal enrichment from seven lakes on Fildes Peninsula. A top-bottom approach was applied to sediment cores from seven lakes in Fildes Peninsula, assessing changes in pre- and post-impact conditions.

Chapter 3: “Reconstructing the effects of anthropogenic activities and climate change in three lakes of the Fildes Peninsula, Maritime Antarctica” The general objective was to develop a comprehensive picture of the effects of human activity in lakes from Fildes Peninsula, evaluating detailed sediment profiles of metal concentrations, bacterial DNA and diatom diversity and teratologies.

Study region

This study was caried out in lakes from the Fildes Peninsula (King George Island, South Shetland Islands; 62° 11' S, 58° 58' W) in Antarctica, which is an approximately 38 km^2 ice-free area at the south-western extreme of King George Island (Fig. 1). As is typical in such ice-free Maritime Antarctic areas, many lakes are present, most of which are of glacial origin having formed following the Late Holocene retreat of the Collins Glacier (to the northeast of Fildes Peninsula). Here, we studied seven lakes. Five of them are closely linked with scientific or logistics stations and they are, or have been, used as freshwater supplies. Two lakes are located further away from infrastructure (~3 km) and were included in the study as sites with the lowest probability of impact. Most of the lakes are shallow, with maximum depths ranging between 3 to 11 m, except for Uruguay Lake that has a maximum depth of 15 m. The lakes remain ice free from the beginning of summer (end of December) until the end of autumn (early April) when they begin to refreeze.

At the beginning of this thesis, many of these lakes were still unstudied and basic limnological parameters were unknown. Several lakes surrounding the airport and the many stations near Fildes Bay have, however, been sampled (Montecino et al., 1991; Préndez et al., 1996; Zhao and Xu, 2000; Préndez and Carrasco, 2003). These studies found the lakes to be circumneutral, with samples taken in 1998 having pH ~6.0, while those from a 1995 study reported weakly alkaline pH of ~7.2-7.9 (Préndez et al., 1996; Préndez and Carrasco, 2003). The lakes sampled were relatively diluted (specific conductivity <500 $\mu S\ cm^{-1}$) and their ionic and trace metal concentrations showed significant interannual variation with high values in certain lakes, such as a small lake near the airport (Hotel Lake) (Préndez et al., 1996; Préndez and Carrasco, 2003). This lake was once used as the potable water supply for the airport, despite elevated concentrations found in 1995 that exceeded Chilean norms for the concentrations of As, Bi, Cd, Cr, Fe and Se (Préndez et al., 1996). Samples from later years found much lower concentrations (Préndez et al., 1999; Préndez and Carrasco, 2003). Hotel Lake was partially drained and refilled in 2011 in an effort to improve its water quality; however, its water is still unsuitable as drinking water (Peter et al., 2013). The precise cause of the spatial and temporal variation in metal concentrations was unclear.

Paleolimnological records of sediment metal concentrations could provide an effective test of the hypothesis that high concentrations are due to human activities by determining whether current levels exceed those prior to the establishment of stations on the Fildes Peninsula, as well as by testing for correlations between trace element concentrations and microbial dynamics.

Chapter 1 Seasonal shifts in microbial diversity in the lakes of the Fildes Peninsula, King George Island, Maritime Antarctica

1.1 Résumé

La péninsule Fildes, sur l'île King George, est fortement influencée par le réchauffement climatique. Nous avons étudié sept lacs pour analyser les variations saisonnières (printemps vs automne) et les différences entre lacs dans les communautés de phytoplancton et de bactérioplancton. Des mesures environnementales, des analyses de pigments (par HPLC) et du séquençage de l'ADN bactérien (gène 16S rRNA) ont été effectués. La saisonnalité a été le principal facteur structurant ces communautés. Les Chlorophyceae dominaient dans tous les lacs, en toutes saisons. Des bactéries identifiées comme indicatrices du printemps (*Flavobacterium, Polaromonas*, Oxalobacteraceae) ont été observées sous la glace lacustre, tandis que Frankiales et Verrucomicrobia étaient associés aux eaux ouvertes de l'automne. Les espèces printaniers sont typiques de conditions oligotrophes, et celles de l'automne sont souvent associées aux sols. Les différences entre lacs étaient plus marquées au printemps. Cette étude constitue une base pour évaluer les futurs changements microbiens aquatiques en Antarctique.

1.2 Abstract

Fildes Peninsula, on King George Island, has been greatly influenced by recent rapid climate warming. Lakes are a pervasive feature of Fildes Peninsula landscapes, some of which are used as water sources for Antarctic stations. We studied seven Fildes Peninsula lakes to explore differences among lakes and between seasons in phytoplankton and bacterioplankton communities. We measured environmental variables, analyzed pigments by high performance liquid chromatography and examined bacterial DNA by high-throughput sequencing of the 16S rRNA gene. The main driver structuring microbial communities was the season (i.e., spring vs. autumn). Chlorophyceae were the dominant phytoplankton group in all lakes and both seasons. Indicator bacteria for each season were identified, including *Flavobacterium*, *Polaromonas* and Oxalobacteraceae as indicators of spring conditions under thick ice, whereas Frankiales and Verrucomicrobia were indicator species of autumn following the ice-free summer. The indicator species for spring are generally observed in oligotrophic conditions, whereas many of the autumn indicators are commonly found in soils. There were lesser between-lake differences in microbial communities in autumn, at the end of the open-water period, than in spring at the end of the ice-covered period. This study will act as the basis for future assessments of changes in aquatic microbial communities.

1.3 Introduction

The Antarctic Peninsula (AP) region has been subjected to some of the most rapid climate warming on Earth during the 20th and 21st centuries, with an increase of more than five times the global mean (0.6 ± 0.2 °C during the 20th century) (Turner et al., 2013). As part of the Maritime Antarctic, the AP region differs from the rest of the continent in many aspects including its climate, which is characterized by higher mean temperature and precipitation (Convey et al., 2014). Numerous ice-free areas with terrestrial habitats are distributed along the coastal fringes of the AP and surrounding islands, as well as in small areas protruding the ice on the AP plateau (Oliva et al., 2017). Ongoing climate change in the region has occurred simultaneously with the intensification of human activities due to increases in scientific research and tourism that impose physical, chemical and biological burdens on the local environment (Schiffer, 2013).

Lakes and ponds are common features of the ice-free areas of Maritime Antarctica. Although these lakes are not subject to the same harsh environments as those in continental Antarctica, they experience pronounced annual cycles of solar irradiance and temperature caused by their high latitudes, and are affected by ice and snow cover during much of the year (Quayle et al., 2003). The less extreme conditions from this region result in lakes that currently lose their ice covers completely each austral summer when they display a primary production peak, and they remain ice-free typically from the end of December to March before refreezing in April (Izaguirre et al., 2021). During these ice-free periods, lakes interact with the atmosphere and receive runoff from local snowmelt and surrounding streams, as well as from permafrost thaw and precipitation (Vincent et al., 2008). These lakes are dominated by the microbial loop, including viruses, bacteria, phytoplankton and protozoa, generally represented by a few species of pigmented zooplankton (Vincent et al., 2008).

Much of our understanding of annual cycles in Maritime Antarctic lakes was developed from studies on Signy Island (South Orkney Islands), which showed marked seasonal and interannual variations in planktonic population dynamics (Quayle et al., 2003). Limnological research is often focused near research stations, with well-studied lake clusters present near Hope Bay and Cierva Point on the Antarctic Peninsula (Izaguirre, 2003; Schiaffino et al., 2009; Allende and Mataloni 2013), Byers Peninsula on Livingston Island (Toro et al., 2007), James Ross Island (Roman et al., 2019) and Potter and Fildes peninsulas on King George Island (Vinocur and Unrein, 2000; Schiaffino et al., 2009; Zhang et al., 2022), where authors have studied phytoplankton, zooplankton and bacterioplankton dynamics. Rochera et al. (2017) recorded major changes in plankton communities on Byers Peninsula (Livingston Island) during the transition to the ice-free period when light availability and allochthonous nutrient fluxes were high. Studies from Byers Peninsula also examined bacterioplankton dynamics using next- generation sequencing (NGS), revealing the dominance of globally distributed freshwater bacterial classes, but also the presence of endemic clades (Picazo et al., 2019). However, bacterioplankton diversity studies employing this approach, which enables the detection of taxa with very low abundances, are not yet common in lakes of the Antarctic Peninsula region.

In this study, we focused on seven lakes on the Fildes Peninsula, an area of King George Island (South Shetland Islands). King George Island is among the areas of Antarctica most impacted by humans due the presence of 12 research stations constructed since 1968. Concern has been raised about environmental contamination near the stations, and studies have observed locally elevated concentrations of heavy metals, due to transportation and oil pollution, as well as polycyclic aromatic hydrocarbons (PAHs) (Chu et al., 2019; Choi et al., 2022). The limited existing limnological research from Fildes Peninsula has focused largely on Kitiesh Lake due in part to its importance as a water supply (e.g., Montecino et al., 1991), or surveys of basic limnological parameters (e.g., Préndez and Carrasco, 2003; Shevnina and Kourzeneva, 2017), although one recent study applied next-generation sequencing to the analysis of microeukaryote communities in five Fildes Peninsula lakes (Zhang et al., 2022). Finally, the only study of bacterioplankton diversity examined three ponds and one lake from Fildes Peninsula during five consecutive summers employing a bacterial culture-dependent approach (Morel et al., 2015).

The aim of our study was to examine phytoplankton and bacterioplankton communities of seven lakes from the Fildes Peninsula and explore compositional differences both among lakes and between seasons (spring and autumn). We address the following research questions:

- Do microbial communities change more between lakes than between seasons?
- How do communities of microorganisms respond to the melting of the ice cover during the summer?

1.4 Materials and Methods

1.4.1 Study region

At 38 km^2, the Fildes Peninsula (62° 11' S, 58° 58' W) is the largest ice-free area of King George Island, in the South Shetland Islands of the Maritime Antarctic region (Fig. 1.1a). The peninsula is the site of numerous lakes and is also among the Antarctic regions with the highest intensity of human activity, including six permanent stations. The Fildes Peninsula also serves as the entry point and logistical hub for the South Shetland Islands, being the site of an airport and a harbor master station; most visitors to these islands and to the Antarctic Peninsula therefore pass through the Fildes Peninsula.

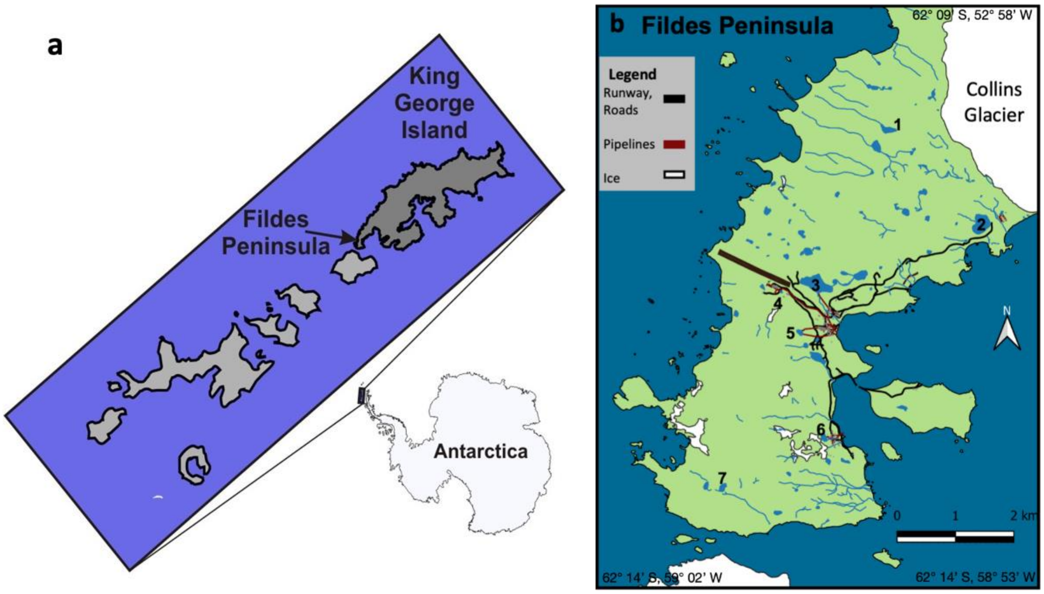

Figure 1.1. Location of the study region. a. King George Island and Fildes Peninsula. b. Map of Fildes Peninsula indicating the study lakes: 1= Mondsee, 2 = Uruguay, 3 = Kitiesh, 4 = Hotel, 5 = Las Estrellas, 6 = Xihu, 7 = Jurasico. Maps were created with geospatial data from the Scientific Committee on Antarctic Research (SCAR) Antarctic Digital Database, accessed 2021.

This study focuses on seven lakes from around the Fildes Peninsula (Fig. 1.1b, Table 1.1). Five of them are or have been used as water supplies for the different stations, and so are located adjacent to infrastructure and associated human activities (Fig. 1.1b). These include Uruguay Lake, which supplies Artigas Station; Kitiesh Lake, which is the source of potable water for the Escudero, Frei and Bellingshausen stations (Chile and Russia); Hotel Lake, which is adjacent to the Teniente Marsh Airport and formerly its water supply, and which has ceased to be used for drinking water due to elevated levels of heavy metals (Peter et al. 2013); Las Estrellas Lake, which previously supplied Escudero Station (Chile); and Xihu Lake, the source of drinking water for Great Wall Station (China). Two lakes more distant from stations were also studied: Mondsee Lake, 3.5 km from Frei Station and 2.6 km from Artigas Station; and Jurasico Lake, located 2.1 km from Great Wall Station and 3.3 km from Escudero Station. The lakes are of glacial origin, having formed following the retreat of the Collins Glacier, which sits at the northeast extreme of the peninsula (Fig. 1.1b). They occur in catchments ranging from 1.71 to 59.71 ha, mostly occupy shallow basins, with depths ranging from 3 to 11 m, except for Uruguay Lake that has a maximum depth of 15 m (Table 1.1). The degree to which anthropogenic activities may have affected these lakes is unclear, as they are largely unstudied and for some even basic limnological parameters are still unknown.

1.4.2 Sampling

Two sampling seasons were covered: austral spring 2017 (from 23 November-17 December), while the lakes were still completely frozen over with thick ice cover (0.80 – 1.60 m; Table 1.1); and autumn 2018 (from 17-23 April), after the austral summer, when lakes were either ice-free or had an ice cover up to 55 cm thick (Table 1.1). Profiles of temperature, pH, specific conductivity and dissolved oxygen in the water column were measured using a YSI 600 QS probe. Dissolved oxygen data in autumn were not included because the probe membrane was damaged in the field.

Water samples for DNA and pigment extractions were collected from the surface at the deepest site in each lake. When lakes were ice-covered, holes were drilled in the ice with a manual auger to collect water samples immediately below the ice layer. Water samples were taken with a 6.2 L Kemmerer sampler, transferred to acid-washed plastic containers, and transported in the dark to the laboratory at Artigas Station. At the lab, the phytoplankton fraction was captured by filtering between 215 and 1000 ml of water per lake through 0.7 µm GF/F glass microfiber filters in duplicate or triplicate depending on the filtered volume. Between 250 and 1000 ml of water (in duplicate or triplicate) were filtered through 0.2 µm nitrate cellulose filters in order to capture the bacterial fraction. Filters were immediately frozen and were transported in a cooler withice packs from King George Island to Uruguay (DNA filters) and Quebec (pigment filters) where they were stored in the dark at -80 °C until analysis. Samples remained frozen at all times. The volumes of water filtered for pigment

Table 1.1. Characteristics and variables measured in the surface water in the study lakes. NA: not available, ND: not detectable. Autumn ice thickness values are approximate. Differences in the means of the temperature, pH and specific conductivity between seasons were not significant ($p = 0.48$, $p = 0.22$ and $p = 0.56$). The difference in the mean of the total chl-*a* between seasons was significant ($p = 0.02$).

Lake	Latitude Longitude	Max. depth (m)	Surface area (ha)	Catchment area (ha)	Season	Temp (°C)	pH	Conductivity (µS cm^{-1})	Dissolved O_2 (%)	Ice thickness (m)	Snow thickness (m)	Total chl-*a* (µg L^{-1})	Total reads
Mondsee	62°10'21.50"S, 58°56'43.26"W	7.0	1.90	10.50	Spring	0.24	6.75	228	67.3	1.06	0.12	1.09	59877
					Autumn	0.64	7.69	172	NA	0.06	0.00	1.03	40944
Jurasico	62°13'00.0"S, 59°00'00.0"W	4.9	1.07	7.45	Spring	0.47	6.90	130	67	0.80	ND	0.24	37134
					Autumn	0.57	7.84	182	NA	0.50	0.00	0.58	49801
Uruguay	62°11'07.4"S, 58°54'39.6"W	15	7.03	18.66	Spring	0.43	7.30	108	55.9	0.96	0.02	0.36	52993
					Autumn	0.27	7.60	136	NA	0.00	0.00	0.66	43957
Kitiesh	62°11' 36.9" S, 58°58' 0.2" W	11	9.43	59.71	Spring	0.28	7.63	156	67.8	0.80	0.05	ND	58380
					Autumn	0.11	7.40	176	NA	0.20	0.00	1.28	35708
Hotel	62°11' 40.4" S, 58°58' 42.2" W	5.0	0.11	1.71	Spring	0.34	6.70	551	23.4	1.60	0.50	0.11	66507
					Autumn	0.52	7.46	461	NA	0.55	0.00	12.76	62617
Las Estrellas	62°12'2.5"S, 58°58'19.4"W	5.6	0.66	4.05	Spring	0.10	7.30	80.0	68.6	0.90	0.05	0.20	43515
					Autumn	0.64	8.15	167	NA	0.20	0.00	0.75	20254
Xihu	62°13'00.80"S, 58°57'56.41"W	10	0.98	39.32	Spring	0.13	7.94	300	69.4	0.95	0.05	0.20	47081
					Autumn	0.29	8.06	173	NA	0.20	0.00	0.82	53928

and DNA extractions are shown in Table A.1 (see below).

1.4.3 Pigment biomass and diversity analyses

We assessed phytoplankton diversity through the analysis of photosynthetic pigments analyzed by high performance liquid chromatography (HPLC), which enables the separation and quantification of individual chlorophylls and carotenoids (Wright and Jeffrey, 2006). Broadly distributed pigments, such as chlorophyll *a* (chl-*a*), serve as indicators of phytoplankton biomass, while other taxonomically diagnostic pigments may be used to examine community composition at the class level (Wright and Jeffrey, 2006). In 2019, pigments were extracted at Université Laval from filters by sonication in 2.5 ml of 95% methanol and incubated under argon gas in darkness, in an ice bath, for 30 min. The extracts were then cleared by centrifugation at 4 °C, filtered through 0.2 µm polytetrafluoroethylene filters into HPLC vials, and placed under argon gas. Shortly following extraction, samples were injected into a Thermo Fisher Accela 600 HPLC equipped with an autosampler, a Hypersil Gold C8 column (3 µm pore size), a photo-diode array (PDA) and a fluorescence detector, using the reverse-phase solvent protocol of Zapata et al. (2000). HPLC peaks were detected by diode-array spectroscopy (350-750 nm) set to a slit width of 1 nm, and absorbance chromatograms were obtained at 450 nm. Chlorophylls were also detected by fluorescence (excitation, 440nm; emission, 650 nm). Pigments were identified and quantified from retention times and absorbance chromatograms using reference standards from Sigma Inc. (St. Louis, MO, USA) and DHI Water and Environment (Hørsholm, Denmark); concentrations of unidentified carotenoids were calculated using the calibration coefficient for β,β-carotene. Derivative products of chl-*a*, including chlorophyllide *a*, pheophorbide *a* and pheophytin a (Table A.2), were measured in the analysis and total chl-*a* was defined as the sum of chl-*a* and chl-*a* derivatives.

Marker pigments were classified into nine major phylogenetic groups at the following division levels: Chlorophyceae, Prasinophyceae, Euglenophyta, Bacillariophyceae, Prymnesiophyceae, Chrysophyceae, Dinophyta, Cryptophyta and Cyanobacteria. Since certain groups share marker pigments, those of the Chlorophyceae, Prasinophyceae and Euglenophyta (MgDVP, 9-cis-neoxanthin, violaxanthin, antheraxanthin, lutein and chlorophyll *b*) were combined, as well as those of the groups Bacillariophyceae, Prymnesiophyceae and Chrysophyceae (chlorophyll c_1, fucoxanthin, 19-hex-fucoxanthin and diadinoxanthin). The pigments dinoxanthin and peridinin were employed as indicators of the Dinophyta, alloxanthin for Cryptophyta, and zeaxanthin for Cyanobacteria (Wright and Jeffrey, 2006). In addition, we found several unidentified carotenoids that were not considered in the statistical analyses; their absorption maxima and retention times can be found in Table A.3. No pigment data is available from Kitiesh Lake during spring, as logistic difficulties prevented sampling the lake. Also, no carotenoid concentrations are available from Hotel Lake in spring, as all carotenoids were below detection limits; however, our more sensitive fluorescence detector enabled the quantification of chl-*a* at that time.

1.4.4 Bacterioplankton composition analyses

Bacterioplankton were examined through high-throughput sequencing of the V4 hypervariable region of the 16S gene, which enables sensitive and accurate molecular detection of bacterial diversity (Picazo et al., 2019). In 2020, at the IIBCE laboratory in Uruguay, DNA was extracted from cellulose filters based on physical disruption of the cells and nucleic acid purification. Filters were cut into small pieces and placed in microcentrifuge tubes containing ceramic beads and an extraction buffer described Martínez de la Escalera et al., (2014) and homogenized with a FastPrep homogenizer. After centrifugation at 12000 g supernatants were subjected 3 times to chloroform:isoamyl alcohol (24:1) extractions and the pellets were precipitated with 0.6 volumes of cold isopropanol at room temperature during 24 h. These precipitates were then subjected to centrifugation for 40 min at 12000 g at room temperature, and the DNA obtained in the pellets was washed with cold ethanol, dried and suspended in water overnight at 4 °C. The concentration and purity of DNA was determined spectrophotometrically at 260 and 280 nm, and its quality was checked by the amplification of a variable region (V4) of the ribosomal 16S gene. PCR products were verified by gel electrophoresis on 1% agarose gel in 0.5X TBE buffer. DNA samples were sent to the University of Minnesota Genomics Center for illumina MiSeq paired-end sequencing of the V4 region, using the primers 515F and 806R (Caporaso et al., 2011).

To correct for sequencing errors and create ASVs (Amplicon Sequence Variants), reads were processed in R using the DADA2 pipeline following a modified version of the DADA2 Bioconductor workflow (Callahan et al., 2017). Briefly, reads were filtered and trimmed by the *filterAndTrim* function based on the quality score which estimates the error probability of the DNA sequence. Reads with a maximum expected error (maxEE) greater than 2 were removed and, based on quality profiles, reads were truncated at 200 and 150 bp for forward and reverse reads, respectively. Sequence variations were inferred with the *learnerrors* and *dada* functions. Chimeric sequences were eliminated, and taxonomy assignments from Kingdom to Genus were performed with *assignTaxonomy* based on the SILVA database (v138) (Quast et al., 2012). ASV sequences assigned as Archaea, Chloroplasts and Mitochondria were removed. Sequences obtained were submitted to the nucleotide archive GenBank with the project id PRJNA848662.

1.4.5 Data analysis

Microbial alpha diversity was calculated and compared between seasons using different indices: richness (S), the Shannon-Weaver index (H), and the Simpson dominance (1-D) and evenness (H/log(S)) indexes, with the function *diversity()* of the R package *vegan* (Oksanen et al., 2020). These indices were calculated for taxonomic pigments and for the ASV composition table. We tested differences in the means of the measured indices as well as the means of the measured environmental variables and in chl-*a* concentrations for each season by Kruskal-Wallis tests. Phytoplankton groups were defined by their taxonomic pigments and their proportions were also compared between seasons and by Kruskal-Wallis tests. In addition to the calculation of alpha diversity indices, alpha diversity for bacterioplankton was analyzed by rarefaction curves of the ASV abundances using the *rarecurve()* function in the R package *vegan* (Oksanen et al., 2020).

As initial bacterioplankton diversity ordinations by db-RDA (distance-based redundancy analysis) indicated that the major similarities were between seasons and not between individual sites across different seasons (see below), we applied the indicator value method (Dufrêne and Legendre, 1997) to identify bioindicator bacterioplankton for a particular season (group). ‘Good’ indicator species are those found only or mostly in a single group of sites (high specificity) as well as those being present at most of the sites belonging to that group (high fidelity). The indicator value (IndVal) of a species is expressed as the degree (%) to which it fulfills the criteria of specificity and fidelity within any group of samples. Finally, permutation tests were used to assess the significance of individual indicator species and the p-values were corrected for multiple testing using Holm’s correction (McGeoch and Chown, 1998). IndVal was calculated by the function *multipatt()* from the R package *indicpecies* (De Cáceres et al., 2010).

Beta diversity was calculated as the variation in the community composition among lakes for each season (spatial beta diversity). The estimation of spatial beta diversity was based on the total variance of the data matrix separately for each season examined for taxonomic pigments and ASVs (Legendre and De Cáceres, 2013), as calculated with Hellinger-transformed data using the *beta.div()* function of the R package *adespatial* (Dray et al., 2022). This function also computed the local contribution to beta diversity (LCBD index) for each sample, and a permutational p-value indicating the significance of each LCBD value. LCBD is calculated as the sum of the variance for each site with respect to the total variance, assessing the individual contribution of each lake to the total spatial beta diversity in the system. The p-values were then corrected for multiple testing using Holm’s correction.

To relate the structure of the microbial community to lake characteristics, db-RDA was employed using the taxonomic pigment concentration matrix as the response variables for phytoplankton pigments and the ASV table for bacteria, with the environmental variable matrix as the explanatory dataset in both cases. The environmental variables considered were temperature, specific conductivity, pH and the ice layer thickness of each lake for both seasons. Dissolved oxygen was not considered in the analysis because it could not be quantified in autumn (Table 1.1). The significance of each db-RDA and each environmental variable was examined, as was the adjusted R^2. db-RDA was performed with the function *dbrda()* in *vegan* (Oksanen et al., 2020) using the percentage difference dissimilarity distances of the raw response matrices.

To explore the association of unknown carotenoids (Table A.3) with taxonomic phytoplankton pigments, we used Spearman correlations to test their relationships with taxonomic marker pigments, and report p-values as well as p-values adjusted for multiple tests using Holm’s correction.

1.5 Results

1.5.1 Environmental characterization

There were differences in the assessed environmental variables (temperature, specific conductivity and pH) between the two sampling seasons and between lakes, however these differences were not significant ($p > 0.05$; Table 1.1, Fig. A.1). The profiles of environmental variables in water column showed only slight variations in most of the lakes and in both seasons, apart from specific conductivity, which increased gradually with depth in spring

(Fig. A.1). Water temperature at the surface ranged from 0.10 to 0.47 °C in spring and between 0.11 and 0.64 °C in autumn. Lakes were circumneutral to weakly alkaline, with pH overall ranging from 6.75 to 8.15 (spring average: 7.22; autumn average: 7.74) (Table 1.1). The pH of two lakes did not change between seasons (lakes Uruguay and Kitiesh), while five had moderate pH increases (average: +0.63 pH) (Table 1.1). The four lakes with the lowest specific conductivities in spring increased somewhat in autumn (spring average: 118 µS cm^{-1}; average increase: 46 µS cm^{-1}), while those with the three highest spring values decreased in autumn (spring average: 360 µS cm^{-1}; average decline: 91 µS cm^{-1}; Table 1.1). The highest specific conductivity was found in Hotel Lake in both seasons, with values of 551 µS cm^{-1} in spring and 461 µS cm^{-1} in autumn (Table 1.1).

1.5.2 Pigment biomass and diversity

The lakes were generally ultraoligotrophic, with chl-*a* concentrations indicating very low phytoplankton biomass (Table 1.1 and A.2). Median total chl-*a* across all lakes was 0.22 µg L^{-1} in spring (min. 0.11, max 1.09) and 0.82 µg L^{-1} in autumn (min. 0.58, max. 12.76), with extreme values found in Hotel Lake both in spring and in autumn (0.11 and 12.76 µg L^{-1} respectively) (Table 1.1). While most lakes had higher chl-*a* concentrations in autumn than spring, Lake Mondsee did not differ appreciably between seasons (1.09 vs. 1.03 µg L^{-1}; Table 1.1).

With the exception of richness, the average indices of all phytoplankton taxonomic pigments decreased in autumn relative to spring, although the differences between seasons were not significant ($p > 0.05$; Fig. 1.2). Chlorophyceae, Prasinophyceae and Euglenophyta pigments dominated in both seasons and in most lakes, representing between 41 and 87% of total marker pigments (Fig. 1.3, Tables A.2 and A.4). Chl-*b* and lutein, mainly derived from Chlorophyceae, were the dominant pigments (Tables A.2 and A.4), and they were more abundant in autumn compared to spring, while in spring the proportion of pigments from Dinophyta and Cyanobacteria was significantly higher ($p < 0.05$), and those from Bacillariophyceae, Prymnesiophyceae, Chrysophyceae and Cryptophyta were also higher but not significantly ($p > 0.05$) (Fig. 1.3). This was accentuated in lakes Las Estrellas, Jurasico and Uruguay due the proportion of pigments from the Bacillariophyceae, Prymnesiophyceae and Chrysophyceae, which reached 52 and 32% in lakes Jurasico and Uruguay, respectively, in spring, while in Las Estrellas Lake, pigments from the Dinophyta represented 26% of total marker pigments in spring (Fig. 1.4). Cyanobacteria, for which zeaxanthin was the only marker pigment identified, represented only a small proportion of pigments in all lakes (between 2 and 7%) (Figs. 1.3 and 1.4).

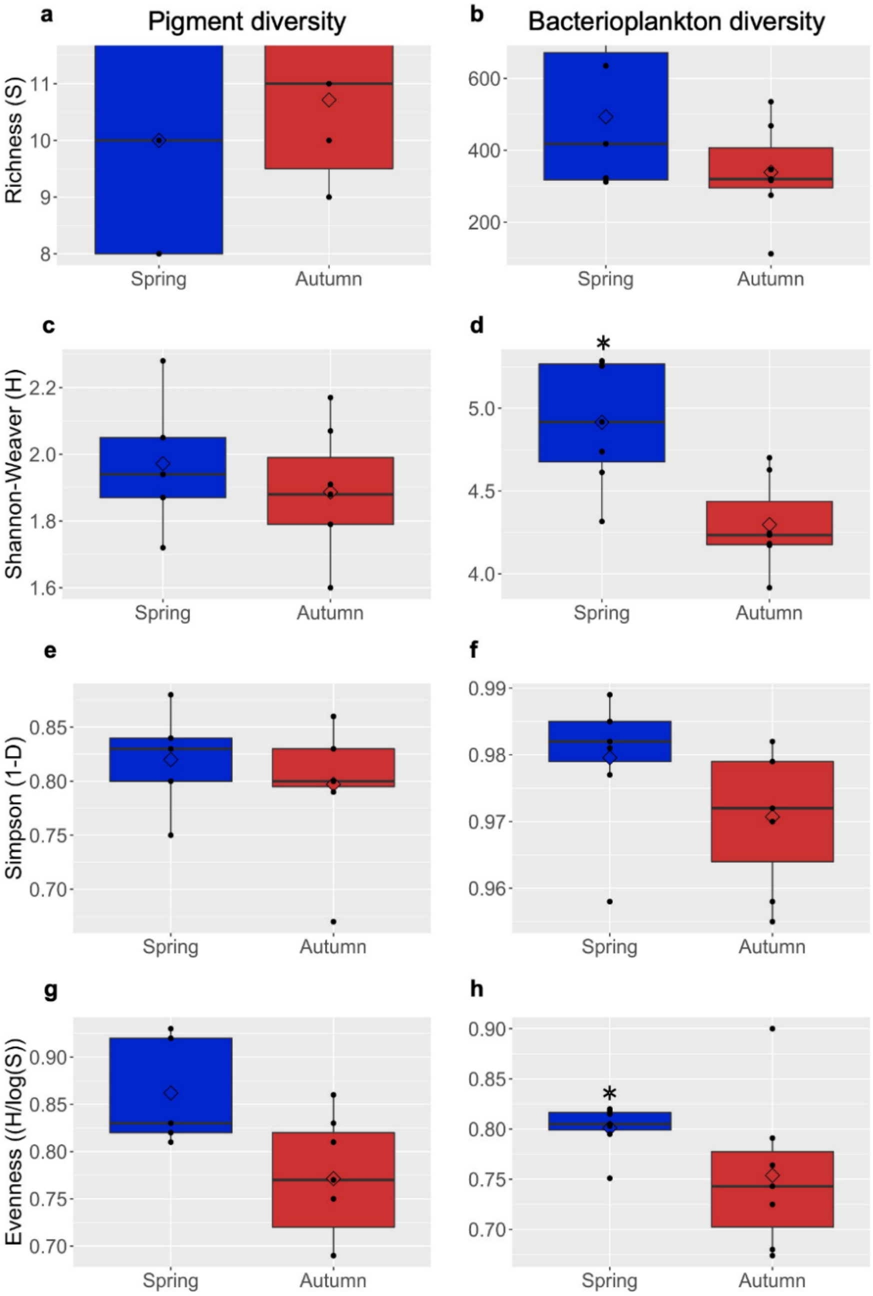

Figure 1.2. Alpha diversity for pigments and bacterioplankton measured as ASVs for the two seasons. a. Pigment richness, b. ASV richness, c. Shannon-Weaver diversity index for pigments, d. Shannon-Weaver diversity index for ASVs, e. Simpson's Index of diversity for pigments, f. Simpson's Index of diversity for ASVs, g. Evenness index for pigments, h. Evenness index for ASVs. Horizontal lines inside the boxplots are median values, boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. Asterisks indicate means with significant differences between seasons ($p < 0.05$).

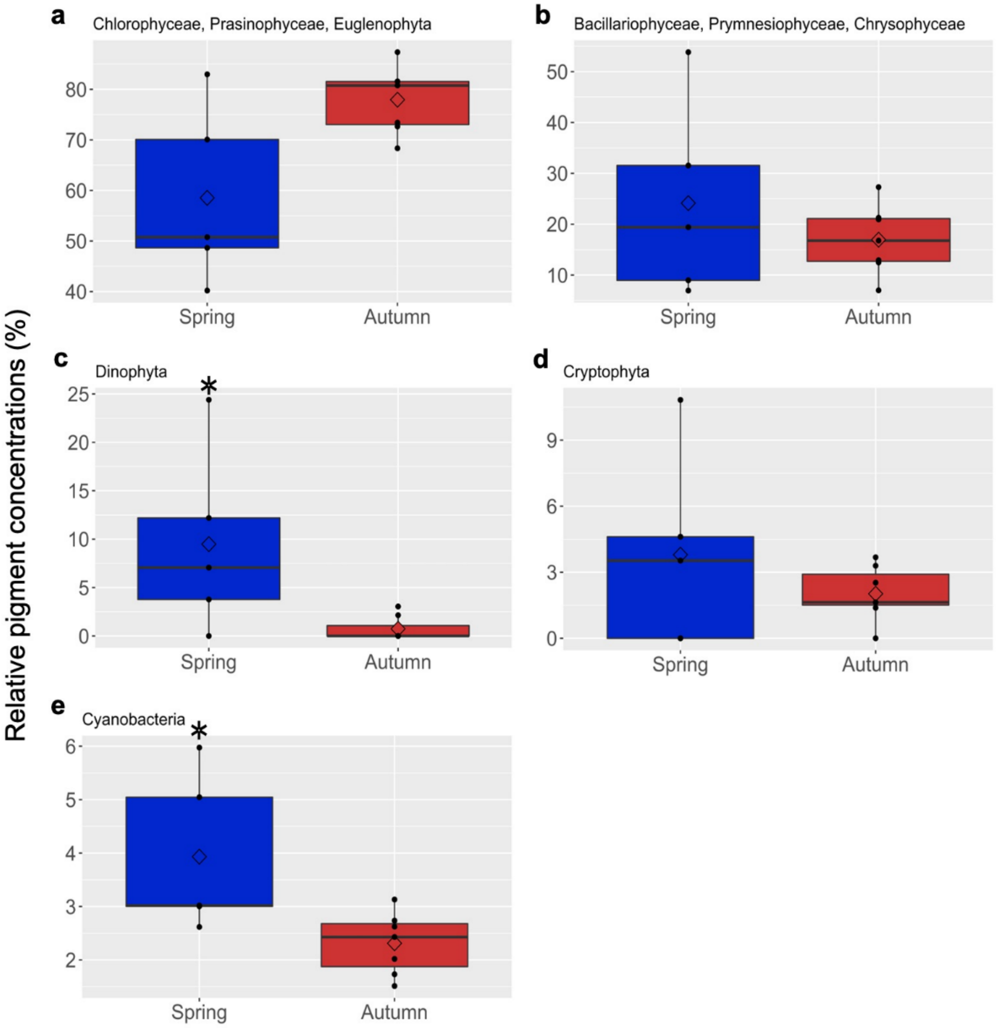

Figure 1.3. Percentages of phytoplankton groups (according to taxonomic pigments) between seasons. Groups were defined according to the combination of the following pigments: a. MgDVP (Mg-3,8-divinyl-pheoporphyrin a_5 monomethyl ester), 9-cis-neoxanthin, violaxanthin, antheraxanthin, lutein and chlorophyll *b* for Chlorophyceae, Prasinophyceae and Euglenophyta; b. Chlorophyll c_1, fucoxanthin, 19-hex-fucoxanthin and diadinoxanthin for Bacillariophyceae, Prymnesiophyceae and Chrysophyceae; c. Dinoxanthin and peridinin for Dinophyta; d. Alloxanthin for Cryptophyta; e. Zeaxanthin for Cyanobacteria. Horizontal lines inside the boxplots are median values, boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. Asterisks indicate means with significant differences between seasons ($p < 0.05$).

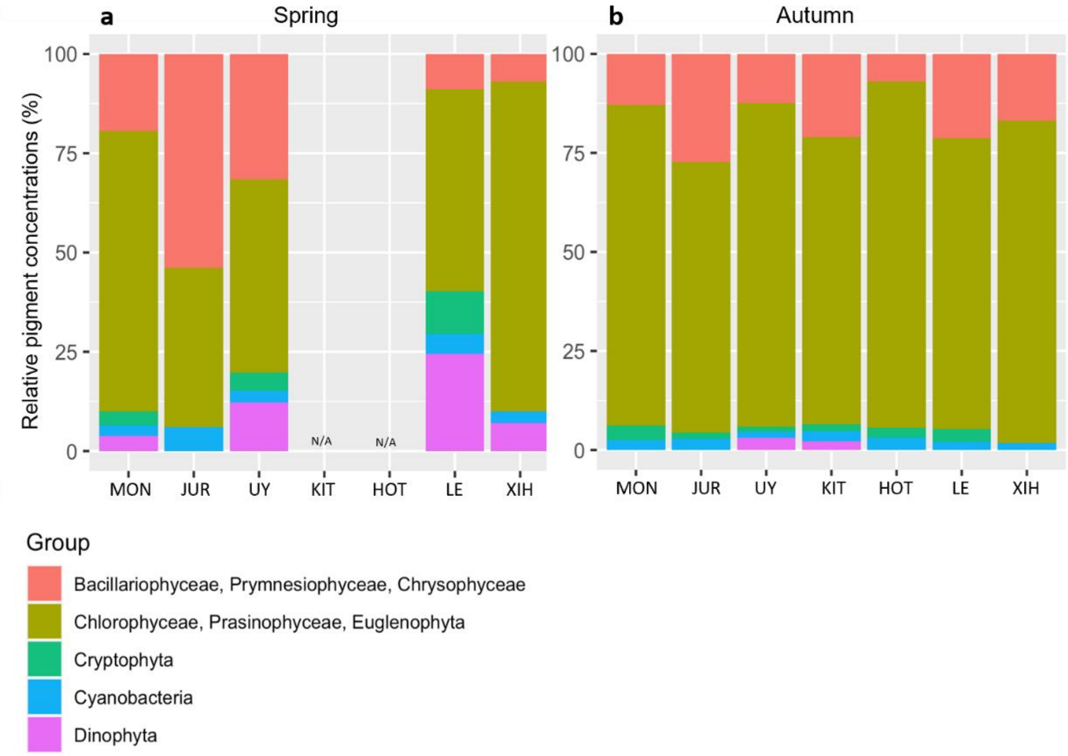

Figure 1.4. Phytoplankton groups by lake between seasons. a. Spring. b. Autumn. N/A: not available. Lake codes: Mondsee: MON, Jurasico: JUR, Uruguay: UY, Kitiesh: KIT, Hotel: HOT, Las Estrellas: LE, Xihu: XIH.

The spatial beta diversity for phytoplankton taxonomic pigments varied slightly between seasons, with a higher value in spring when all lakes were ice-covered (0.21) that decreased in autumn (0.10). In spring, Las Estrellas Lake had significantly higher LCBD (local contribution to beta diversity) relative to the other lakes ($p < 0.05$), while Jurasico Lake also had a high LCBD but the difference was not significant after Holm correction ($p > 0.05$) (Table A.5). In autumn, Hotel Lake had the highest LCBD but the difference with the other lakes was not significant after Holm correction, if only marginally ($p = 0.08$) (Table A.5).

The db-RDA for phytoplankton taxonomic pigments explained 48.51% of the variance, of which 26.36% was explained by the first axis and 15.85% by the second axis (Fig. 1.5a). The overall permutation test of the analysis was significant ($p < 0.05$) and the adjusted R^2 was 0.19. Specific conductivity was the only environmental variable with a significant contribution to the model ($p < 0.05$) and ice thickness was marginally non-significant ($p = 0.08$). Specific conductivity was related with the first db-RDA axis, largely controlled by the influence of Hotel Lake in autumn (Fig. 1.5a). Ice thickness and temperature had the largest influence on the dispersion of the sites along the second db-RDA axis (Fig. 1.5a). Finally, pH had a very weak relationship with both axes (Fig. 1.5a).

The results of the multiple Spearman correlations performed between the unknown carotenoids and the taxonomic pigments are shown in Table A.6. From the eleven unknown carotenoids found (Table A.3), three of them had high correlation coefficients (0.93, 0.66 and 0.54) with the pigment peridinin, a further unknown carotenoid with the pigments diadinoxanthin, antheraxanthin and alloxanthin (0.66, 0.63 and 0.67 respectively), another with chl-c_1 (0.74) and a last one with violaxanthin (0.58). All these correlations were significant ($p < 0.05$), however, after the multiple testing Holm correction only one correlation was significant (the one between one unknown pigment and peridinin). In addition, some of the different unknown pigments were highly and significantly correlated with each other even after Holm corrections ($p < 0.05$) (Table A.7).

1.5.3 Bacterioplankton diversity

A total of 672606 reads were obtained, representing 3349 ASVs. Rarefaction curves based on the observed ASVs (richness) reached plateaus (Fig. A.2), indicating that sequencing depth was sufficient to capture the overall diversity of all lakes. Bacterioplankton alpha diversity was higher in spring (when all lakes were ice-covered) than in autumn (Fig. 1.2). Shannon-Weaver diversity and evenness were significantly higher in spring relative to autumn ($p < 0.05$), while between-season differences were not significant for richness and Simpson diversity (Fig. 1.2).

Total bacterioplankton beta diversity was higher than that observed for phytoplankton pigments and was higher in spring than in autumn (0.54 and 0.37, respectively). The largest contributions to this beta diversity were from lakes Hotel and Las Estrellas, which showed significantly higher LCBDs in spring ($p < 0.05$) (Table A.5). Hotel Lake also had significantly higher LCBD in autumn than in spring ($p < 0.05$). The phyla Bacteroidota (26.24%), Proteobacteria (25.68%), Actinobacteriota (25.27%), Verrucomicrobiota (14.32%) and Planctomycetota (3.12%) had the highest total relative abundances across all samples. The classes Bacteroidia, Gammaproteobacteria (including the former Betaproteobacteria class now within the Gammaproteobacteria in SILVA), Actinobacteria and Verrucomicrobiota were the most abundant members from these phyla respectively.

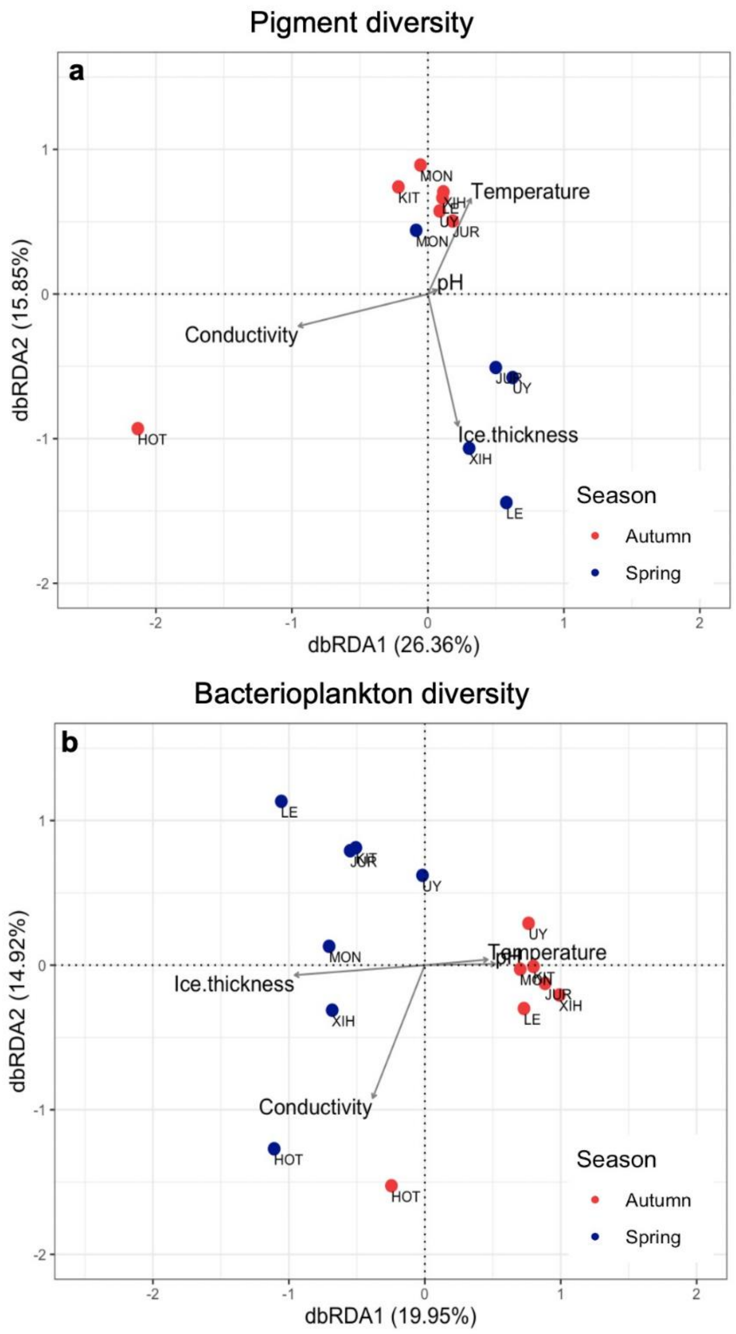

Figure 1.5. Distance-based redundancy analysis (db-RDA). a. db-RDA for pigments, p value = 0.040, $R^2 = 0.19$. b. db-RDA for bacterioplankton, p value = 0.003, $R^2 = 0.17$. Lake codes: Mondsee: MON, Jurasico: JUR, Uruguay: UY, Kitiesh: KIT, Hotel: HOT, Las Estrellas: LE, Xihu: XIH.

The db-RDA model for bacterioplankton explained 42.57% of the variance, with the first and second axes explaining 19.95 and 14.92%, respectively. The overall permutation test of the analysis was significant ($p < 0.05$) and the adjusted R^2 was 0.17. Similar to phytoplankton pigments, the samples were separated according to season along the first axis, but with significant dispersion during spring along the second axis (Fig. 1.5b). The variables that contributed significantly to the model were ice thickness and specific conductivity ($p < 0.05$). The first db-RDA axis was controlled by ice thickness, which had a high influence on the dispersion of spring samples, as well as by temperature and pH which appeared to influence the dispersion of autumn samples. Finally, specific conductivity was the variable most strongly related with the second db-RDA axis, largely reflecting the higher values in Hotel Lake (Fig. 1.5b).

Bacterioplankton indicator species analysis selected 42 indicator ASVs in spring, with the prominence of the classes Gammaproteobacteria (14 ASVs), Alphaproteobacteria (7 ASVs) (both phylum Proteobacteria), and Bacteroidia (9 ASVs) (phylum Bacteroidota) (Fig. 1.6a and Table A.8). These indicator taxa included the genera *Rhodoferax*, *Aquaspirillum arcticum* group and *Polaromonas,* all from the order Burkholderiales of the Gammaproteobacteria, the genus *Flavobacterium* from the order Flavobacteriales, the genus *Algoriphagus* from the order Cytophagales and the order Chitinophagales, all from the Bacteroidia, and members from the class Alphaproteobacteria including the orders Rhodobacterales (genus *Pseudorhodobacter*), Sphingomonadales (genus *Polymorphobacter*), Reyranellales (genus *Reyranella*) and Acetobacterales (genus *Rhodovastum*) (Fig. 1.6a and Table A.8).

For autumn, 17 indicator ASVs were identified, represented by the classes Bacteroidia (8 ASVs), Verrucomicrobiae (5 ASVs) and Actinobacteria (4 ASVs), from the phylum Bacteroidota, Verrucomicrobiota and Actinobacteriota, respectively (Fig. 1.6b and Table A.9). Although members of the class Bacteroidia were also identified as indicator species for spring, different taxa were selected in autumn, including the orders Cytophagales (genus *Pseudarcicella*), Chitinophagales (genus *Sediminibacterium*) and Sphingobacteriales (Fig. 1.6b). Indicators of autumn from the class Actinobacteria were from the orders Micrococcales and Frankiales (Table A.9). Finally, most of the autumn indicator taxa from the class Verrucomicrobiae were not assigned to any order (Fig. 1.6b and Table A.9).

While the composition of the bacterioplankton was mainly structured according to season, the presence of certain groups was notable in some individual lakes, in particular lakes Hotel and Las Estrellas, as reflected in the db-RDA and LCBD values (Fig. 1.5b and Table A.5). For example, methane oxidizing bacteria (genus *Methylobacter* from the family

Methylomonadaceae) and sulfur bacteria (sulfate-reducing forms from the families Geobacteraceae, Desulfurivibrionaceae and Desulfocapsaceae; and sulfur oxidizers from the families Rhodobacteraceae, Rhodocyclaceae and Sulfurimonadaceae), were relatively more abundant in Hotel Lake in spring. These groups were observed at low relative abundances, with proportions of 0.05% for *Methylobacter*, 0.01% and 0.20% for sulfate-reducing and sulfur-oxidizing bacteria, respectively. Moreover, the greater variance in community composition observed in Las Estrellas lake in spring (Fig. 1.5b and Table A.5) was due to the presence of some bacteria families with relative abundances between 0.002 and 0.040%, including Acidobacteriaceae, Blastocatellaceae, Chthoniobacteraceae, Frankiaceae and Xanthobacteraceae.

1.6 Discussion

Chl-*a* concentrations in the Fildes Peninsula lakes here studied were comparable to most oligotrophic lakes from Maritime and continental Antarctica (typically <3 µg L^{-1}), with the exception of autumn concentrations in Hotel Lake (12.76 µg L^{-1}). Conversely, eutrophic lakes described in the region are generally close to the sea and are enriched by excreta from birds and marine mammals, with concentrations in extreme cases exceeding 100 µg L^{-1} chl-*a* (Izaguirre et al., 1998). The higher chl-*a* concentrations found in autumn relative to spring (Table 1.1) indicate higher phytoplankton biomass. However, we note that chl-*a* concentrations in Mondsee Lake did not vary between seasons (Table 1.1), and that it was the only lake whose autumn phytoplankton community clustered together with the spring samples from the other lakes (Fig. 1.5a). Its microclimate is likely strongly influenced by the adjacent Collins Glacier (Fig. 1.1b), which may also dampen the amplitude of seasonal phytoplankton shifts. The extremely low chl-*a* concentration found in spring for Hotel Lake (0.11 µg L^{-1}) was unexpected given that the lake water during spring was visibly green on the lake's snow and ice. Despite this, in spring carotenoids were below the detection limit of our HPLC. This may be related to clogging of the filter with particulate matter (only 250 ml were filtered before the filter became clogged; Table A.1), that may have resulted in an underestimation of chl-*a*. While the volume of water filtered in Hotel Lake in autumn was also low, and even lower (215 ml) than that filtered in spring (Table A.1), it was enough for the detection of high concentrations of pigments.

Phytoplankton such as small flagellates from the Cryptophyceae, Chrysophyceae and Dinophyta are dominant in most Antarctic lakes, both in the Antarctic Peninsula when they are ice-covered (Izaguirre et al., 1998) as well as perennially ice-covered lakes (Lizotte and Priscu, 1998). Their dominance under ice is attributed to the presence of shade-adapted species capable of free movement in water columns where wind-induced mixing is absent (Priddle et al., 1986). However, we found Chlorophyceae, Prasinophyceae and Euglenophyta, and particularly the Chlorophyceae, to be dominant during both seasons, although Chrysophyceae, Cryptophyta and Dinophyta were more abundant in spring (Figs. 1.3 and 1.4), when lakes were still ice-covered.

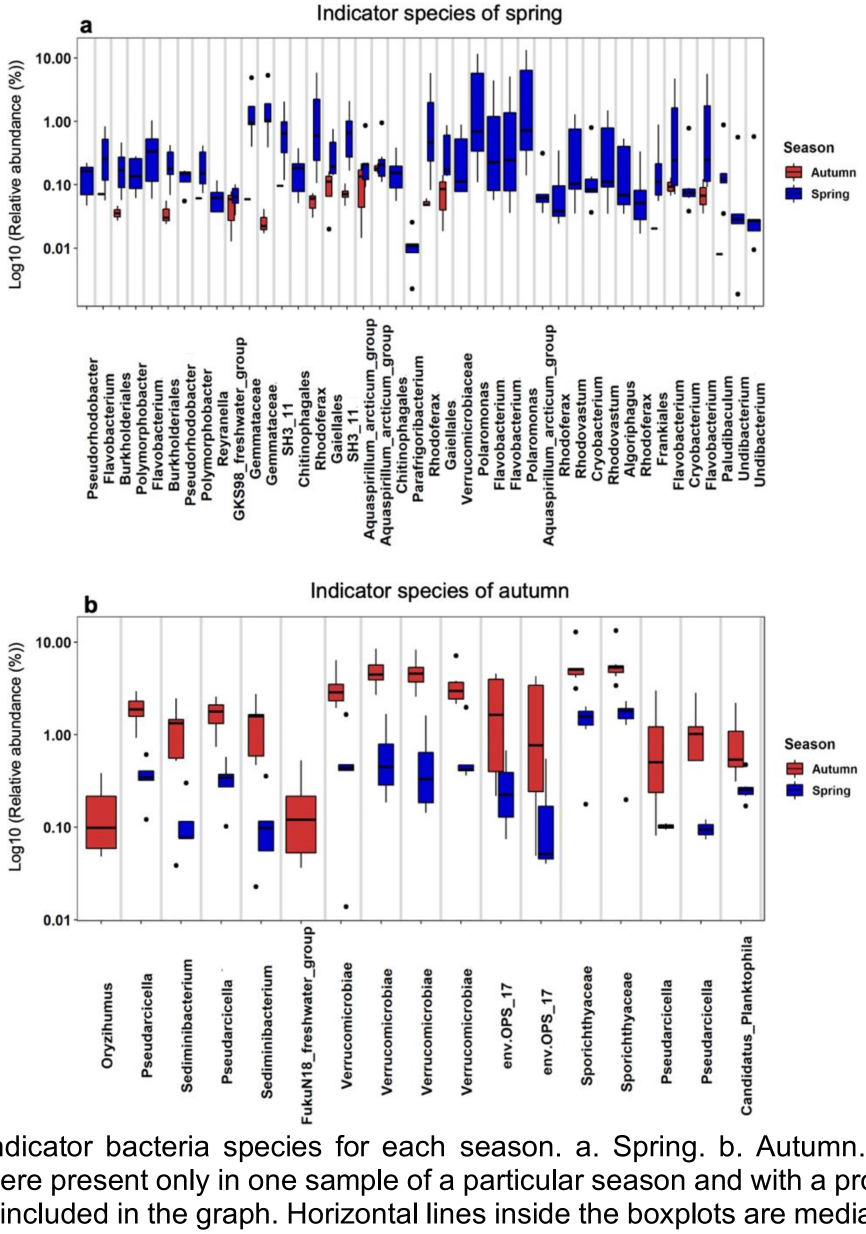

Figure 1.6. Indicator bacteria species for each season. a. Spring. b. Autumn. Indicator species that were present only in one sample of a particular season and with a proportion ≤ 0.09 were not included in the graph. Horizontal lines inside the boxplots are median values, boundaries indicate the 25th and 75th percentiles. Vertical lines above and below indicate the 10th and 90th percentiles. The name of each ASV is according to the highest taxonomic rank identified.

Chlorophytes have also been shown to dominate in Antarctic lakes but usually under relatively higher nutrient concentrations, such as those found in summer when lakes are ice-free (Izaguirre et al., 1998; Rochera et al., 2013). Nevertheless, a recent study performed in Fildes Peninsula during the ice-free period found Chrysophyta to be dominant, while Chlorophyta were the second most abundant group (Zhang et al., 2022). Zhang et al. (2022) is the first study in the region using next-generation sequencing (NGS) for phytoplankton analysis and differences in the methods employed could be a key factor explaining differences in the results. We recognize that the chemotaxonomic approach we employed by analyzing pigments does not enable high resolution determinations of phytoplankton taxa. HPLC analysis nonetheless enabled the detection of different phytoplankton pigment assemblages between seasons, similar to that observed for bacterioplankton diversity. The more notable differences in alpha diversity between seasons for bacterioplankton compared to phytoplankton (Fig. 1.2) may be due to a higher taxonomic resolution achieved for detecting bacterial diversity compared with HPLC, and also because different phytoplankton groups share the same pigments.

The unidentified carotenoids in our dataset are likely degradation products of several marker carotenoids, as suggested by their high correlations. During degradation, structural alterations of pigment molecules result in modified retention times and absorption spectra that therefore did not match those of our pigment standards. Additionally, these unidentified carotenoids were strongly related to peridinin, violaxanthin and diadinoxanthin, all highly labile pigments prone to rapid degradation in the water column (Wright and Jeffrey, 2006).

Our analysis based on ASVs shows the dominance of phyla previously reported from high latitude systems in studies employing NGS, for example in ice-free lakes from the Maritime Antarctic region (Byers Peninsula) (Picazo et al., 2019), ice-covered Laurentian Great Lakes (North America) (Beall et al., 2016) and the Canadian High Arctic (Marois et al., 2022). Also, some of the most representative phyla in our lakes were reported to be abundant in marine and freshwater systems, including in the Antarctic, such as members of Proteobacteria and Bacteroidota (Rochera and Camacho, 2019). Their dominance in oligotrophic lakes may be related to their high biogeochemical and physiological diversity that allows for adaptation to nutrient scarcity, among other stressors (Newton et al., 2011).

The identification of indicator bacterioplankton species by the IndVal approach suggests that there are unique portions of the microbial community in each season, likely related to confined niches under selection pressure. Both groups of indicator species showed high gene sequence similarity with relatives found in GenBank from environments similar to those of the Fildes Peninsula, including microbial mats from King George Island, Antarctic and Arctic soil and sediment, glacier ice, cryoconite holes, permafrost, lakes from Patagonia, Antarctic lichens, and lakes from high latitudes (Table A.8 and A.9). Indicator species from the phylum Proteobacteria were exclusive to spring samples, which could be related to more oligotrophic conditions reflecting winter conditions. On the other hand, indicator species of Bacteroidota were present in both seasons, but taxa from the genus *Flavobacterium* were indicators only in spring (Tables A.8 and A.9). *Flavobacterium* and *Polaromonas* (order Burkholderiales), both indicator species of spring, appear to be common in nutrient-limited Antarctic lakes, as reported in lakes from Byers Peninsula (Livingston Island, South Shetland Islands) (Rochera and Camacho, 2019). Also, the fact that *Polaromonas* has been reported as a dominant bacterium in lake ice (Veillette et al., 2011), may suggest that this bacterium indicates ice presence. There were other indicator species of spring from the family Oxalobacteraceae (also from the order Burkholderiales; Table A.8), which contains a mix of psychrophilic species and genera adapted to oligotrophic conditions (Baldani et al., 2014). By comparison, Actinobacteria are commonly found in soils and their presence in lakes is attributed primarily to run-off and aeolian deposition (Beall et al., 2016). The identification of members of the soil Actinobacteria Frankiales as indicator species of autumn and not of spring (Table A.8 and A.9), may be because in spring there has been neither runoff for approximately eight months nor delivery of aeolian particles, and soils have been snow covered. By contrast, in autumn there has been runoff and aeolian delivery to the lakes for at least three months, with exposed soils, and their identification as autumn indicators may therefore indicate that Actinobacteria are being deposited allochthonously from soils. Verrucomicrobiota was also well represented in our lakes and the indicator species of Verrucomicrobiota were exclusively from autumn (Table A.9). Verrucomicrobia is another important representative of soil communities, but it is also present in aquatic environments where it metabolizes diverse polysaccharides (Martinez-Garcia et al., 2012). Finally, the unassigned members of Verrucomicrobiae (Table A.9), suggest yet undescribed taxa that could imply bacterial groups with new adaptations, potentially related with the hydrolysis of compounds which could have entered lakes during the summer when they were ice-free.

The microbial community (both phytoplankton and bacterioplankton) in Hotel Lake differed from the rest of the lakes (Fig. 1.5 and Table A.5). This lake was characterized by greater specific conductivity, which showed a significant relationship with its microbial community (Fig. 1.5) and its chl-*a* concentration in autumn was extremely elevated compared to the rest of the lakes (Table 1.1). This lake has been the focus of particular attention due to evidence of high concentrations of heavy metals that caused it to be abandoned as a drinking water source (Peter et al., 2013). Transportation is among the human activities that has most strongly altered natural environments in the Fildes Peninsula (Peter et al., 2013), and the lake is situated adjacent to the airport as well as the peninsula's most traveled road. The only group of Methanotrophs we identified was classified as Methylobacter (family Methylomonadaceae) and was almost exclusively found in this lake during spring. While this bacterium is aerobic, a previous study suggested that these methanotrophs are microaerophilic, and they were found throughout the water column under ice cover in a dimictic Arctic Lake (Schütte et al., 2016). The sequences that we identified from this group have high gene similarities with relatives from potentially anoxic environments, including sediments, tailings pond water, a stratified freshwater lake and a methane seep (GeneBank Accession numbers MT067475, OK135604, AB753945 and MN602493). The sulfur reducing and oxidizing bacteria found in Hotel Lake indicate that they might be important for the sulfur cycle during spring. Sulfate-reducing bacteria reduce sulfate to sulfide by using a variety of electron donors, including H_2, fatty acid, alcohols, metals and aromatic compounds such as those found in organic contaminants (e.g., petroleum compounds) (George et al., 2011). Also, because most of these bacteria are obligate anaerobes, such as members of the families Geobacteraceae and Desulfurivibrionaceae (Kuever 2014), they may indicate anoxia under spring ice, which is further supported by the presence of *Methylobacter*.

Several factors may be hypothesized to explain the fact that Hotel Lake was distinguished from the other lakes in terms of phytoplankton, bacterioplankton and the environmental variables that we measured. This lake is considerably smaller than the others, had the thickest ice cover in both spring and autumn, and had the highest specific conductivity and lowest dissolved oxygen among all lakes. The lake is also adjacent to the airport and its access road, and was previously noted to be strongly contaminated with heavy metals (Peter et al. 2013). As such, this lake merits further investigation to discern the precise causes of its disparate microbial assemblages.

The decrease in the variance between the microbial community composition of the lakes in autumn (Fig. 1.5 and Table A.5) indicated that communities were more similar at the end of the summer open-water period, whereas in spring, following the prolonged, dark winter period, lakes differed more. We hypothesize that in spring, the thick ice cover that isolated the lakes from the atmosphere for several months may have engendered variable environments conditioned by lake-specific factors. In autumn, by contrast, the more homogenous microbial communities may have resulted from summer conditions that resulted in free exchange with the atmosphere and more similar irradiance regimes between ice-free lakes.

1.7 Conclusions

This study explored the diversity of microbial communities in two seasons in lakes strongly affected by global warming and located near an area with high human impacts. This is the first study in lakes from the Fildes Peninsula employing HPLC for phytoplankton analysis and NGS for the analysis of bacterial diversity, including under-ice communities.

We found that lakes from Fildes Peninsula showed less variation between each other than between seasons, and that the main seasonal differences from spring to autumn were decreased bacterioplankton richness and evenness, increased phytoplankton biomass and changes in the community composition of both communities. Lakes were more similar in autumn, at the end of the summer open water, while in spring, at the end of the winter period lakes differed more.

These results have important consequences given that changes in bacterial communities imply changes in ecosystem function. Moreover, longer ice-free periods such as those predicted due to future warming, in conjunction with the presence of pollutants because of the increases in human activities in the area, will generate further microbial changes that we cannot yet predict. The matching responses of phytoplankton and bacterioplankton highlighted the importance of both communities as bioindicators that reflect environmental changes. These aquatic ecosystems and their microbial assemblages are thus sentinels of adaptive responses to environmental change in this rapidly warming area.

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Chapter 2 Sedimentary indicators of anthropogenic impact in Fildes Peninsula lakes (King George Island, Maritime Antarctica)

2.1 Résumé

La péninsule Fildes est l'une des zones antarctiques les plus soumises à l'activité humaine et est également fortement influencée par le réchauffement climatique. Nous avons étudié sept lacs pour évaluer les effets anthropiques sur leurs processus écologiques en analysant le métabarcoding 16S rRNA, les pigments photosynthétiques et les métaux dans les sédiments d'avant et d'après l'établissement des bases en utilisant une approche haut-bas. Des différences spécifiques aux lacs ont été observées dans les communautés bactériennes entre les deux périodes. Dans deux lacs proches des routes et de l'aéroport, nous avons trouvé des bactéries indicatrices de milieux pollués, associées à une augmentation des métaux, suggérant des impacts récents liés aux dérivés du pétrole. Bien que l'ADN bactérien soit bien préservé, des processus diagénétiques pourraient avoir affecté les pigments. Nos résultats indiquent que l'ADN bactérien peut servir de proxy sédimentaire pour reconstituer et évaluer les impacts anthropiques dans les lacs antarctiques.

2.2 Abstract

Fildes Peninsula, on King George Island, is among the Antarctic sites with the most intense human activity and is located in a region strongly influenced by global warming. While alterations to its once pristine environments have been noted, there is a lack of data concerning the region's natural state before the increased human presence (~1968). We studied seven lakes from Fildes Peninsula to assess anthropogenic effects on their ecological processes by studying pre- and post-anthropic sediments with a top-bottom approach. We examined differences in bacterial and phytoplankton communities using 16S rRNA metabarcoding, HPLC (high performance liquid chromatography) and analysis of sediment metals. We observed lake-specific differences in bacterial communities between pre- and post-anthropic samples. Using indicator species analysis, we identified bacteria associated with polluted environments (e.g., KD4–96, Bacteroidetes vadinHA17*,* Hungateiclostridiaceae and *Leptolinea*) in post-anthropic sediments from two lakes that showed notable increases of metals. As both lakes are very close to roads and airport infrastructure, these associations may imply the greater recent presence of compounds including petroleum derivatives. Results indicated good preservation of bacterial DNA, but also that diagenetic processes may have affected pigment concentrations. Our data suggest that bacterial DNA may be used as a sedimentary proxy to reconstruct environmental changes including anthropogenic impacts in Antarctic lakes.

2.3 Introduction

Since the discovery of Antarctica in the early 19th century, the number of humans arriving to the continent has progressively increased. Scientific pursuits have involved the establishment of stations that permitted year-round human presence, and a long-term increasing trend implies that over 100,000 tourists now visit per year (Schiffer, 2013; IAATO, 2024). The construction of the Chilean airport in 1980 on King George Island (South Shetland archipelago), together with the proximity of this island to the South American continent, make it the major logistical hub for this area to both scientific research and tourism (Braun et al., 2014; Braun at al., 2020). As a result, the impacts on the natural environment have grown, altering terrestrial and aquatic environments adjacent to areas of high human traffic (Martins et al., 2010, Choi et al., 2022;). Fildes Peninsula (King George Island) has become one of the Antarctic regions with the highest intensity of human activity since Bellingshausen Station (Russia) was built in 1968, with twelve stations on the island and six on Fildes Peninsula alone. Consequently, alterations to the environment have been observed, for example due to transportation and oil pollution (Braun et al., 2012). The increased human presence on King George Island has occurred within the context of global climate warming, which is amplified in high-latitude regions (Meredith et al., 2019). In Maritime Antarctica, warming during the past 50 years has affected all aspects of the cryosphere, including the collapse of ice shelves (Seroussi et al., 2020), the retreat of glacier fronts (Potocka et al., 2019) and alterations to lakes including lake expansions at the glacier fronts (Rosa et al., 2022), decreases in the duration of ice cover and increases in nutrient concentrations and production (Camacho et al., 2012; Izaguirre et al., 2021).

Although increased anthropogenic impacts are well recognized in many parts of the Antarctic Peninsula, there is a paucity of historical data concerning the region's natural state, and the pre-impact conditions of most Antarctic ecosystems are poorly constrained. One of the most important drawbacks when assessing the impact of anthropogenic processes on ecosystems is the difficulty of determining their status prior to the intensification of local human activities. Paleoenvironmental reconstructions, which provide an understanding of natural cycles and baseline conditions and thus allow the discrimination of effects caused by human activity from natural processes, are a powerful tool to overcome this limitation.

Different sedimentary proxies can be analyzed to reconstruct past conditions. Diatoms are frequently used as bioindicators because they can be identified to the species level and their environmental preferences are generally well defined (Smol and Stoermer, 2010). The potential of diatoms as indicators of changing environmental conditions in Fildes Peninsula is well established, as they have been used to reconstruct events such as the beginning of deglaciation ~8000 before the present (BP), with lakes forming between 6000 and 4000 years BP (Camacho et al., 2012; Izaguirre et al., 2021). Other sedimentary proxies include pigments and biomolecular signatures derived from organisms that are not themselves preserved in sediments (Hodgson and Smol, 2008). Fossil pigments have been used to reconstruct phytoplankton dynamics related to numerous natural and anthropogenic processes, including climate change, UV effects and eutrophication (Leavitt and Hodgson, 2001; Leavitt et al., 2003) and they have recorded shifts induced by anthropogenic and other environmental factors in high-latitude freshwater photosynthetic communities (Antoniades et al., 2011; Squier et al., 2002). The analysis of sedimentary DNA represents an important source of information about past organismal diversity (Coolen et al., 2004; Anderson-Carpenter et al., 2011), allowing the detection of changes in microbial communities and in multicellular organisms (Pedersen et al., 2015). Bacteria have qualities of good bioindicators, as they have high growth and metabolic rates and respond rapidly to environmental changes, including those related to anthropogenic activity (Hartmann et al., 2012). Because bacteria do not leave morphological fossils, using their DNA as a biomarker permits the reconstruction of their populations, including paleo-microbial community dynamics in aquatic ecosystems (Ravasi et al., 2012), although most recovered sedimentary DNA is derived from indigenous sediment bacteria (Torti et. al 2018, Garner et al., 2020). Sedimentary DNA have been studied in Antarctic lakes (Fernandez-Carazo et al., 2013), including one lake on Fildes Peninsula in order to characterize biodiversity changes during large climate fluctuations (García-Rodríguez et al., 2021; Piccini et al., 2024). Existing paleolimnological studies of Fildes Peninsula lakes have only focused on long-term regional climate and landscape evolution, and they have therefore lacked sufficient temporal resolution to assess changes caused by human activities during the past several decades (e.g., Lee et al., 2007; Tatur et al., 1991; Matthies et al., 1990). The aim of the present study is to fill this knowledge gap determining the extent of anthropogenic impact on aquatic ecosystems through the assessment of microbial community structure and metal enrichment from seven lakes on Fildes Peninsula. We address the following research questions:

- Are there differences between the pre-anthropic microbial communities of Fildes Peninsula and those of the present in the seven study lakes?

- Are there increases in metal concentrations in the recent sediments compared with the pre-anthropic sediments?
- If any changes have occurred, are these changes related to the human activities in the region?

We applied a “top-bottom” snapshot approach, which is conceived to assess changes in pre- and post-impact conditions across multiple sites (Smol, 2008), such that the surface, or ‘top’ section represents modern conditions, while the bottom core section predated the construction of the research stations. We employed ancient sedimentary DNA and bacterial 16S rRNA metabarcoding, sedimentary pigments and metal concentrations to determine the potential human impacts on limnological conditions.

2.4 Materials and Methods

2.4.1 Study area

Fildes Peninsula (62°11' S, 58°58' W) is a 38 km² ice-free area at the south-western extreme of King George Island, in the South Shetland Islands of the Maritime Antarctic region (Fig. 2.1a). The bedrock geology of this peninsula consists of a suite of subalkaline volcanic, plutonic and volcaniclastic rocks from Late Cretaceous to Miocene (Bastías et al., 2023). In addition to being one of the Antarctic regions with the greatest human presence due to multiple research stations, in 1980 the Teniente Rodolfo Marsh Martin Airfield was constructed with a gravel runway for the transport of cargo, station personnel, and tourists in the South Shetland Islands (Harris, 1991; Braun et al., 2020). The presence of the Fildes harbor master station also implies that supply, research, patrol and tourist vessels frequently anchor in Maxwell Bay (Cai et al., 2012). The concentration and intensity of all such activities in a relatively small geographic region implies an elevated likelihood of impacts in the region’s natural ecosystems.

Lakes are a characteristic feature of Fildes Peninsula, most of which are of glacial origin having formed following the Late Holocene retreat of the Collins Glacier (Fig. 2.1b); some of these are used as water sources for various stations. During summer, the lakes remain ice-free typically from the end of December to March, whereas their surface freezes at the end of autumn (March–April) (Izaguirre et al., 2021). Our study included seven lakes from around the Fildes Peninsula (Fig. 2.1b; Table 1); five are closely linked to research stations and they are or have been used as water supplies, while two lakes are more distant from the stations. The lakes include Uruguay Lake, which supplies Artigas Station (Uruguay); Kitiesh Lake, which is the source of potable water for the Escudero, Frei and Bellingshausen stations (Chile and Russia); Hotel Lake, which is adjacent to the Teniente Marsh Airport and was formerly its water supply, and which has ceased to be used for drinking water due to elevated levels of heavy metals (Peter et al., 2013); Las Estrellas Lake, which previously supplied water to Escudero Station (Chile); and Xihu Lake, the source of drinking water for Great Wall Station (China) (Fig. 2.1b). The more remote lakes in our study were Mondsee Lake, 3.5 km from Frei Station and 2.6 km from Artigas Station; and Jurasico Lake, located 2.1 km from Great Wall Station and 3.3 km from Escudero Station (Fig. 2.1b; Table 2.1).

Little is known about most of the lakes of the Fildes Peninsula. One of the most studied is Kitiesh Lake, where limnological work (Galaz and Préndez, 1999; Préndez and Carrasco, 2003; Shevnina and Kourzeneva, 2017) has classified it as an oligotrophic lake by chlorophyll *a* concentration (Cabrera and Montecino, 1987) and with a low ionic and metal concentrations (Préndez et al., 1996). In addition, studies have demonstrated that it has low zooplankton diversity, with one copepod and one anostracan species (Montecino et al., 1991). Special emphasis has also been placed on Hotel Lake, a small lake adjacent to the airport, where elevated concentrations of metals exceeding Chilean norms were found that caused it to be abandoned as a source of potable water (Préndez et al., 1996; Peter et al., 2013). It was hypothesized that the elevated metal and ionic concentrations in Hotel Lake may have been derived from localized geological factors (Préndez et al., 1996), however no evidence was provided to support this assertion. Later studies found much lower levels and suggested several hypotheses, including climatic variables and phytoplankton uptake, to explain the discrepancy between sampling years (Préndez et al., 1999; Préndez and Carrasco, 2003). Finally, a recent limnological study noted that differences of phytoplankton and bacterioplankton communities in our study sites were greater between seasons than between lakes, and that microbial communities in Hotel Lake differed from the rest of the lakes (Bertoglio et al., 2023).

2.4.2 Sampling

Sediment cores were taken from the seven study lakes (Fig. 2.1b) during November and December 2016 at the deepest known point of each lake, except for Hotel Lake that was

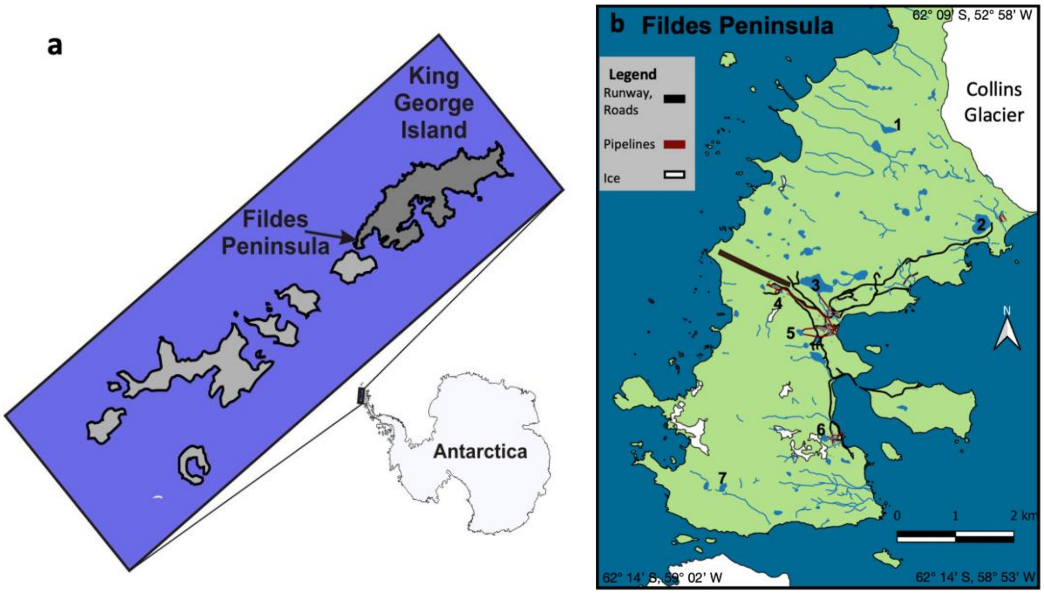


Figure 2.1. Location of the study region. a. King George Island and Fildes Peninsula. b. Map of Fildes Peninsula indicating the study lakes: 1= Mondsee, 2 = Uruguay, 3 = Kitiesh, 4 = Hotel, 5 = Las Estrellas, 6 = Xihu, 7 = Jurasico. Maps were created with geospatial data from the Scientific Committee on Antarctic Research (SCAR) Antarctic Digital Database, accessed 2021.

cored in December 2013. Holes were drilled in the lake ice with a manual ice auger, and cores were recovered using a universal corer with tenite butyrate core tubes of either 67 or 95 mm internal diameter (Aquatic Research Instruments).

Based on sedimentation rates obtained from earlier studies, a general target length of 30 cm was established for the sediment cores, which was long enough to far exceed the period of human presence on Fildes Peninsula. The sediment-water interfaces were stabilized with sodium polyacrylate (Tomkins et al., 2008), and cores were then sealed and transported whole, cold and in the dark to Canada. The cores were then split lengthwise, and one half was stored as an archive while the other half was used for analysis. All cores were visually laminated, and all subsampling followed existing laminations. The uppermost stratigraphic layer was collected as the “top” sample, beginning at the sediment surface (i.e., 0.0 cm) and continuing to the base of the first clearly defined sediment lamination, ranging between 0.20–0.60 cm thick. For the bottom sample, we attempted to maintain a roughly consistent age across lakes while pre-dating human presence on Fildes Peninsula (midpoints between 4.10–4.70 cm). However, in certain lakes the bottom samples were taken from depths of 3.25, and 2.55 cm (Mondsee and Jurasico) because it was not possible to recover sequences from deeper samples for bacteria analysis (see Table 2.2 and the chronology section below for details).

Table 2.1. Characteristics of the study lakes.

Lake	Latitude	Longitude	Maximum depth (m)	Surface area (ha)	Catchment area (ha)	Distance to nearest infrastructure (km)
Mondsee	62°10'21.50"S	58°56'43.26"W	7.0	1.90	10.50	2.60
Uruguay	62°11'07.4"S	58°54'39.6"W	15	7.03	18.66	0.34
Kitiesh	62°11' 36.9" S	58°58' 0.2" W	11	9.43	59.71	0.53
Hotel	62°11' 40.4" S	58°58' 42.2" W	5.0	0.11	1.71	0.10
Las Estrellas	62°12'2.5"S	58°58'19.4"W	5.6	0.66	4.05	0.37
Xihu	62°13'00.80"S	58°57'56.41"W	10	0.98	39.32	0.14
Jurasico	62°13'00.0"S	59°00'00.0"W	4.9	1.07	7.45	2.10

All subsampling was carried out under sterile protocols to preclude contamination, and separate subsamples were taken from each top and bottom sample for analysis of photosynthetic pigments, DNA and radioisotopic dating, placed in sterile Whirl-Pak bags, and kept frozen at –80 °C until analysis.

2.4.3 Dating

To approximate the age of the bottom samples, ^{210}Pb activities were measured by alpha spectroscopy at Chronos Scientific Inc. in Ottawa, Canada. Freeze-dried samples were ground and spiked with ^{209}Po, after which HNO_3 (nitric acid) and HCl (hydrochloric acid) were added, and the samples were heated at 80 °C for 16 hours. The solutions were then centrifuged and evaporated to dryness three times, with HCl added after each cycle. Finally, the Po isotopes were electroplated on silver disks and measured using alpha spectroscopy. The approximate ages of the bottom samples were then calculated according to the equation:

$$t_x = k^{-1} \ln(C_0 \cdot C_x^{-1})$$

where tx is the time, in years, elapsed since the coring date, k is the ^{210}Pb decay constant (i.e., 0.0312 y^{-1}), C_0 is the unsupported 'Top' sample ^{210}Pb activity (in Bq kg^{-1}), and C_x is the unsupported ‘Bottom’ sample ^{210}Pb activity, with unsupported ^{210}Pb activity estimated as (total ^{210}Pb activity) – (^{214}Bi activity) (Binford, 1990; Table 2.2). The error range of the bottom ages was calculated according to the same equation after adding and subtracting the 1-sigma counting error of the ^{210}Pb activities (Table 2.2).

2.4.4 Metals

Metal activities in the sediment cores were determined using µ-XRF (micro-X-ray fluorescence) on an Itrax core scanner located at the INRS (Quebec, Canada) and equipped with a molybdenum-anode X-ray tube, with measurements taken at 500 µm intervals for 20 s at 40 kV and 10 mA. This method provides semi-quantitative data about elemental concentrations and has been shown to accurately reflect changes in sediment metals over time (Zilkey et al., 2024). We focused on the metals chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn) and arsenic (As) as potential indicators of local anthropogenic impact.

2.4.5 DNA extraction, sequencing and taxonomic assignment

To detect changes in bacterial community composition, bacterial diversity was analyzed by 16S rRNA amplicon sequencing. All material employed for manipulation of DNA sediment subsamples, as well as the vertical laminar flow cabinet (ESCO Class II, Type A2) where the DNA was extracted, was previously sterilized by irradiation with UV light. 0.5 g of sediment were aseptically transferred to sterile microtubes containing ceramic beads and an extraction buffer composed of 1% CTAB (Hexadecyltrimethylammonium bromide) and EDTA (Ethylenediaminetetraacetic acid) and homogenized with a FastPrep homogenizer (MP). After centrifugation at 12000 g the supernatants were subjected 3 times to chloroform:isoamyl alcohol (24:1) extractions and the pellets were precipitated with 0.6 volumes of cold isopropanol at room temperature during 24 h. These precipitates were then subjected to centrifugation for 40 min at 12000 g at room temperature, and the DNA obtained in the pellets was washed with cold ethanol, dried and suspended in ultrapure water overnight at 4 °C. The concentration and purity of DNA was determined spectrophotometrically at 260 and 280 nm, and its quality was checked by the amplification of a variable region (V4) of the ribosomal 16S gene. PCR (polymerase chain reaction) products were verified by gel electrophoresis on 1% agarose gel in 0.5X TBE buffer. DNA samples were sent to Novogene for Illumina MiSeq paired-end sequencing of the V4 region, using the 515 F and 806 R primers (Caporaso et al., 2011).

Table 2.2. Dating results. ^{210}Pb activities measured by alpha spectroscopy. C_0: unsupported 'Top' sample ^{210}Pb activity, C_x: unsupported 'Bottom' sample ^{210}Pb activity. N/A: it was not possible to estimate a lower age limit due to extremely low ^{210}Pb activities in Xihu Lake.

Lake	**Coring date**	**C_0 (Bq kg^{-1})**	**C_x (Bq kg^{-1})**	**Top sample midpoint (cm)**	**Bottom sample midpoint (cm)**	**Bottom age (Yr CE)**	**Bottom age error range (Yr CE)**
Mondsee	6 Dec 2016	96.80	16.14	0.10	3.25	1960	1954-1964
Uruguay	9 Dec 2016	16.91	6.10	0.30	4.70	1984	1976-1991
Kitiesh	1 Dec 2016	31.15	3.46	0.25	4.55	1947	1935-1955
Hotel	14 Dec 2013	189.18	3.59	0.10	4.35	1887	Pre.1850-1922
Las Estrellas	28 Nov 2016	60.63	0.40	0.13	4.10	1856	Pre.1850-1901
Xihu	4 Dec 2016	2.46	1.11	0.10	4.65	1991	N/A-2014
Jurasico	11 Dec 2016	33.10	4.07	0.12	2.55	1950	1944-1955

To correct for sequencing errors and create Amplicon Sequence Variants (ASVs), reads were processed in R using the DADA2 pipeline following a modified version of the DADA2 Bioconductor workflow (Callahan et al., 2017). Briefly, reads were filtered and trimmed by the filterAndTrim function based on the quality score which estimates the error probability of the DNA sequence. Reads with a maximum expected error (maxEE) greater than 2 were removed and based on quality profiles, reads were truncated at 200 and 190 bp for forward and reverse reads, respectively. Sequence variations were inferred with the learnerrors and dada functions. Chimeric sequences were eliminated, and taxonomy assignments from Kingdom to Genus were performed with assignTaxonomy based on the SILVA database (v138) (Quast et al., 2012). ASV sequences assigned as Archaea, Chloroplasts and Mitochondria were removed. Sequences obtained were submitted to the nucleotide archive GenBank with the project id PRJNA1013445.

2.4.6 Photosynthetic pigment analysis

Sedimentary photosynthetic pigment concentrations were assessed by high performance liquid chromatography (HPLC). Sediment samples were freeze dried for 24 h, and pigments were extracted from 0.1 to 0.3 g (dry weight) samples by sonication in 2.5 mL 90% acetone under argon gas and incubated for 24 h in a freezer. Samples were then cleared by centrifugation at 4 °C, filtered through 0.2 μm polytetrafluoroethylene filters into HPLC vials and argon gas was again added. Extracts were immediately injected into a Thermo Fisher Accela 600 HPLC equipped with an autosampler, a Hypersil Gold C8 column (3 µm pore size), a photo-diode array (PDA) and a fluorescence detector, using the reverse-phase solvent protocol of Zapata et al. (2000). Pigments were detected by diode-array spectroscopy (350–750 nm) set to a slit width of 1 nm, and absorbance chromatograms were obtained at 450 nm. Chlorophylls were also detected by fluorescence (excitation, 440 nm; emission, 650 nm). Pigments were identified and quantified from retention times and absorbance chromatograms using reference standards from Sigma Inc. (St. Louis, MO, USA) and DHI Water and Environment (Hørsholm, Denmark). Pigment concentrations were expressed relative to sedimentary dry mass and normalized to organic matter content (ng g OM^{-1}).

2.4.7 Data analysis

To assess metal enrichment over time, changes between pre- and post-anthropic sediments were calculated as the ratio of the surface XRF activities to those of the bottom samples. All metal activities are expressed as ratios to titanium (Ti) to account for changes in background sedimentation and diagenesis.

To explore potential microbial responses to metal enrichment, we applied the indicator value method of Dufrêne and Legendre (1997) to identify ASVs most strongly associated with metal-enriched sediments. Because there were notable increases of certain heavy metals in post-anthropic sediments from Hotel and Las Estrellas lakes (see below), the top sediment samples from both lakes (metal-enriched group) were compared against all other samples using the R package indicspecies (De Cáceres et al., 2010). Significant indicators ($p < 0.05$) were retained for interpretation.

Total spatial beta diversity was calculated as the total variance in the bacterial community composition among lakes for each time point (i.e., the pre- and post-anthropic samples) (Legendre and De Cáceres, 2013), calculated by the Odum’s percentage difference (Bray-Curtis) dissimilarity of the ASV matrix by the R package adespatial (Dray et al., 2022). The local contribution to beta-diversity (LCBD) index for each sample was also estimated as the sum of the variance for each site with respect to the total variance in the dataset, as well as a permutational p value indicating the significance of each LCBD value.

To analyze the degree of change in bacteria community composition through time we calculated the temporal beta diversity index (TBI) (Legendre and De Cáceres, 2013) for each top-bottom pair using the R package adespatial (Dray et al., 2022). TBI was estimated using the Odum's percentage difference index applied to the ASV matrix at the two different time points within each lake with significance assessed by permutational p values. TBI values range from 0 to 1, with higher values for greater differences in bacterial community between both times.

To explore relationships among environmental data, a standardized principal component analysis (PCA) was performed with the variables: metal enrichment (ratios of top to bottom samples XRF counts), TBI values, lake areas, catchment areas and lake depths. We also carried out a PCA of the Hellinger-transformed ASV matrix to examine similarities in bacterial composition between all samples from both time periods. A permutational multivariate analysis of variance (PERMANOVA) test was run to establish the statistical significance of the differences in bacterial communities of each time (pre- vs post-anthropic) by the R package vegan (Oksanen et al., 2020).

We explored bacterial compositional variation across time separately for each lake using similarity percentage analysis (SIMPER) of vegan (Oksanen et al., 2020). SIMPER identified the taxa (i.e., ASVs) with the greatest contribution to the percentage difference dissimilarity between the two different time points for each lake, thus determining the ASVs that changed the most between the pre-anthropic period and the present.

Finally, to determine changes in trophic state we used the sum of chlorophyll *a* (chl-*a*) and its degradation products as total chl-*a* (Leavitt and Hodgson, 2001). Post-anthropic values of total chl-*a* concentrations were expressed as ratios to pre-anthropic concentrations.

2.5 Results

2.5.1 Dating

The average age of the bottom samples was 1939 CE, and the bottom samples in all lakes but two pre-dated permanent human presence on Fildes Peninsula, enabling inferences of pre-impacted conditions (Table 2.2). The bottom sample from Uruguay Lake was dated at 1984, the same year as the inauguration of the adjacent Artigas Base, while that of Xihu

Lake was dated at 1991, shortly following the 1985 establishment of nearby Great Wall Station.

2.5.2 Changes in metals and pollution-sensitive taxa

Metal enrichment over time, expressed as the ratios of top to bottom samples, was close to 1 (i.e., no change) in most lakes and for most of the metals examined (Fig. 2.2a). Values for Cr and Ni were less variable across the lakes, ranging from 0.63 to 3.72 (top:bottom), while Cu, Zn and As showed greater variations with extreme values of recent enrichment found in Hotel Lake (10.38, 21.21 and 33.99 respectively). Las Estrellas Lake also showed Zn enrichment (5.74), while for the rest of the lakes Zn ratios ranged from 0.52 to 2.22. Then, except for Hotel Lake, Cu ratios ranged from 0.68 to 2.87 and As from 1.42 to 2.41 (Fig. 2.2a).

Indicator ASVs for the post-anthropic samples from Hotel and Las Estrellas lakes (the lakes with notable metal increases) are shown in Table B.1. These ASVs (with significant indicator values, $p < 0.05$), showed specificity values ≥ 0.8 and a fidelity value of 1. The predominance of certain taxonomic groups was observed, for example, the class KD4–96, the families Bacteroidetes vadinHA17 and Hungateiclostridiaceae and the genus *Leptolinea*. These taxa were indicator bacteria of contamination with metals or oil as discussed below.

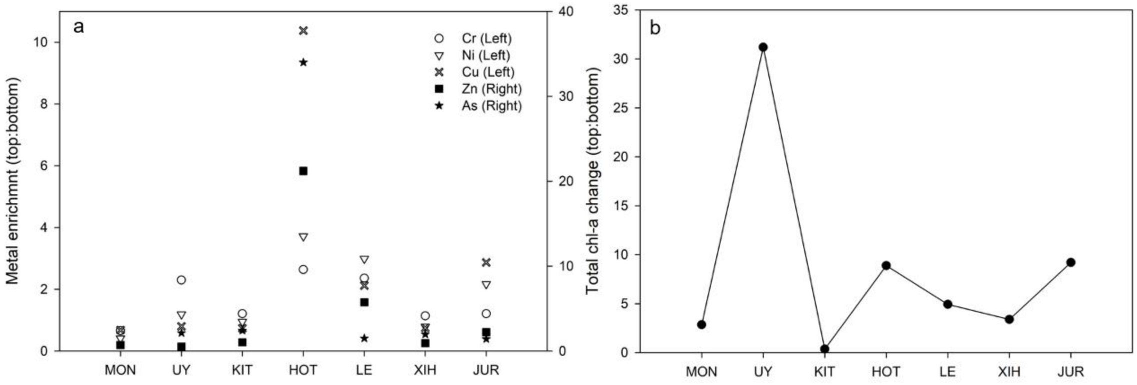


Figure 2.2. a. Changes in metals from XRF data. Note the different y-axis for Zn and As (right) vs the left y-axis for Cr, Ni and Cu. b. Total chl-*a*, calculated as the ratio between top and bottom samples values for each core. Lake names are abbreviated as MON: Mondsee; UY: Uruguay; KIT: Kitiesh; HOT: Hotel; LE: Las Estrellas; XIH: Xihu; JUR: Jurasico.

2.5.3 Bacterial beta diversity

2.5.3.1 Spatial beta diversity

We obtained a total of 3,934,606 reads with a mean of 281,043 reads per sample, which were categorized into 11,650 ASVs. Rarefaction curves based on the observed ASVs reached plateaus (Fig. B.1), indicating that sequencing depth was adequate to capture the overall diversity of all lakes.

Proteobacteria was the dominant phylum in all samples, with relative abundances that ranged from 25% to 50%. Other groups including the phyla Chloroflexi, Desulfobacterota, Acidobacteriota, Actinobacteriota, Bacteroidota, Planctomycetota, Gemmatimonadota, Nitrospirota, Firmicutes and MBNT15 generally ranged between 1.2% and 23% relative abundance per sample, while certain phyla such as Zixibacteria, Methylomirabilota, Latescibacterota and Nitrospinota were also among the most frequent, but at low relative abundances ranging between 0.03% and 5.09% (Fig. 2.3). The bacterial community from each lake was composed of a few very abundant ASVs (e.g., *Rhodoferax*, Pseudomonas and *Janthinobacterium*) while most taxa had low abundances in all lakes for both pre- and post-anthropic sediments.

Although no significant differences were detected between the total spatial beta diversity of the pre- and post-anthropic sediments (value: 0.188), the pre-anthropic sample of Jurasico Lake had a significantly higher LCBD index ($p < 0.05$) (Table 2.3).

2.5.3.2 Temporal beta diversity

The overall bacterial communities from the pre-anthropic sediments did not differ significantly from those of the post-anthropic sediments (Fig. B.2; PERMANOVA: R^2: 0.07; p value: 0.538), instead the bacterial community varied between both sediments according to the lake (Fig. 2.4).

TBI indices indicated that bacterial communities in each lake differed on average by ~50% between top and bottom samples. The highest TBI values were found in Kitiesh and in Xihu lakes (0.63 and 0.61 respectively), reflecting statistically significant bacterial community changes between the pre- and post-anthropic sediments in both lakes ($p < 0.05$; Table 2.3). The top-bottom TBI differences in the other five lakes were not statistically significant.

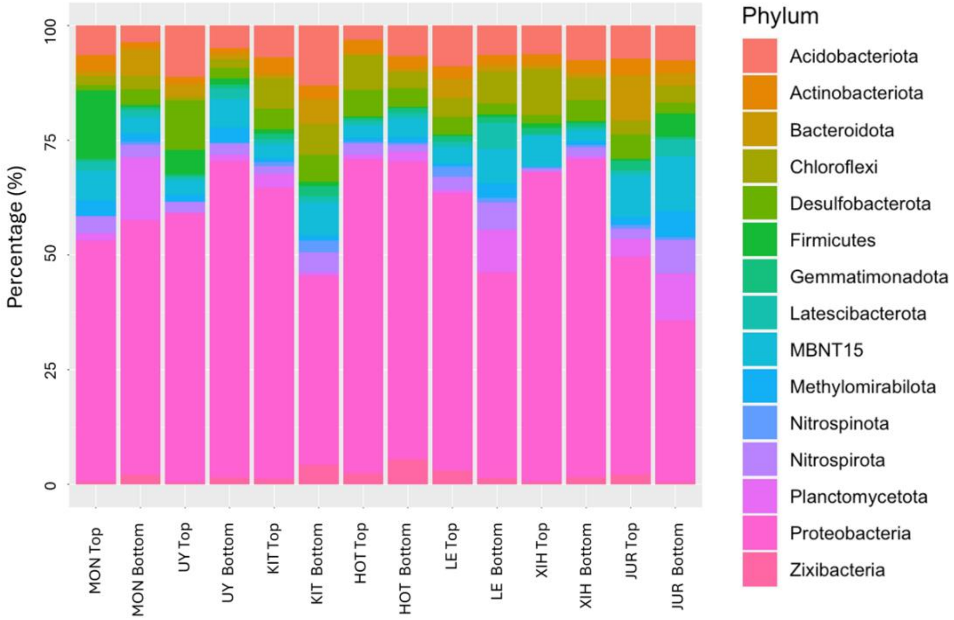


Figure 2.3. Most abundant and frequent phyla. The percentages were calculated only from phyla that were present in all samples and with read abundances higher than 6000 over the total of the samples. Lake names are abbreviated as MON: Mondsee; UY: Uruguay; KIT: Kitiesh; HOT: Hotel; LE: Las Estrellas; XIH: XIhu; JUR: Jurasico.

Table 2.3. Local contribution to spatial beta diversity (LCBD) for the pre-anthropic (bottom) and post-anthropic (top) sediments (estimated as the sum of the variance for each lake with respect to the total spatial beta diversity (total variance); and temporal beta diversity indices (TBI) estimated by the percentage difference dissimilarity between the top and bottom samples. Values with asterisks indicate significant values ($p \leq 0.05$).

Lake	Pre-anthropic LCBD	Post-anthropic LCBD	TBI
Mondsee	0.12	0.16	0.58
Uruguay	0.09	0.14	0.52
Kitiesh	0.15	0.13	0.63*
Hotel	0.15	0.16	0.50
Las Estrellas	0.13	0.12	0.51
Xihu	0.16	0.15	0.61*
Jurasico	0.19*	0.13	0.51

The two first axes of the PCA for metal enrichment, TBI values, lake areas, lake area catchments and lake depths explained 79.93% of the variance, of which 62.83% was explained by the first axis and 17.10% by the second (Fig. 2.5). The first axis was positively correlated with metal enrichment and negatively correlated with TBI and lake areas, catchments and depths. Hotel and Las Estrellas lakes were strongly associated to increases in metals (Fig. 2.5).

SIMPER results revealed that the ASVs which contributed most to the dissimilarity between pre- and post-anthropic sediments followed different patterns in each lake (i.e., increases or decreases; Fig. 2.4). For example, the taxa *Delftia*, *Desulfosporosinus*, *Desulfurivibrio*, and *Geothrix* increased in the top samples of Mondsee and Uruguay lakes; *Sulfuricella* and Anaerolineaceae in Kitiesh Lake, *Desulfatirhabdium*, *Polaromonas* and *Leptolinea* increased in Hotel Lake; BSV26 and Zixibacteria increased in Las Estrellas Lake; *Rugamonas*, MBNT15 and *KD4–96* increased in Xihu Lake and BSV26 increased in Jurasico Lake (Fig. 2.4). We also observed taxa that were very abundant in pre-anthropic samples but decreased in modern sediments. Many of these taxa were novel groups such as SG8–4 (phylum Planctomycetota) in Mondsee Lake; GIF9 (phylum Chloroflexi) and 4–29–1 (phylum Nitrospirota) in Kitiesh and Hotel lakes; CCM11a (phylum Planctomycetota) and the candidate phylum MBNT15 in Las Estrellas Lake; and CCM11a and P2–11E (phylum Chloroflexi) in Jurasico Lake (Fig. 2.4).

2.5.4 Photosynthetic pigments

Total chl-*a* concentrations in post-anthropic sediments increased relative to pre-anthropic samples in 6 out of 7 lakes. The highest ratio was found in Uruguay Lake (31.18), followed by Jurasico and Hotel lakes (9.21 and 8.89) whereas Las Estrellas, Xihu and Mondsee lakes exhibited more moderate chl-*a* increases (ratios: 4.92, 3.38 and 2.85) (Fig. 2.2b). In Kitiesh Lake, there was a chl-*a* decrease in the top sample relative to the bottom (top-bottom ratio: 0.34, Fig. 2.2b).

Chl-*a* preservation, as estimated from the total concentration of undegraded chl-*a* relative to the total of all chl-*a* degradation products, was considerably lower in the bottom samples (mean: 0.07) than the top samples (mean: 0.85) (Table B.2).

Taxonomic pigments used to infer phytoplankton diversity changes between pre- and post-anthropic sediments indicated higher phytoplankton diversity in post-anthropic sediments of all lakes compared with pre-anthropic sediments (Table B.3). Pigments derived from Chlorophyceae (chl-*b* and lutein) increased in post-anthropic sediments compared with pre-anthropic sediments of all lakes except in Kitiesh Lake where the opposite was found. Cyanobacteria were inferred from the pigments canthaxanthin, zeaxanthin and echinenone, however the abundances of these pigments were not always correlated. For example, zeaxanthin was abundant in the post-anthropic sediment of Hotel Lake, but echinenone was not detected and canthaxanthin had a low concentration. Other more labile pigments were identified, including pigments derived from Bacillariophyceae*,* Chrysophyceae and Dinophyta*,* such as fucoxanthin, chl-c_1, chl-c_2 and diatoxanthin, which dominated in post-anthropic sediments (Table B.3).

2.6 Discussion

We assessed the impact of human activities associated with research stations (such as roads, hydraulic changes, dust pollution, hydrocarbon use, wastewater and garbage), on the bacterial diversity of lakes. Since microbes are the key drivers of biogeochemical cycles and ecosystem functioning (Treseder et al., 2012), the shifts of bacterial community composition between the present (current activities) and the past (before human impact), were used as a proxy of alterations to lake ecology.

The two lakes where we found pronounced metal enrichments are very close to roads and airport infrastructure (especially Hotel Lake), and we therefore hypothesize that contaminants associated with transportation (i.e., emissions from fuel burning, dust from roads and the airport) may have structured bacterial communities in these lakes. Other factors, such as greater lake area and volume, likely contribute to diminishing the signal of anthropogenic processes, which may explain why metals did not increase in the post-anthropic sediments of the much larger Kitiesh Lake despite its relative proximity to the airport (Fig. 2.2b). The lake has a much greater volume than those of the other lakes near the airport (i.e., ~86 and ~14x the surface area of lakes Hotel and Las Estrellas, respectively, and ~2x their maximum depth; see Table 2.1), which would dilute inputs of contaminants and contribute to greater ecosystem inertia in the face of changing environments.

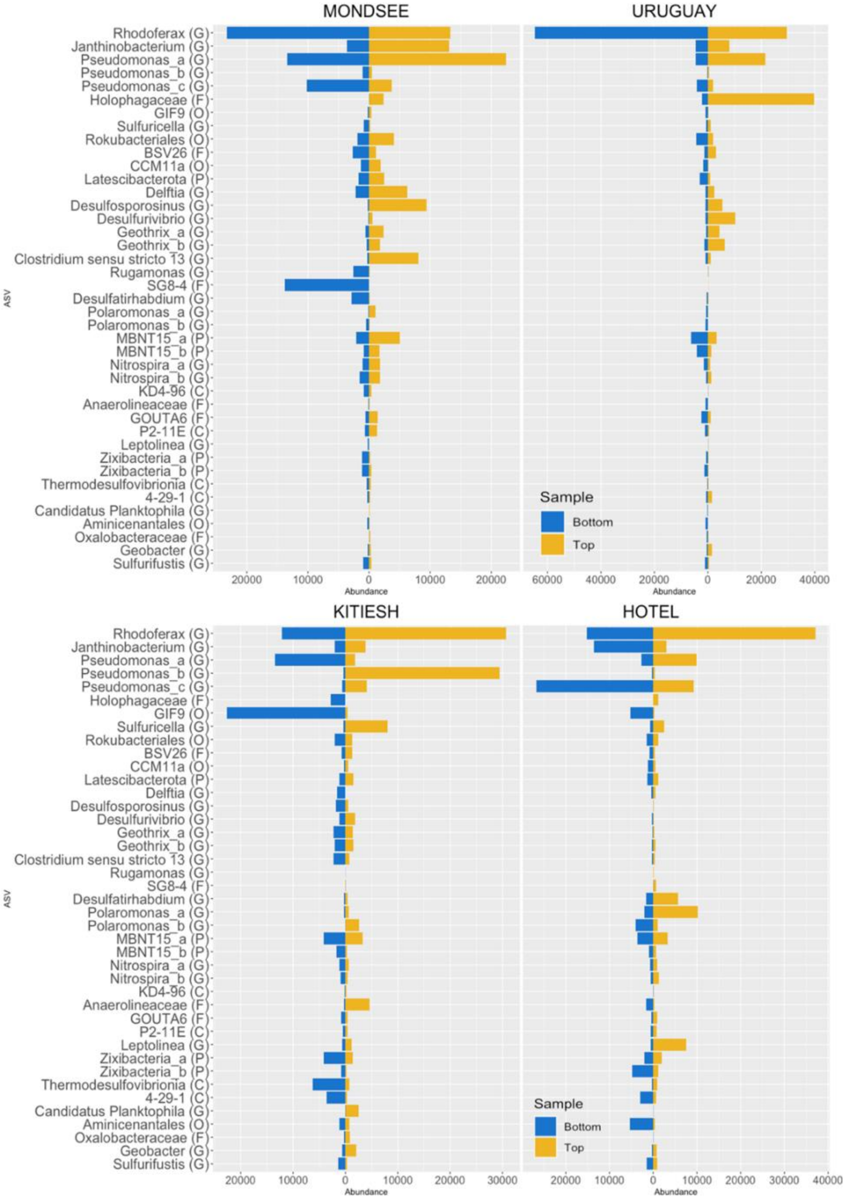
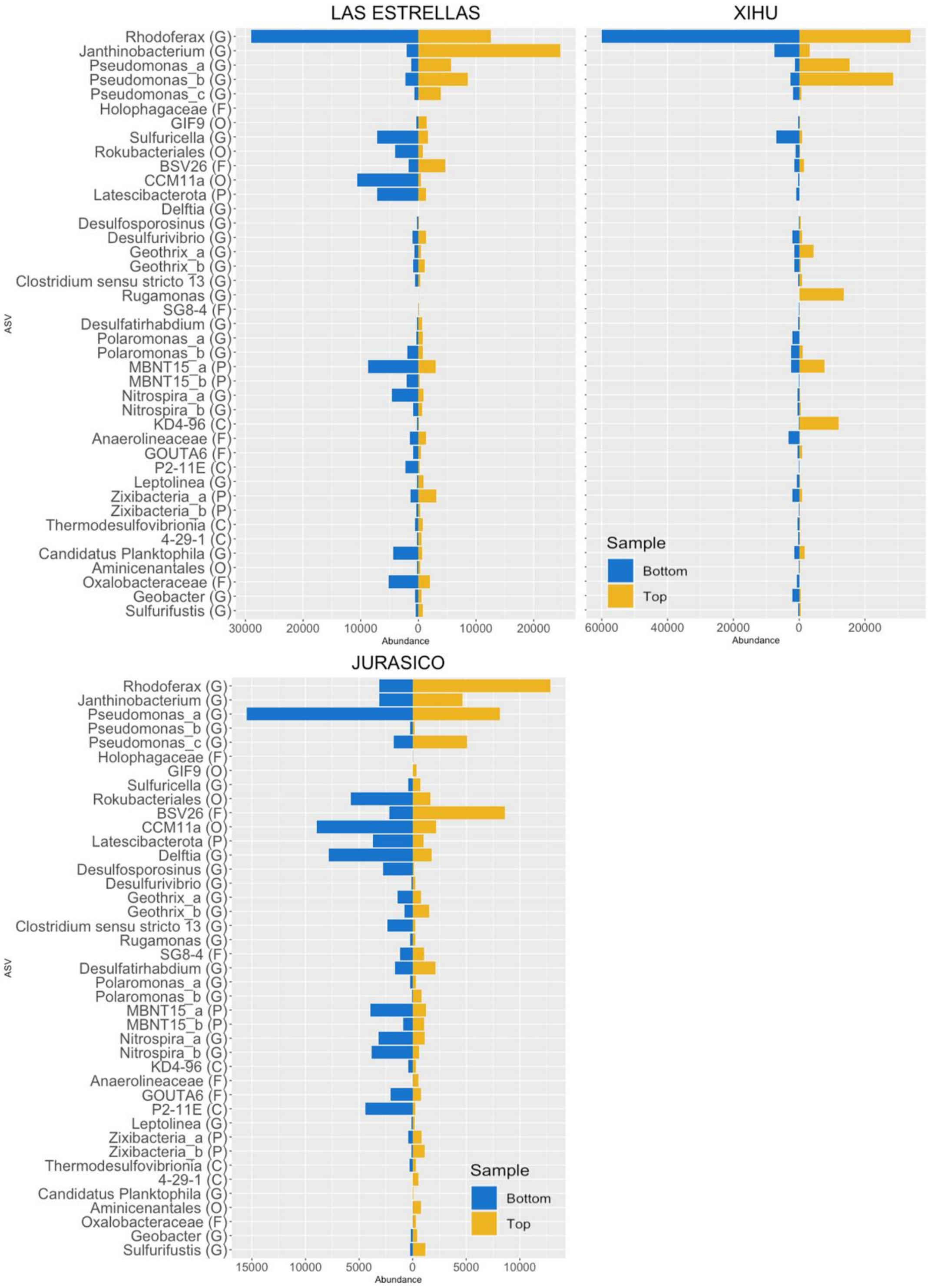


Figure 2.4. ASVs with abundances > 10,000 reads across all samples which accounted for the 40 % of the dissimilarity of the post-anthropic (top) and pre-anthropic (bottom) samples based on the SIMPER analysis for each lake. Taxa follow the same order in each figure panel; the length of the blue bars represents the abundance (number of reads) in the bottom sample while the yellow bars represent the top sample. Note the differing x-axis scales between lakes. Where the identification of the genus (G) was not possible, the name of family (F), order (O), class (C), or phylum (P) is given instead. Where different ASVs with the same taxonomic affiliation were identified they are denoted as a, b or c.


LAS ESTRELLAS

XIHU

JURASICO

Figure 2.4. (continued).

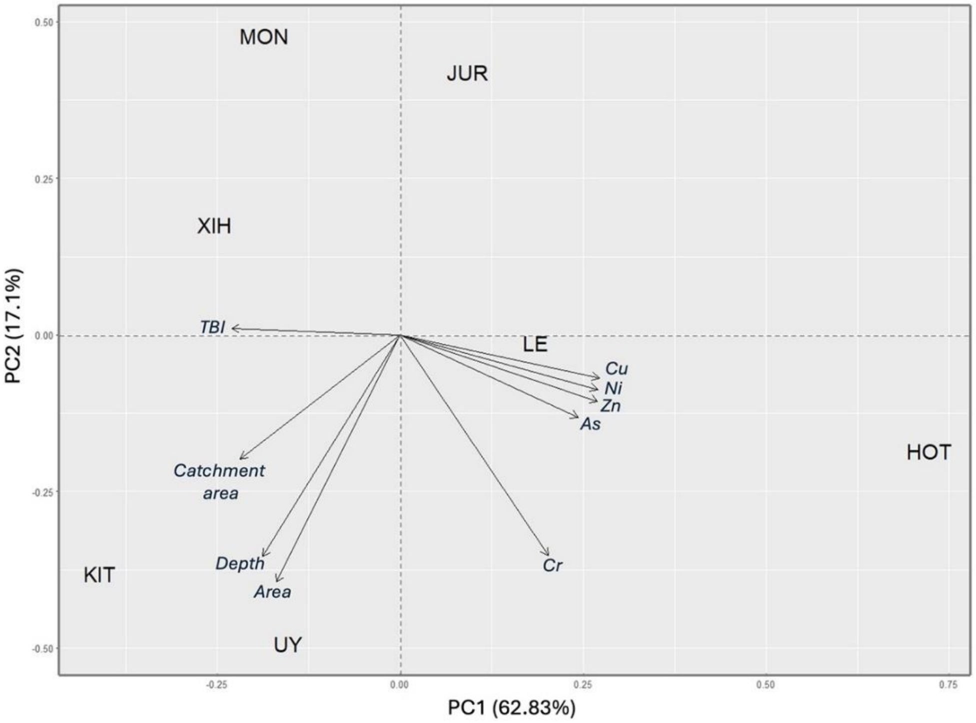


Figure 2.5. First two axes of the PCA for the environmental data: TBI (temporal beta diversity index), metal enrichments (As, Zn, Cu, Ni and Cr), lake areas, catchment areas and lake depths. The first axis explained 62.83 % of the variance while the second explained 17.10 %. Lake names are abbreviated as MON: Mondsee; UY: Uruguay; KIT: Kitiesh; HOT: Hotel; LE: Las Estrellas; XIH: Xihu; JUR: Jurasico.

2.6.1 Bacterial changes between pre- and post-anthropic sediments

DNA was equally amplifiable in both pre- and post-anthropic sediment samples, indicating good DNA preservation and confirming that the observed changes in bacterial diversity over time reflected real responses to environmental factors rather than taphonomic changes (Fig. B.3).

Two of the most representative ASVs in our samples are common taxa in environments exposed to high light: *Rhodoferax* which can grow photoheterotrophically and can be found in glacial environments (Junge et al., 2019), and *Janthinobacterium*, which is a widespread genus found in soils and freshwater ecosystems. The latter was previously isolated from lichen samples from Collins Glacier, which produce violacein, a pigment involved in different bacterial survival mechanisms such as resistance to UV irradiation (Alem et al., 2020).

Increasing UV radiation has become a greater stress factor in recent times due to shorter lake ice duration in Antarctic Peninsula lakes as well as due to regional ozone thinning (Uchino et al., 1999; Convey and Peck; 2019,). This may explain the increase of *Janthinobacterium* in post-anthropic sediments in most of the studied lakes (Fig. 2.4). Additionally, *Pseudomonas*, also one of the most representative taxa in our samples, is a widespread group important in freshwater systems elsewhere in the world, including polar lakes and glaciers (Michaud et al., 2012).

We detected several novel bacteria in our samples. One of these (P2–11E, belonging to the phylum Chloroflexi) was previously detected in soil from Byers Peninsula (da Silva et al., 2024); GIF9, from the class Dehalococcoidia, also within the phylum Chloroflexi, was previously isolated from hot spring sediments revealing heterotrophic activity on aromatics and plant-associated compounds (Palmer et al., 2023); and CCM11a, from the phylum Planctomycetes, has a broad metabolic function allowing chemolithotrophic growth involving iron oxidation and methane oxidation (Storesund and Øvreås, 2013). Due to the poorly defined ecological affinities of these novel bacteria, the ecosystemic significance of their recent decrease observed in certain lakes is unclear.

Differences in the ages of the pre-impacted sediment samples may potentially have influenced the degree of bacterial change between top-bottom pairs. The bottom sediments from Uruguay and Xihu lakes were the youngest in our dataset and they did not predate the establishment of their respective adjacent bases. However, Xihu Lake was amongst the lakes with the greatest bacterial community change between pre- and post-anthropic sediments. Other environmental factors, such as the pronounced warming that has affected the region since the middle of the 20th century with an increase in average annual air temperature of 2.5 °C since 1969, may also have influenced this pronounced change in the bacterial community (Carrasco et al., 2021; READER, 2024).

We are aware that sedimentary DNA may come from both dead and active microbial communities (Ellegaard et al., 2020), and evidence suggests that a low composition of sediment DNA is of historical origin (Garner et al., 2020). While we acknowledge the challenge of this methodological approach, evidence suggests that the signal of the sedimentary microbial community, regardless they come from the water column or are active sediment communities, is influenced by conditions prevalent in the water-sediment interface and thus reflects lake ecosystem changes (Møller et al., 2020).

2.6.2 Bacterial taxa indicating human presence

A number of species that were identified as indicator species of recent samples in the two lakes with the greatest metal enrichment (Hotel and Las Estrellas lake) are known to degrade complex organic compounds and be found in polluted environments. These included *Leptolinea*, from which sequences have been previously recovered from anaerobic sludge (Rivière et al., 2009) and contaminated aquifer sediments (Winderl et al., 2008); Bacteroidetes vadinHA17, which can degrade phenols (Fan et al., 2022); Hungateiclostridiaceae, a xylan-degrading bacterium isolated from an oilfield (Zhang et al., 2018); KD4–96, which was detected in contaminated soil where its abundance was correlated with iron and aluminum concentrations (Wegner and Liesack, 2017); OPB41, which was previously isolated from subsurface environments in a naturally petroleum-rich region (Khomyakova et al., 2022); *Pseudorhodoplanes*, isolated from an oil-contaminated soil sample (Tirandaz et al., 2015); and *Anaerovorax*, which ferments putrescine (Matthies et al., 2000). The proximity of both lakes to roads and airport infrastructure may imply greater pollution due to inputs of dust, vehicle and aircraft emissions, and compounds such as petroleum derivatives in the Hotel and Las Estrellas lake catchments.

2.6.3 Changes in photosynthetic pigments

Concentrations of total chl-*a*, estimated as the sum of chl-*a* and its degradation products, suggested an increase in phototrophic biomass since the research stations were established (Fig. 2.2b). It is possible, however, that this may be a consequence of recent warming, as many Antarctic Peninsula lakes have shown increases in nutrient concentrations and production due to decreases in ice cover duration (Camacho et al., 2012). Moreover, the relatively low preservation of chl-*a* in the pre-anthropic samples (Table B.2), implies that the influence of post-depositional diagenetic processes on the pigment differences between top-bottom pairs may have been considerable.

The preservation of sedimentary pigments is affected by various factors, including temperature, light and oxygen (Leavitt et al., 1997). Pigment degradation in sediments is often less rapid than in the water column, but also depends on other factors such as light penetration (Leavitt and Carpenter, 1989), which is generally high in polar lakes due to low concentrations of UV-absorbing dissolved organic matter (Gibson et al., 2000). Pronounced depletion of stratospheric ozone has also occurred in the Antarctic (Smith et al., 1992), leading to greater incident UV radiation. Previous studies from the same lakes showed no evidence of anoxia in deep water samples (Bertoglio et al., 2023), although these data were a single water column profile during spring. Changes in redox conditions estimated by changes in Fe/Mn between pre- and post-anthropic sediments suggest that redox conditions likely stayed relatively constant on the time scales considered in our study. Changing redox conditions could also affect metal diagenesis over time. However, the relative stability of the Fe/Mn ratio between top and bottom samples (average ratio for the seven lakes = 1.2) suggests limited variation in redox conditions at the time scale of our study (Table B.4), and the metals we considered (i.e., Cr, Ni, Cu, Zn and As) are not amongst the most sensitive to redox changes (Bennett and Canfield, 2020).

Although many carotenoids are more stable than chl-*a*, such as the stable taxonomically diagnostic carotenoids lutein, echinenone, canthaxanthin and zeaxanthin (Leavitt and Hodgson, 2001), we cannot definitively state that the decrease in pigment diversity and concentrations observed in pre-anthropic sediments does not result from post-depositional alteration rather than reflecting responses to environmental changes. As such, we are reluctant to draw conclusions about changes in biomass based on simple top-bottom pigment analysis.

2.7 Conclusion

Based on our original three research questions we draw the following conclusions:

Question 1. Are there differences between the pre-anthropic microbial communities of Fildes Peninsula and those of the present in the seven study lakes? We found differences between the bacterial communities that existed prior to the construction of the research stations compared and those of modern samples, with different degrees of difference between lakes.

Question 2. Are there increases in metal concentrations in the recent sediments compared with the pre-anthropic sediments? We found marked increases in metals in recent sediments relative to pre-anthropic samples in Hotel and Las Estrellas lakes, two small lakes in close proximity to infrastructure and intense human activity.

Question 3. If any changes have occurred, are these changes related to the human activities in the region? The increase of bacteria taxa sensitive to pollution in post-impacted sediments, as well as greater metal enrichment over time, was most pronounced in lakes near to transportation infrastructure, whereas the degree of change in the bacterial community composition over time was related to intra-lake characteristics such as their surface areas and catchment sizes.

Bacteria are a sedimentary proxy that can be used to reconstruct environmental changes in lakes, including anthropogenic impacts. Here we demonstrated that anthropogenic processes over the past ~half century have impacted at least two of the seven studied lakes from Fildes Peninsula based on metal enrichment and the association of bacterial taxa with tolerance to contaminants to recent samples from the metal-enriched lakes. The indicator species method employed here proved to be appropriate for targeting specific environmental processes, selecting bacteria that were more specific to given samples, even if they were not the most abundant. This is important given that bacteria with relatively minor abundances, but that play key roles, could be favored in response to environmental stressors such as pollution, and be used to detect alterations to important ecosystem functions in which they are involved. This study established the short-term variability of bacterial communities in a highly sensitive region under human and climate pressure. Further research is necessary to disentangle the relative importance of climate change and anthropogenic factors in driving changes in bacterial communities and other limnological characteristics.

2.8 Acknowledgements

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Chapter 3 Reconstructing the effects of anthropogenic activities and climate change in three lakes of the Fildes Peninsula, Maritime Antarctica

3.1 Résumé

Les lacs de la péninsule Fildes (Antarctique Maritime) subissent les effets du réchauffement climatique prononcé ainsi que des activités humaines locales, en étant l'une des régions antarctiques les plus touchées par la présence humaine. Afin d'évaluer ces impacts, nous avons analysé les diatomées, les bactéries (via le métabarcodage 16S rRNA) et les métaux dans les sédiments de trois lacs (deux proches des stations et un plus éloigné). Les lacs proches aux installations anthropiques ont montré des enrichissements en métaux, des tératologies de diatomées, et des changements marqués depuis les années 1970, date de construction de plusieurs stations. Certaines bactéries (Hungateiclostridiaceae, OPB41, *Anaerovorax* et *Leptolinea*) étaient identifiées comme indicatrices de la pollution métallique. Le lac éloigné a surtout révélé des changements liés au climat. Ces résultats indiquent le rôle important des transports comme source de contamination, et fournissent des preuves importantes sur les effets combinés des activités humaines et du changement climatique.

3.2 Abstract

The Fildes Peninsula (Maritime Antarctica) is greatly affected by global warming and local human impacts since it is in one of the Antarctic regions with the highest intensity of human activity. To establish the effect of human activities on Fildes Peninsula lakes, we compared trends in diatom assemblages, bacterial communities and metal concentrations in sediment cores from two lakes close to human infrastructure with those in a more remote lake. In the two lakes close to stations and the airport, we found heavy metal enrichments and diatom teratologies, as well as notable changes in diatom assemblages in one of these lakes, roughly coincident with the time when the first two stations were built (~1970). Due to the known association between diatom teratologies and metal enrichment, metal stress is a convincing explanation for these changes. Certain bacterial taxa determined to be indicators of pollution were also found to be more abundant in the impacted lakes in recent sediments (i.e., Hungateiclostridiaceae, OPB41, *Anaerovorax* and *Leptolinea).* Metal, diatom and bacteria changes observed in the lake more distant to infrastructure were more subtle and are likely related to climate change alone. Given the proximity of the affected lakes to the airport and roads, our data suggests that transportation infrastructure and activity on Fildes Peninsula is likely a key cause of contamination in the region's ecosystems. This study provides important insights into how human activities and climate change have affected Fildes Peninsula aquatic ecosystems and how they may respond to future stressors.

3.3 Introduction

The geography of the Antarctic Peninsula (AP) distinguishes it from the rest of the continent in aspects such as climate and human activities. The AP has experienced accelerated climate warming during the past 50 years and is amongst the most rapidly warming regions in the Southern Hemisphere (Turner et al., 2020). Maritime Antarctic temperatures show strong interannual variability and are highly dependent on seawater temperature and annual changes in sea ice extent (Meredith and King, 2005; Kejna et al., 2013). Superimposed on this interannual variability, Bellingshausen Station temperatures showed a significant ($p < 0.05$) increasing trend of 0.23 °C per decade between 1969 and 2024 (READER, 2025).

Scientific pursuits became an important component of anthropogenic Antarctic activities with the establishment of stations that permitted year-round human presence (Schiffer, 2013). For example, since Bellingshausen Station (Russia) was built in 1968, King George Island (situated in the AP) has seen a surge in the construction and expansion of research and other infrastructure, with twelve stations present on the island (COMNAP, 2025). These developments have placed increasing strain on sensitive polar ecosystems and led to a growing concern about the threats, including contamination of terrestrial and aquatic environments, modification of animal behavior and the introduction of non-indigenous species, that the increased human traffic may pose to the continent's natural systems (Holmes et al., 2006; Tin et al., 2009; Martins et al., 2010; Cowan et al., 2011; Choi et al., 2022). However, monitoring programs in general have only been undertaken since the agreement of the Protocol on Environmental Protection to the Antarctic Treaty, which came into effect in 1998. Paleolimnological techniques, by which lake sediments are used to reconstruct changes over time in lakes and their catchments, can be applied retrospectively to understand impacts caused by human activities (Smol, 2008). In Antarctic lakes, such approaches permit comparisons of present conditions with those of the past several decades and enable the determination of pristine baseline conditions by reaching samples prior to the onset of local human activities (Hodgson et al., 2004).

We studied the Fildes Peninsula, which in addition to being the largest ice-free area of King George Island and the site of numerous lakes is also amongst the areas of Antarctica most impacted by humans (Peter et al., 2013). Since the construction of Bellingshausen Station in 1968 the population of the peninsula has gradually increased with six permanent stations built between 1968 and 1994, belonging to Chile, China, Russia and Uruguay, that accommodate up to 375 people at peak occupation in the austral summer (COMNAP, 2025). An airfield constructed in 1980 and a harbor master station also serve as logistics hubs for entry by scientists and tourists to the South Shetland Islands (Braun et al., 2020). The strong human presence resulting from tourism and the high density of bases implies an accrued risk of environmental contamination, including from fuel spills, poor waste management, habitat destruction and damage to vegetation by vehicles, and indeed the ground around many stations has been suggested to show traces of contamination (Bargagli, 2008; Braun et al., 2012; Peter et al., 2013).

Several studies have focused on long-term regional climate and landscape evolution in the Fildes Peninsula (Tatur et al., 1991; García-Rodríguez et al., 2021; Oliva et al., 2023; Piccini et al., 2024), while one assessed changes on shorter time scales caused by human activities (Bertoglio et al., 2025). This study demonstrated that DNA was well preserved in sediments, allowing the detection of changes in bacterial communities over time, and identified indicator bacteria of human presence related to lakes impacted by metals, enabling their use as sedimentary proxies to reconstruct anthropogenic impacts. The only available assessment of recent changes in pre- and post-impact conditions used a top-bottom approach (Bertoglio et al., 2025); here we evaluated detailed profiles in three lakes of metal concentrations, bacterial DNA and diatom assemblages and teratologies in the sedimentary record to develop a comprehensive picture of the effects of human activities. We compared trends over the past century in microbial community composition in two lakes close to stations (Las Estrellas and Hotel lakes) to those in one more remote lake (Mondsee Lake) to assess the hypothesis that perturbations related to the stations have altered microbial diversity in lakes close to human activities.

3.4 Methods

3.4.1 Study area

Fildes Peninsula (62°11′ S, 58°58′ W) is located at the southwestern tip of King George Island in the South Shetland Islands of the maritime Antarctic region (Fig. 3.1). The 38 km^2 peninsula is surrounded by the waters of the Drake Passage, Fildes Strait and Maxwell Bay. It is free of ice except for its northeast end which is covered by the Collins Glacier (Fig. 3.1b).

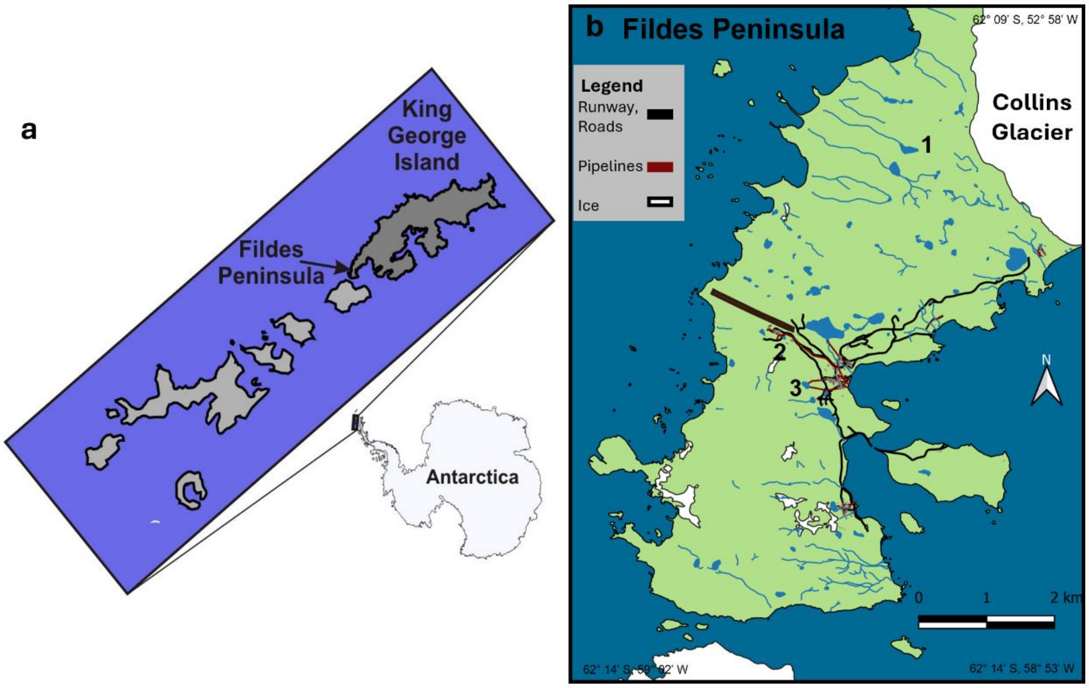

Figure 3.1. Location of the study region. a. King George Island and the Fildes Peninsula. b. Map of Fildes Peninsula indicating the study lakes: 1 = Mondsee, 2 = Hotel and 3 = Las Estrellas. Maps were created with geospatial data from the SCAR Antarctic Digital database, accessed 2021.

Numerous lakes that formed following glacial recession during the Late Holocene are present on the peninsula (Simonov, 1977), some of which are used as water sources for various stations. Our study included three Fildes Peninsula lakes (Fig. 3.1b): two are situated adjacent to stations, while one is more distant from human infrastructure. The lakes included Hotel Lake, which is adjacent to the Teniente Marsh Aerodrome and was formerly its water supply, and which has ceased to be used for drinking water due to elevated levels of heavy metals (Peter et al., 2013); Las Estrellas Lake, which previously supplied water to Escudero Station (Chile), and is ~200 m from the nearest roads and infrastructure; and Mondsee Lake, situated 3.5 km from Frei Station (Chile) and 2.6 km from Artigas Station (Uruguay).

3.4.2 Sampling

Sediment cores were taken in November-December 2016 (Las Estrellas and Mondsee lakes) and in December 2013 (Hotel Lake), at the deepest known point of each lake. Holes were drilled in the lake ice with a manual ice auger, and cores were recovered using a universal corer (Aquatic Research Instruments) with tenite butyrate core tubes of either 67 or 95 mm internal diameter. Based on sedimentation rates obtained from earlier studies, a general target length of 30 cm was established for the sediment cores, which was long enough to far exceed the period of human presence on Fildes Peninsula. The sediment-water interfaces were stabilized with sodium polyacrylate (Tomkins et al., 2008), and cores were then sealed and transported whole, cold and in the dark to Université Laval (Canada). The cores were then split lengthwise and sectioned at fine intervals for the analysis of diatoms, DNA, metals and radioisotopes. Subsamples followed visible layers in the stratigraphy of each sedimentary record and ranged from 0.1 to 0.5 cm thickness. Samples were studied to sufficient depths to reach at least the mid-20th century, in order to encompass the entire period of human influence on Fildes Peninsula and reach baseline conditions prior to local anthropogenic impacts. More details about the number of samples analyzed for each proxy as well as the sediment range included are available in Table C.1. All subsampling was carried out under sterile protocols to preclude contamination, and separate subsamples were taken for analysis of diatoms, DNA and radioisotopic dating, placed in sterile Whirl-Pak bags, and kept frozen at -80 °C until analysis.

3.4.3 Chronologies

Sediment chronologies were generated from ^{210}Pb activities measured by alpha spectroscopy at Chronos Scientific Inc. in Ottawa, Canada. Freeze-dried samples sectioned at fine intervals (average ~4 mm) were ground and spiked with ^{209}Po, after which HNO_3 (nitric acid) and HCl (hydrochloric acid) were added, and the samples were heated at 80 °C for 16 hours. The solutions were then centrifuged and evaporated to dryness three times, with HCl added after each cycle. Finally, the Po isotopes were electroplated on silver disks and measured using alpha spectroscopy. Age-depth models were generated with the R package serac using the constant rate of supply (CRS) model, with interpolated ages generated to cover the specific depths of the subsamples for all lakes and indicators (Appleby, 2001; Bruel and Sabatier, 2020).

3.4.4 Metals

Metal concentrations (i.e., Cd, Ni, Cr, Pb, Co, Zn, Cu, Fe, Mn, As, Se, Ti) were measured on ~1 g (dry weight) sediment subsamples using inductively coupled plasma-mass spectrometry (ICP-MS) in the Laboratory for the Analysis of Natural and Synthetic Environmental Toxins (LANSET) at the University of Ottawa, Canada. Duplicates, blanks and standard reference materials were used for quality assurance/quality control. Values are expressed in milligrams per kilogram of dry sediment (mg kg^{-1}).

Metal enrichment factors (EF) were calculated in each lake using the following equation:

$$\mathrm{EF}(x) = \frac{\left(\frac{x}{\mathrm{Ti}}\right)\mathrm{sample}}{\left(\frac{x}{\mathrm{Ti}}\right)\mathrm{reference}}$$

where x refers to the metal measured by ICP-MS; Ti is titanium, a reliable indicator of changes in allochthonous sedimentation (Davies et al., 2015) used to normalize metal concentrations; and reference represents pre-human background values, calculated using the last sample in each core that age models indicated to be from the 19th century (i.e., 1890, 1895, and 1876 CE in Mondsee, Hotel and Las Estrellas lakes, respectively). EF values around 1 show no sign of enrichment, while values over 3 and 10 show moderate and severe enrichment, respectively (Chen et al., 2007). EFs for Ti were calculated as concentrations relative to those of the reference sample.

3.4.5 Diatom analysis

0.1 g of dried sediment was prepared for diatom analysis by oxidizing organic matter with 30% H_2O_2. Following digestion, samples were allowed to settle for 24 h, the supernatants aspirated, and slurries were rinsed several times with distilled water to return them to neutral pH. Slurries were then placed on cover slips to dry on in a dust-free environment and fixed on microscope slides using Naphrax mounting medium. A minimum of 300 valves were counted in each sample using a Zeiss Axioscop 2 microscope equipped with differential interference contrast (DIC) optics at 1000x magnification under immersion oil. Primary taxonomic references for the identification of the diatom flora included Zidarova et al. (2016), Van de Vijver and Kopalová (2014), Sterken et al. (2015) and Wetzel et al. (2015). The identification of some species was verified using a scanning electron microscope (SEM) at

Centres Cientifics i Tecnològics of the Universitat de Barcelona (CCiTUB), Spain. In addition to taxonomic identifications, teratologies were enumerated in separate scans of 500 valves to determine the proportion of diatom deformities in each lake. Scans to determine the proportion of diatom deformities in each lake began with the surface sample and continued downcore until 3 consecutive samples showed an absence of teratologies after 200 valves were counted. The teratology percentage was based only on specimens found in valve view, as teratologies are generally not visible in girdle view (Lavoie et al., 2017).

3.4.6 DNA extraction, sequencing and taxonomic Assignment

To detect changes in bacterial community composition, bacterial diversity was analyzed by 16S rRNA amplicon sequencing. All material employed for manipulation of DNA sediment subsamples, as well as the vertical laminar flow cabinet (ESCO Class II, Type A2) where the DNA was extracted, was previously sterilized by irradiation with UV light. 0.5 g of wet sediment were aseptically transferred to sterile microtubes containing ceramic beads and an extraction buffer composed of 1% CTAB (Hexadecyltrimethylammonium bromide) and EDTA (Ethylenediaminetetraacetic acid) and homogenized with a FastPrep homogenizer (MP). After centrifugation at 12000 g the supernatants were subjected 3 times to chloroform:isoamyl alcohol (24:1) extractions and the pellets were precipitated with 0.6 volumes of cold isopropanol at room temperature during 24 h. These precipitates were then subjected to centrifugation for 40 min at 12000 g at room temperature, and the DNA obtained in the pellets was washed with cold ethanol, dried and suspended in ultrapure water overnight at 4 °C. The concentration and purity of DNA was determined spectrophotometrically at 260 and 280 nm, and its quality was checked by the amplification of a variable region (V4) of the ribosomal 16S gene. PCR (polymerase chain reaction) products were verified by gel electrophoresis on 1% agarose gel in 0.5X TBE buffer. DNA samples were sent to Novogene for Illumina MiSeq paired-end sequencing of the V4 region, using the 515F and 806R primers (Caporaso et al., 2011).

To correct for sequencing errors and create Amplicon Sequence Variants (ASVs), reads were processed in R using the DADA2 pipeline following a modified version of the DADA2 Bioconductor workflow (Callahan et al., 2017). Briefly, reads were filtered and trimmed by the *filterAndTrim* function based on the quality score which estimates the error probability of the DNA sequence. Reads with a maximum expected error (maxEE) greater than 2 were removed and based on quality profiles, reads were truncated at 200 and 190 bp for forward and reverse reads, respectively. Sequence variations were inferred with the *learnerrors* and *dada* functions. Chimeric sequences were eliminated, and taxonomy assignments from kingdom to genus were performed with *assignTaxonomy* based on the SILVA database (v138) (Quast et al., 2012). ASV sequences assigned as Archaea, Chloroplasts and Mitochondria were removed. Sequences obtained were submitted to the nucleotide archive GenBank with the BioProject ID PRJNA1365190.

3.4.7 Data analysis

Multivariate analyses were used to explore patterns in the variation over time of metals, diatoms and bacteria. Principal component analysis (PCA) was performed separately for each proxy (metals, diatoms and bacteria); the ICP-MS metals data were *log* transformed prior to PCA while the diatom and bacteria datasets were Hellinger transformed.

The relationship between shifts in metals and those of bacteria identified as indicators of human presence (Bertoglio et al., 2025) was explored by calculating Spearman correlations between EF values and the read abundances of the most abundant indicator bacteria.

3.5 Results and discussion

3.5.1 Chronology

Log^{210}Pb activities were low and showed roughly linear decreasing trends over time in each lake, with activities in surface sediments of 95.4, 61.2, and 189.7 Bq kg^{-1}, and reaching background at 55, 54 and 31 mm depth in Mondsee, Las Estrellas and Hotel lakes, respectively (Fig. 3.2). CRS models indicated that the year 1968 CE, when Bellingshausen base was established, corresponded to ~21, ~23, and ~5.7 mm depth in Mondsee, Las Estrellas and Hotel lakes, respectively, according to interpolated ages (Fig. 3.2).

3.5.2 Metal concentrations and enrichment factors

The metals with the greatest variation throughout the three sediment cores were Pb, Zn, As, Cu, and Cr (Fig. C.1). Mondsee Lake showed little change, with concentrations that ranged between 6.7 and 7.5 mg kg^{-1} for Pb, 51.4 and 59.9 mg kg^{-1} for Zn, As between 3.3 and 8.2 mg kg^{-1}, Cu between 84.4 and 111.5 mg kg^{-1} and Cr between 7.9 and 10.2 mg kg^{-1} (Fig. C.1). There was greater variation in metals in Las Estrellas Lake, with concentrations ranging between 5.2-10.6 mg kg^{-1} for Pb, 39.8-148.8 mg kg^{-1} for Zn, 2.6-9.7 mg kg^{-1} for As, 44-107.2 mg kg^{-1} for Cu and 10.6-18.3 mg kg^{-1} for Cr (Fig. C.1).

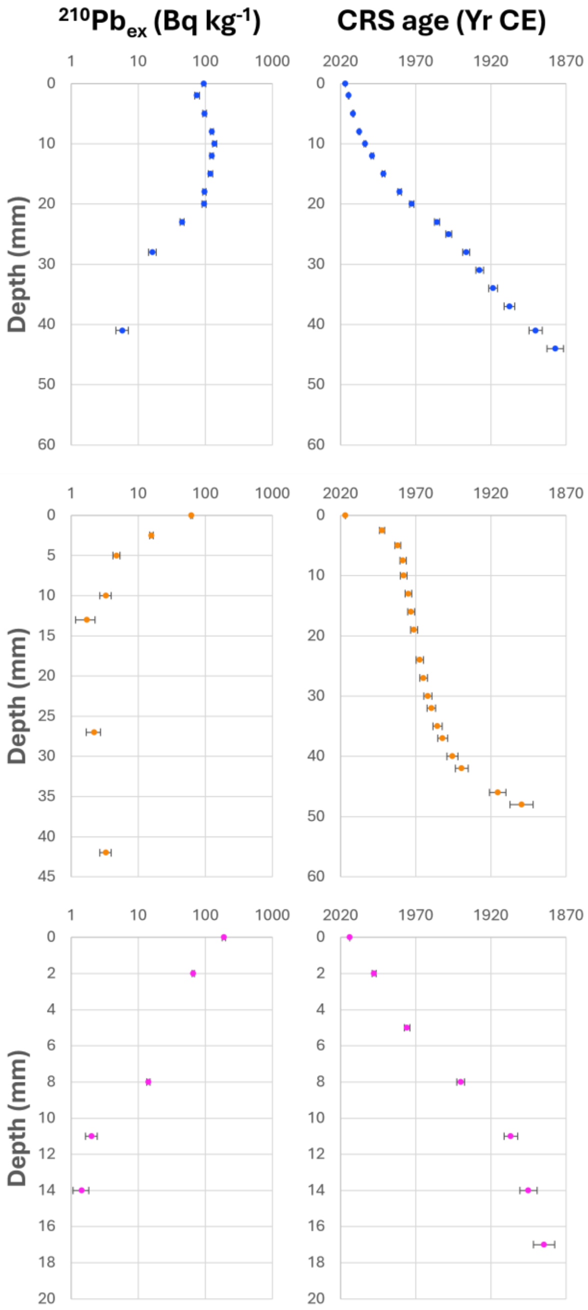

Figure 3.2. Unsupported ^{210}Pb activities ($^{210}Pb_{ex}$) and age-depth relationships established with the constant rate of supply (CRS) model for the three study lakes. Top: Mondsee Lake; middle: Las Estrellas Lake; bottom: Hotel Lake.

Hotel Lake showed by far the highest metal concentrations as well as the greatest variation, varying between 5.4-683.9 mg kg^{-1} for Pb, 47.3-350.7 mg kg^{-1} for Zn, 1.8-5.4 mg kg^{-1} for As, 62-189.4 mg kg^{-1} for Cu and 9.9-30.5 mg kg^{-1} for Cr (Fig. C.1).

The first two axes of the PCA of metal concentrations explained 80.0% of the variance in the dataset, with PC1 explaining 62.1% and PC2 explaining 17.9%. First axis scores for Mondsee Lake did not change through the core, while Las Estrellas Lake PC1 values undulated slightly between 1950 and the present, and Hotel Lake PC1 values changed markedly beginning between 1940 and 1998 (Fig. 3.3).

Enrichment factors for Ti were close to 1 and showed little variation throughout all three sediment cores, indicating relatively constant supplies of allochthonous sedimentation over time (Fig. 3.4). The highest EFs were observed for Pb, Zn, As, Cu, Cr and Cd (Fig. 3.4, Table C.2), apart from Mondsee Lake where EFs throughout the core were close to 1 (i.e., no enrichment) except for As, which increased moderately between 2012 and 2017 reaching a maximum EF of 3.0 in 2015 (Fig. 3.4). EFs for Pb, Cu Cr and Cd in Las Estrellas Lake were close to 1 and varied little in the core, except in 2017 for Pb which was moderately enriched (EF: 2.9) and Cr that increased somewhat but at 1.9 remained below threshold for moderate enrichment (i.e., 3; Chen et al., 2007) (Fig. 3.4). The metal showing the greatest enrichment in Las Estrellas Lake was Zn, which increased upwards from 1974 to the surface, with moderately enriched values in 1982 and a maximum at the surface of the core (2017) of 5.7 (Fig. 3.4). As was moderately enriched from 1962 to the most recent sample (2017), with the maximum values in 1974 and 1978 (EFs: 4.9 and 5.6 respectively; Fig. 3.4). EFs of many metals in Hotel Lake showed pronounced enrichment and had similar trends, increasing slightly in the early 20th century, and greatly after ~1976 to a maximum in 1998, and then decreasing somewhat in the surface sediment (Fig. 3.4). Pb, Zn and Cd showed severe enrichment in 1998 (maximum EFs of 146.2, 14.9 and 10.6) while As, Cu and Cr showed moderate enrichment at this point (maximum EFs: 6.1, 5.5 and 6.2) (Fig. 3.4).

3.5.3 Diatom composition

Ninety-one diatom taxa belonging to 30 genera were identified in the three study lakes (Table C.3, Fig. C.2). In general, assemblages were dominated by small benthic diatoms

(<20 µm), mainly from the genera *Achnanthidium*, *Psammothidium*, *Planothidium*, *Sellaphora* and *Staurosirella*.

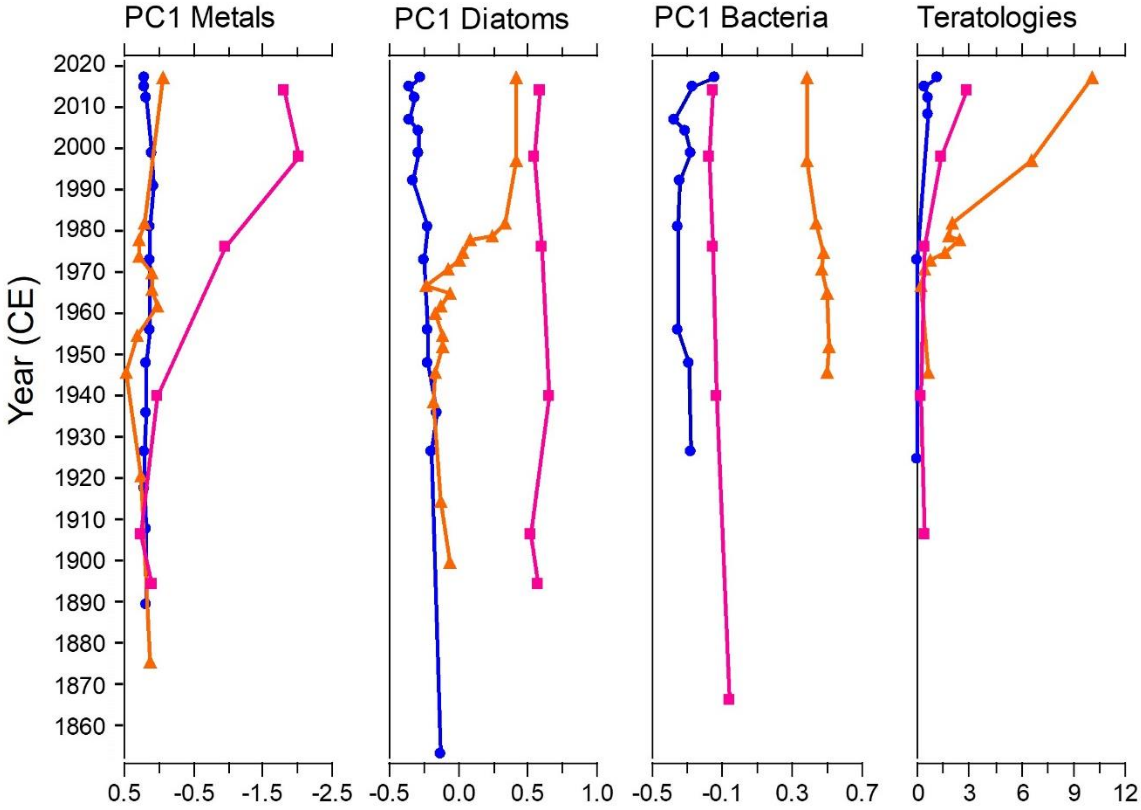

Figure 3.3. PCA axis 1 scores (PC1) for metals, diatoms and bacteria, and the frequency of diatom teratologies in the three study lakes. Blue lines: Mondsee Lake; orange lines: Las Estrellas Lake; pink lines: Hotel lake.

A total of 78 species from 25 different genera was identified in Mondsee Lake. Some species showed pronounced stratigraphic changes. There was a step-change in *Achnanthidium indistinctum,* which averaged 4.2% relative abundance prior to ~1991 and 26.5% thereafter. This was coincident with an increase of *Planothidium frequentissimum* and decreases in *Staurosirella antarctica* and other, less abundant species including *Chamaepinnularia australomediocris* and *Psammothidium confusoneglectum* (Fig. 3.5).

Seventy-four diatom species from 28 genera were identified in the Las Estrellas Lake core. There were three horizons of pronounced assemblage change in the core: at ~1955, ~1968 and ~1976. After ~1955, assemblages that had shown relative stability were marked by increases in *Achnanthidium indistinctum*, *Staurosirella antarctica* and *Psammothidium incognitum*, and the period had low abundances of *Planothidium renei*. Between ~1968 and ~1976, there was an abrupt decrease of *Achnanthidium indistinctum* from 22% relative abundance to values ~1%, an abrupt increase of *Staurosirella antarctica*, and gradual increases of *Planothidium renei*, *Stauroneis sofia* and *Psammothidium abundans*. Around 1975, there were sharp decreases of *Staurosirella antarctica* and *Planothidium renei* coincident with sharp increases in *Sellaphora nigri* and *Cavinula pseudoscutiformis* (Fig. 3.5).

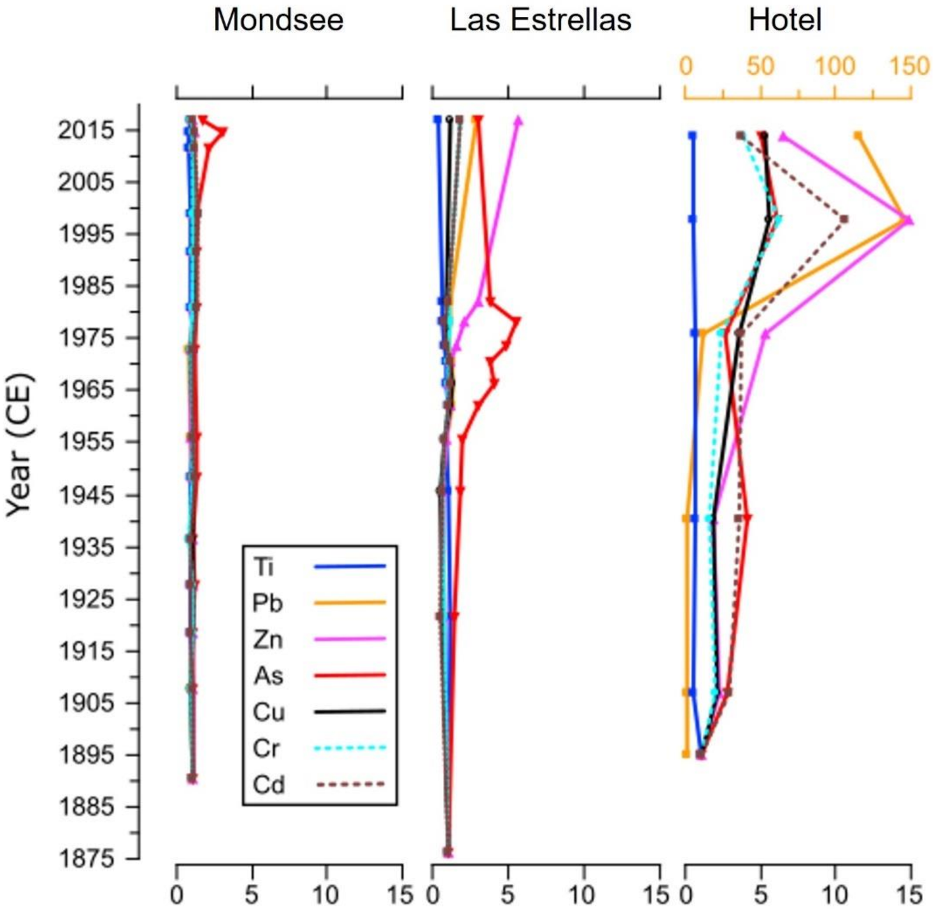

Figure 3.4. Enrichment factors (EFs) for metals: Ti, Pb, Zn, As, Cu, Cr and Cd in the three study lakes. Note the different x-axis for Pb in Hotel Lake (10x exaggeration, top).

A total of 50 species from 18 genera was identified in the Hotel Lake core, with no taxa having an average abundance exceeding 20%, representing the lowest diversity among the three lakes. The species that showed the most evident trend was *Psammothidium papilio*, which was abundant in the lower part of the core (average 10.3%, 1867-1907) and then decreased dramatically above this horizon (average 0.8%), as well as *Psammothidium abundans* which also peaked ~1907 but had a higher abundance in the upper section of the core (between 10 and 18%). *Sellaphora nigri* was present at generally low relative abundances during most of the 20th century until the most recent sample (2014) when it increased to a relative abundance of 14% (Fig. 3.5), and *Achnanthidium maritimo-antarcticum* increased after 1976, but with lower relative abundances (between 0.3 and 5%) (Fig. 3.5).

The first axis of the diatom PCA explained 33.8% of the variation in the diatom data while the second axis explained 16.6%. Based on first axis scores, the most pronounced change in diatom composition occurred in Las Estrellas Lake from 1967 to 1979, while compositional changes in Mondsee and Hotel lakes were less notable (Fig. 3.3).

3.5.4 Bacterial composition

We detected a total of 14,567 ASVs across the three cores. Rarefaction curves based on the observed ASVs reached plateaus, indicating that sequencing depth was adequate to capture the overall diversity of all lakes (Fig. C.3). As was observed in our previous study (Bertoglio et al., 2025), bacterial communities from each lake were composed of a few very abundant ASVs while most taxa had low abundances. In Mondsee Lake the taxa that showed the most evident trends over time were *Sulfurifustis* and Phycisphaerae, which decreased after ~1956 (Fig. 3.6). Additionally, certain species dominated in the most recent sample in Mondsee Lake (2017) while others decreased dramatically. For example, *Janthinobacterium,* Rokubacteriales, *Delftia*, *Clostridium* sensu stricto 13, and *Desulfosporosinus* were more abundant in this sample, whereas *Desulfatirhabdium* and P9X2b3D02 (phylum Nitrospinota) decreased (Fig. 3.6).

The abundances of several bacteria in Las Estrellas and Hotel lakes changed notably after ∼1970 (Fig. 3.6). For example, Holophagaceae and *Desulfatirhabdium* increased while Phycisphaerae and CCM11a decreased in both lakes (Fig. 3.6). Other groups also showed considerable shifts but with different patterns in each lake, such as *Pseudomonas* and Zixibacteria that increased after ∼1970 in Las Estrellas Lake while Phycisphaerae decreased, and MBNT15, Latescibacterota, Rokubacteriales and Zixibacteria decreased in Hotel Lake after ~1976.

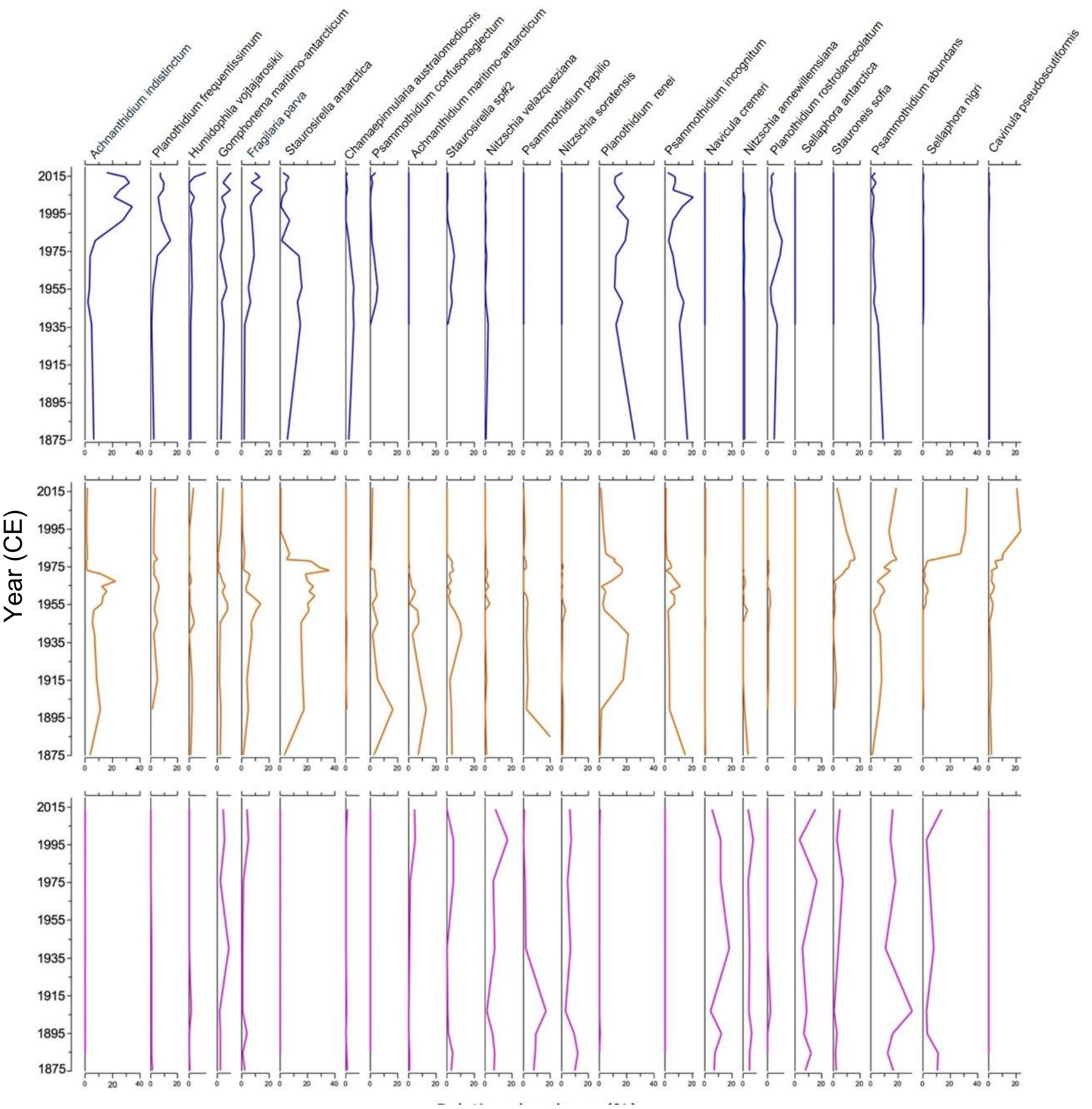

Figure 3.5. Relative abundances of the most abundant diatoms and those that changed the most over time. Top: Mondsee Lake; middle: Las Estrellas Lake; bottom: Hotel Lake.

The first axis of the bacteria PCA explained 28.9% of the variation in bacterial composition data while the second axis explained 18.0%. In addition, based on first axis scores, the most pronounced change was observed in the most recent sample of Mondsee Lake (2017), whereas changes in Las Estrellas and Hotel lakes were more muted (Fig. 3.3).

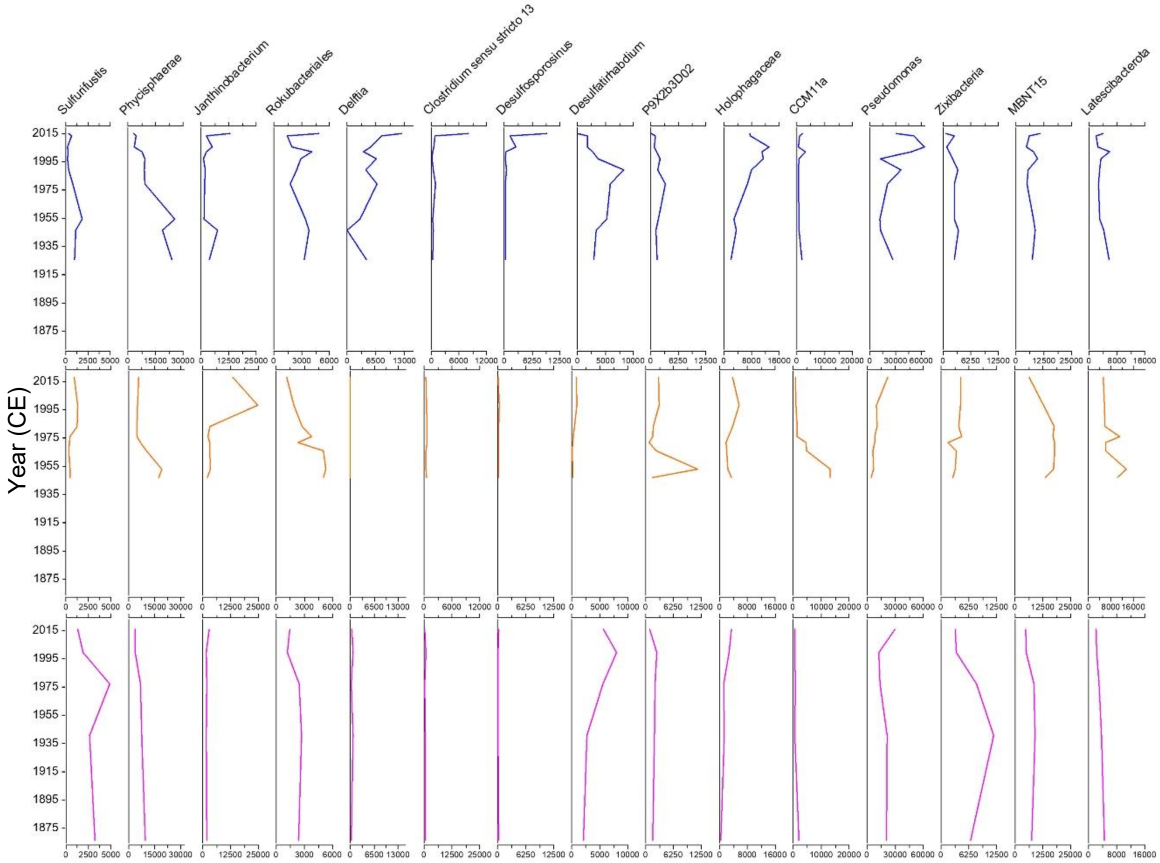

Figure 3.6. Read abundances of the most abundant bacteria and those that changed the most over time. Top: Mondsee Lake; middle: Las Estrellas Lake; bottom: Hotel Lake.

3.5.5 Diatom teratologies and bacterial indicators of contamination

Diatom teratologies are cell morphology deformations that have been observed to increase in contaminated water bodies, particularly those with high concentrations of heavy metals and pesticides (Falasco et al., 2021). In Mondsee Lake they had a maximum occurrence of 1.2% (Fig. 3.3). Teratologies were most notable in Las Estrellas Lake, where they increased between 1975-1982 to ~2% of the enumerated valves, and sharply thereafter with frequencies of 6.6 and 10.0% (in 1997 and 2017, respectively), including 30% of the valves *of P. abundans* (Fig. 3.3). The surface sample of Hotel Lake also had more prevalent teratologies, increasing from 1998 to a maximum of 2.9% deformed valves in the surface sample (Fig. 3.3).

We tested correlations between different metal EFs and the abundances of certain bacteria that were identified as indicators of contamination (i.e., *Anaerovorax,* Hungateiclostridiaceae, OPB41, *Pseudorhodoplanes*, and *Leptolinea*; Bertoglio et al., 2025). Of the five taxa evaluated, all except *Pseudorhodoplanes* were positively correlated with enrichments of multiple metals (Table 3.1). Pb, Zn, Cu, Cr and Cd had positive, significant and high correlation coefficients (i.e., R^2 = 0.40 - 0.70) with between two and four taxa each (Table 3.1).

Table 3.1. Spearman correlations between indicator bacteria and metal EFs. R^2 values are shown in the table, and significant correlations ($p < 0.05$) are indicated by bold values.

Indicator bacteria	Ti	Pb	Zn	As	Cu	Cr	Cd
Anaerovorax	0	**0.53**	**0.53**	0	**0.54**	**0.57**	**0.70**
Hungateiclostridiaceae	0	0	0	0	**0.59**	**0.40**	**0.61**
OBP41	0	0	0	0	**0.48**	**0.45**	**0.66**
Pseudorhodoplanes	0	0	0	0	0	0	0
Leptolinea	**-0.52**	**0.51**	**0.58**	0	**0.54**	**0.60**	**0.69**

3.5.6 Separating the effects of environmental change and anthropogenic activities

Fildes Peninsula lakes are subject to multiple stressors, including rapidly rising temperatures and strong UV exposure due to ozone depletion, in addition to any anthropogenic impacts. We hypothesized that, due to its remoteness from infrastructure, Mondsee Lake could be used as a control site to determine changes in diatom and bacterial communities due primarily to natural environmental change, including ongoing warming. This hypothesis was supported by the differing trends in metal enrichment between Mondsee Lake and the other two sites (Fig. 3.4).

The limnological consequences of recent warming in maritime Antarctica include changing lake ice cover and thermal stratification regimes (Izaguirre et al., 2021). Decreases in ice cover duration influence diatom communities, as has been observed in many lakes in the Northern Hemisphere (Lotter and Bigler, 2000; Sorvari et al., 2002; Smol et al., 2005; Rühland et al., 2015). Although somewhat muted, recent diatom shifts in Mondsee Lake likely reflect changes to ice cover duration and extent, including declines of *Staurosirella antarctica*, which is associated with colder conditions and prolonged ice cover (Lotter and

Bigler 2000; Keatley et al., 2008), and increases after ~1991 of *Achnanthidium indistinctum*, a taxon that thrives in nearshore lake environments where external disturbances such as wind are common (McCabe and Cyr 2006; Cyr, 2016). A coincident decline in *Staurosirella antarctica* in Las Estrellas Lake (after the mid-1970s) also likely reflects the broader effects of climate warming on ice cover duration and thickness, although the species was absent in the 20th century in Hotel Lake. In fact, diatom assemblages in Hotel Lake showed no systematic changes, either in overall assemblage composition or in the abundances of most taxa. Hotel Lake had much thicker ice cover than the other two lakes during spring 2017 and autumn 2018 (1.60 and 0.55 m vs. 0.98 and 0.13 m on average for both seasons, respectively; Bertoglio et al., 2023). Although the precise reasons are uncertain, Hotel Lake is located in the upper section of the Grande Valley, which appears to channel cold winds between the Drake Passage to the west and Maxwell Bay to the east (pers. obs.). Its thick ice may therefore reflect a colder microclimate and imply the delayed onset of the effects of warming in this lake.

Certain bacteria found with higher abundances in the most recent sample of Mondsee Lake may potentially be associated with the consequences of warming. For example, *Janthinobacterium* produce the pigment violacein which confers resistance to UV radiation (Alem et al., 2020), which is expected to increase as ice cover thins. Moreover, Rokubacteriales and Desulfosporosinus are common soil taxa that were also found in peatlands (Pester et al., 2010; Ivanova et al., 2021), and their increases could be related to increasing runoff and aeolian deposition in longer melting seasons. The increase of *Janthinobacterium* after ~1995 in Las Estrellas Lake may also be a consequence of climate warming; it also increases in Hotel Lake but much more subtly. This reaffirms the muted nature of climate change effects in Hotel Lake as suggested by diatom trends. The lack of increases in other bacteria taxa reflecting climate change in Hotel and Las Estrellas lakes may be because the influence of climate change in shaping communities in these lakes is overshadowed by the pronounced impact of anthropogenic activities. Indeed, in the presence of multiple stressors related to climate change and human activities, lakes may differ in responses compared with those that only experience single stressors (Jackson et al., 2016).

The increase in metal EFs in Las Estrellas and Hotel lakes since the establishment of bases on the peninsula is consistent with our hypothesis that the sites nearest human activities would show increases in contaminants over time, and the contrast with the lack of changes in Mondsee Lake suggests that these increases cannot be ascribed to natural environmental changes. The changes we observed in Las Estrellas and Hotel Lakes therefore represent the cumulative effects of climate change and human impacts. There is growing evidence for human impacts in terrestrial and marine ecosystems in Maritime Antarctica. For example, elevated Cu, Zn, Cd and Pb have been found in marine sediments, soil, lichens and mosses from Fildes Peninsula close to stations and contaminated sites (Aronson et al., 2011; Lu et al., 2012; Padeiro et al., 2016; Pereira et al., 2017; Fabri-Jr et al., 2018). These results are comparable to our findings, since heavy metal contamination may be related to intense human activity such as transportation, fossil fuel combustion, accidental oil spills, waste incineration and sewage disposal (Chu et al., 2019). Our results give temporal context to these findings, showing that the affected sites not only had high metal concentrations but that they increased over time, while those in our remote site showed little to no change over the same period.

Biological proxies also showed greater changes in the proximal vs. remote lake and provided evidence of the ecological effects of anthropogenic impact. While shifts in overall community composition of diatoms and bacteria did not differ markedly between the three lakes, we found trends in taxa that were indicators of pollution and may thus have adaptive advantages in metal-impacted environments. The diatom *Sellaphora nigri* is an indicator species that is found in greater abundances in environments where eutrophication, organic contaminants or pollution by pesticides and heavy metals are observed (Morin et al., 2012, 2014; Wetzel et al., 2015). Given its tolerance to pollution, the increase in *S. nigri* relative abundances in Las Estrellas Lake around 1975, coincident with increasing human activities as well as with the construction of numerous stations in the subsequent years (Braun et al., 2012; Peter et al. 2013) may therefore reflect contamination due to human activity. The increase of this species in the surface sediments of Hotel Lake, while less than that observed in Las Estrellas Lake, may also reflect such impacts. By comparison, *S. nigri* always had abundances <1% in Mondsee Lake, which reinforces the hypothesis of the pristine nature of the lake relative to our other sites.

Diatom teratologies represent an individual-level response to environmental stress (Falasco et al., 2021). Deformed frustules associated with heavy metal stress have been reported in various studies (Falasco et al., 2009; Cantonati et al., 2014; Pandey and Bergey, 2018).

While the exact mechanism of the deformations has not been demonstrated, it has been suggested that contaminants alter cell membrane polarity and cause cytoplasmic acidification, leading to disruption of cytoplasmic homeostasis (Pinto et al., 2003). While some authors have attributed diatom teratologies to high UV exposure (summarized in Falasco et al., 2021), such as that due to thinning of the stratospheric ozone layer in the Antarctic, this mechanism cannot explain the differing trends between Mondsee Lake and the other two sites. The increase in teratologies in Las Estrellas and Hotel lakes, and their absence in Mondsee Lake, however, is consistent with the observed changes in metal enrichment and thus demonstrates the ecological effects of pollution.

Within bacterial communities, particular taxa with higher tolerances to contaminants became more abundant over time in Hotel and Las Estrellas lakes, such as *Sulfurifustis* and *Desulfatirhabdium*. These sulfur bacteria may be related to the presence of pollutants as they can employ a variety of electron donors or inorganic sulfur compounds as electron acceptors (Balk et al., 2008, Kojima and Fukui 2015), such as those found in contaminants originating from the burning of fossil fuels. However, we do not exclude that the presence of these bacteria may be related to changes in sediment habitats, for example due to anoxia, influencing active bacteria rather than reflecting historical changes. *Desulfatirhabdium* increased in both Las Estrellas and Hotel lakes after ~1982, while *Sulfurifustis* also increased in Las Estrellas Lake over the same period. Neither increased in abundance in Mondsee Lake where their abundances in fact decreased there after ~1956 and 1991, respectively. Several groups of sulfur bacteria (e.g. Geobacteraceae, Desulfurivibrionaceae and Rhodobacteraceae) were also previously observed in the bacterial community in water samples from Hotel Lake (Bertoglio et al., 2023). Moreover, the significant relationships between bacterial indicators of pollution and metal EFs (i.e., Hungateiclostridiaceae, OPB41, *Anaerovorax* and *Leptolinea*; Bertoglio et al., 2025), provide further evidence that human impacts are modifying aquatic communities in Fildes Peninsula's lakes.

3.6 Conclusions

Lakes in maritime Antarctica are subject to stress from rapidly warming climates, and, in some cases, from anthropogenic activities in their catchments. We analyzed sedimentary proxies (metals, diatoms and bacterial DNA) in three lakes and showed notable changes in metal enrichment, diatom teratologies and bacteria indicators of pollution in two (Las Estrellas and Hotel lakes) that were located near to logistics infrastructure. The changes of the same indicators in Mondsee Lake, more distant from human activities, were muted by comparison. We conclude that Mondsee Lake represents the baseline of recent changes due to climate change, and that the more pronounced shifts in the other two lakes can therefore not be attributed to warming alone. Although based on our study we cannot draw direct causal links between changes in lake sediments and anthropogenic activities, the trends we observed provide strong evidence for significant human effects on aquatic ecosystems. Further studies are needed to better quantify the effects and prevent further deterioration of these sensitive Antarctic environments.

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General conclusion

This thesis explored the influence of environmental variations caused by human activities, including climate change, on the lakes of the Fildes Peninsula. This was possible through the analysis of changes in microorganism diversity in sediments and in the water column, representing one of the few existing studies where the subject has been explored at fine temporal scales.

The first chapter highlighted the importance of ice cover in such lakes, where summer melt each year alters microbial diversity mainly by a decrease in bacterioplankton diversity. Runoff from lake basins during the ice-free period selecting only a few bacteria species could explain this. These results underline the importance of understanding bacteria responses to warming, such as longer lake ice-free periods.

The second chapter analyzed the same lakes but using a top-bottom paleolimnological approach through the analysis of sedimentary metals and microbial communities, elucidating the extent of anthropogenic impacts in the region. This chapter demonstrated that bacteria are also a good proxy of environmental changes related to human impact. However, due to issues with the preservation of sedimentary pigments, it was not possible to reconstruct phytoplankton community changes from pigment diversity. Based on certain bacteria with environmental affinities with two lakes which showed metal enrichment, we established a method for targeting specific bacteria as indicators of human activities. This study revealed that the main contaminants affecting the lakes may be associated with transportation.

The third chapter explored in detail bacterial, diatom and metal dynamics in sedimentary profiles in two impacted lakes as well as one non-impacted lake more distant from human activities. This study complemented the second chapter in several aspects, for example by the absolute quantification of metal concentrations, and suggested that transport could be the main cause of metal contamination in the lakes. The strong relationship between the indicator bacteria identified in the second chapter and changes in metal concentrations reinforced their utility as indicators of human activities. Likewise, the analysis of diatoms represented an excellent complement to bacteria as they were good indicators of environmental change not only through changes in species abundances, but also through the frequency of teratologies observed in their valves.

In this thesis I concluded that the main changes associated with anthropogenic effects on the evaluated communities were at the species or individual level, and were reflected in the selection of certain species of bacteria as well as the presence of teratologies in diatoms. Furthermore, the changes observed at the community level in diatoms could be also due to the effects of local human activities in the area, but also due to the rapid climate change experienced in the region during the last several decades. As increases in both temperature and traffic are expected in Antarctica, the diversity of microorganisms in the future may be affected to a greater extent and because microorganisms perform key functions for ecosystems, this could result in serious consequences.

One of the main limitations in the evaluated sedimentary proxies is the preservation of sedimentary pigments and DNA. This thesis demonstrated that there was good preservation of the DNA throughout the cores since DNA was well and equally amplified in the different sediment layers, as compared to indications of poor pigment preservation. A further potential limitation for sedimentary DNA is the distinction between past DNA deposited in sediments from that of extant sediment bacterial communities. In my thesis it was not possible to distinguish the two. However, the changes detected in bacterial communities through sedimentary DNA appear to indicate that they have responded to changes in the environment in a consistent manner.

Perspectives

This thesis constitutes a contribution to the awareness of the impact that humans are having on Antarctica. The need to enhance the protection of Antarctica is clear, and because the main source of anthropic pollution is related to transportation, decreasing human access through flights should be an urgent measure. This includes tourism, which although there are protocols for environmentally friendly tourism the measures should be as extreme as prohibiting it or restricting numbers, but also to be more conscientious when carrying out research activities. Avoidance of overlapping methodological approaches by different research projects, and encouraging cooperation between scientists, including exchanges of data, should be emphasized. Also, research projects that do not require access to Antarctica should be considered, such that research can be continued with samples previously taken and preserved for future analysis. The environmental impact of local activities could clearly be greatly reduced by improving practices such as waste management, fuel handling and building construction, but other measures limiting visitors' access are necessary.

The results here obtained suggest that the use of environmental bacterial DNA should be considered as reliable indicators for use in Antarctic monitoring programs. Also, at a scientific level, considering different populations of microorganisms in addition to bacteria using DNA should prove useful but may also lead to different methodological challenges. For example, shotgun metagenomics (i.e., the sequencing of the total DNA from environmental samples) also allows functional inferences as functional genes are also included in the sequencing.

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Appendix A: Supplementary material of chapter 1

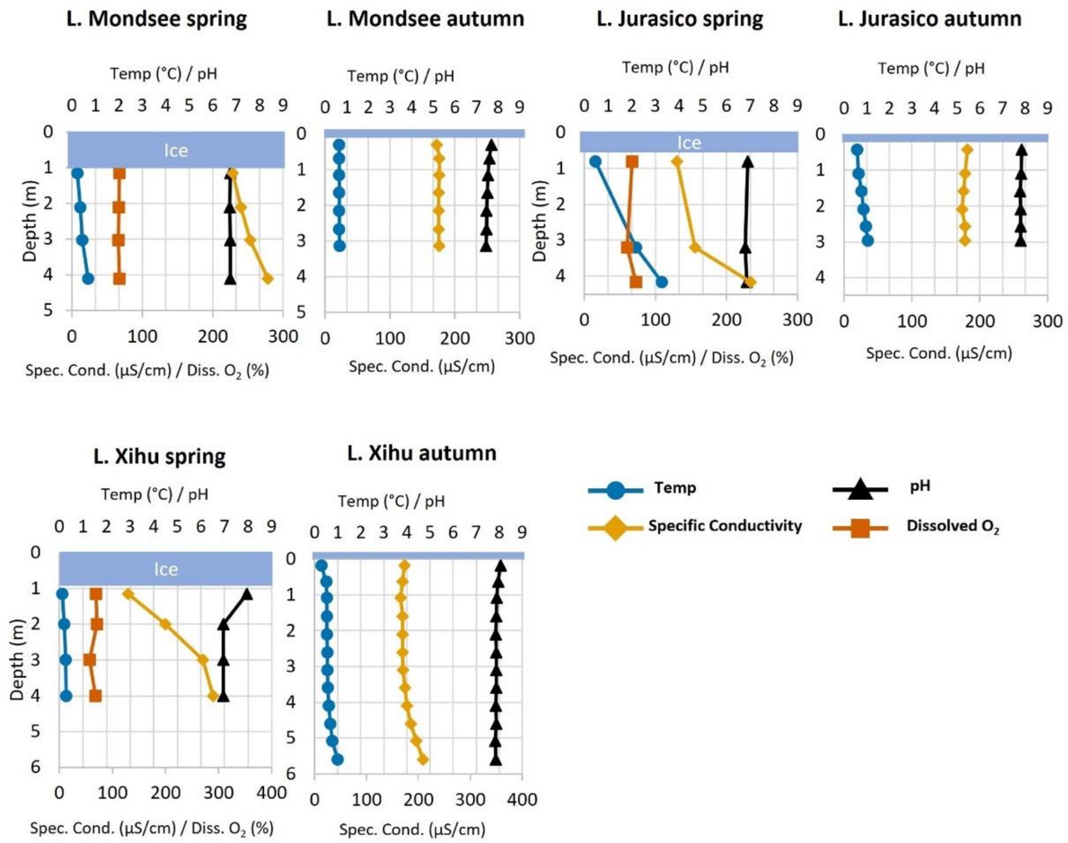


Figure A.1. Water column profiles of Fildes Peninsula lakes. Temp = temperature. Spec. cond. = specific conductivity. Diss. O_2 = dissolved oxygen. Note the differing x and y axis scales.

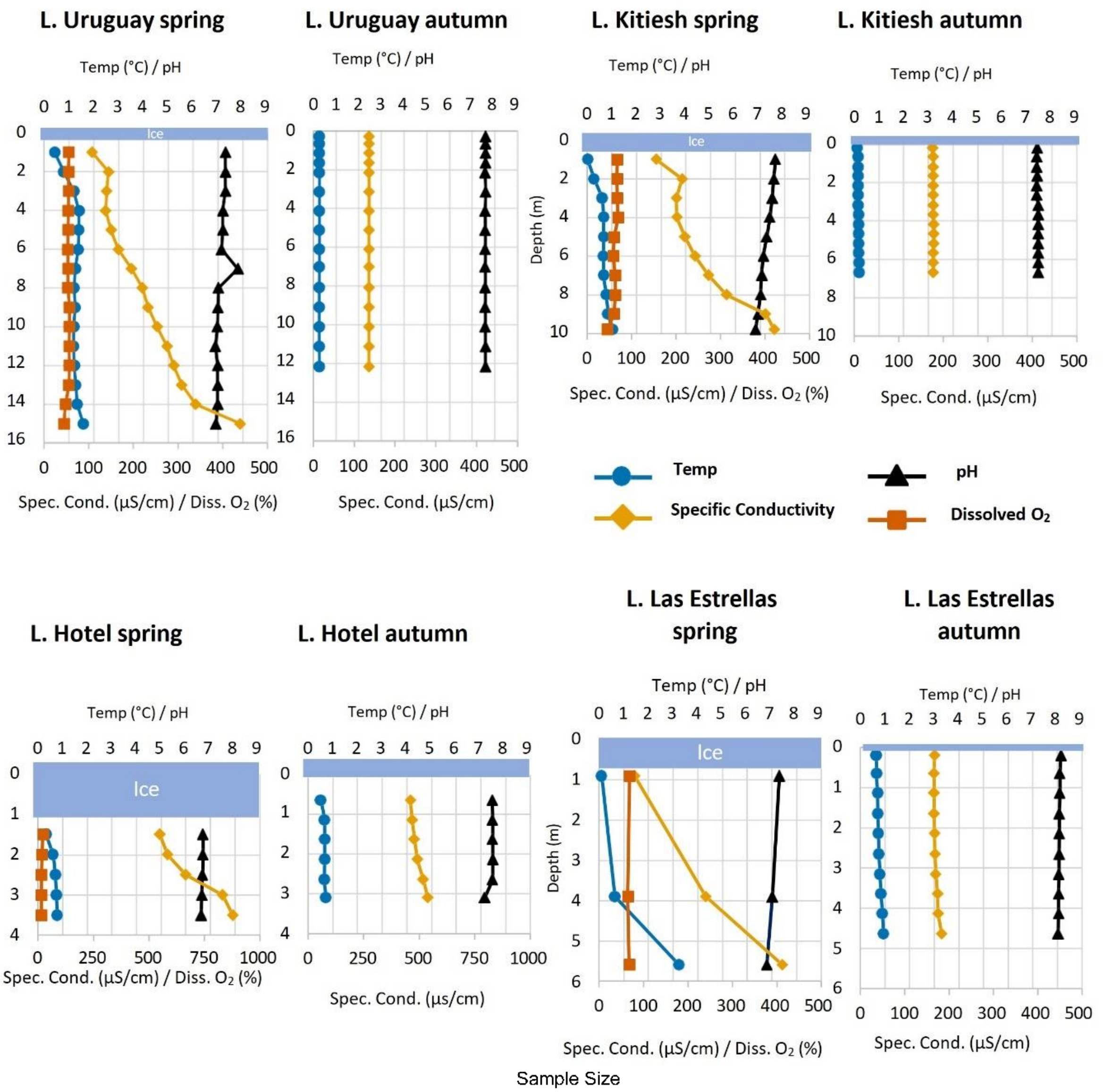


Figure A.1 cont'd. Water column profiles of Fildes Peninsula lakes. Temp = temperature. Spec. cond. = specific conductivity. Diss. O2 = dissolved oxygen. Note the differing x and y axis scales.

Table A.1. Volume of water filtered for pigment and DNA extraction. NA: not available.

Lake	Season	Volume filtered for phytoplankton (ml)	Volume filtered for bacterioplankton (ml)
Mondsee	Spring	700	1000
	Autumn	345	588
Jurasico	Spring	700	1000
	Autumn	1000	1000
Uruguay	Spring	700	1000
	Autumn	690	1000
Kitiesh	Spring	NA	1000
	Autumn	420	725
Hotel	Spring	250	750
	Autumn	215	250
Las Estrellas	Spring	500	1000
	Autumn	950	1000
Xihu	Spring	500	750
	Autumn	500	1000

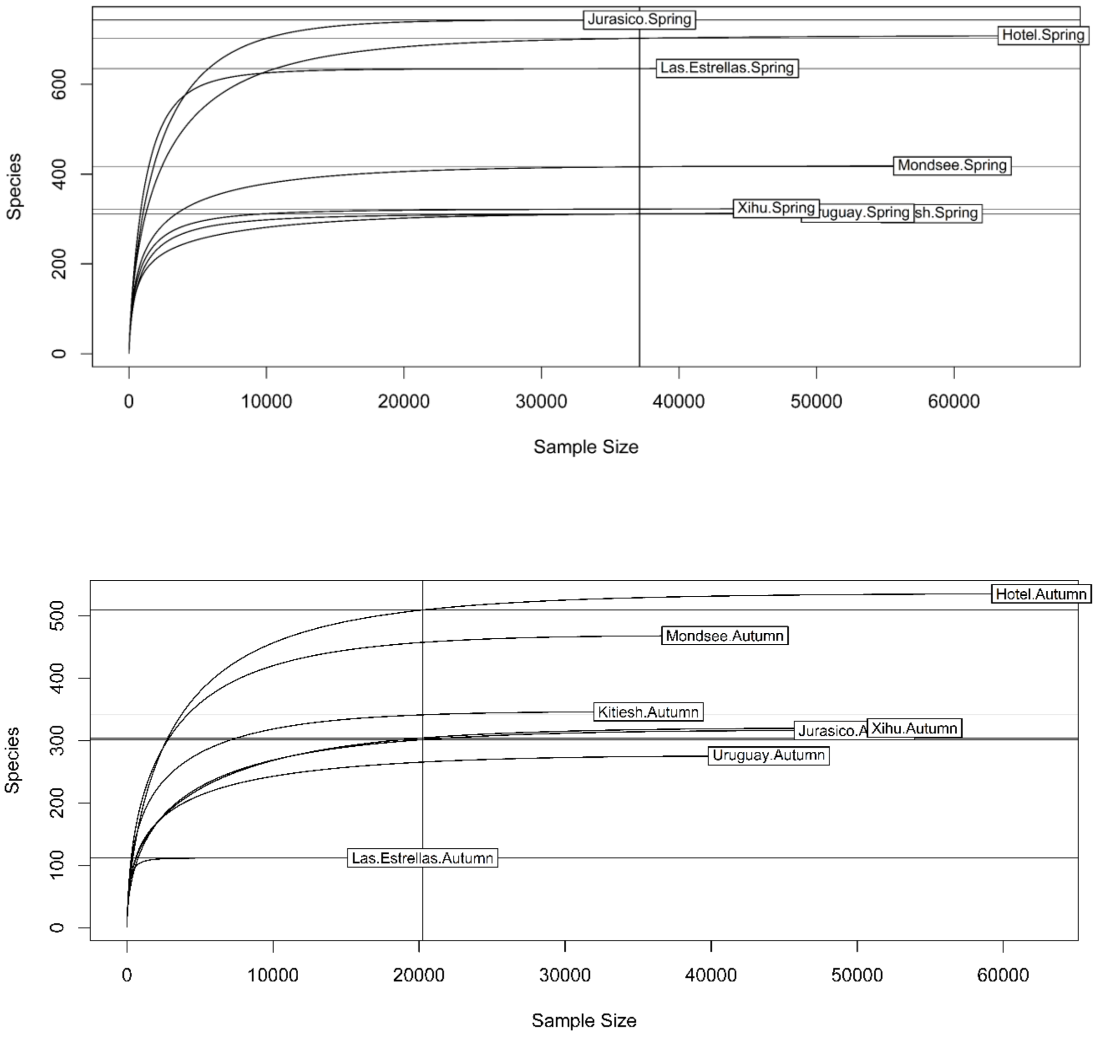


Figure A.2. Rarefaction curves for spring (a) and autumn (b).

Table A.2. Chlorophyll concentration (ng L^{-1}). Chl-*a*: Chlorophyll *a*, Chl-*b*: Chlorophyll *b*, Chl-c_1: Chlorophyll c_1, MgDVP: Mg-3,8-divinyl-pheoporphyrin a_5 monomethyl ester. NA: not available.

Lake	Season	Chl-a	Chlorophyllide a	Chl-b	Chlorophyllide b	Chl-c1	MgDVP	Pheophorbide a	Pheophytin a
Mondsee	Spring	649.97	179.78	48.87	4.10	0.00	0.00	0.00	128.58
	Autumn	657.06	71.63	44.72	0.00	0.00	2.41	87.47	150.03
Jurasico	Spring	132.24	17.50	5.92	0.00	0.00	0.00	0.00	45.91
	Autumn	433.82	60.43	40.31	3.38	7.44	0.90	0.00	42.44
Uruguay	Spring	213.69	72.02	18.33	0.00	0.00	0.00	0.00	38.49
	Autumn	440.85	87.86	32.61	7.12	0.00	0.00	0.00	64.91
Kitiesh	Spring	NA	NA	NA	NA	NA	NA	NA	NA
	Autumn	783.40	83.93	45.18	0.00	0.00	10.78	0.00	205.74
Hotel	Spring	45.85	3.28	13.80	0.00	0.00	0.00	0.00	29.29
	Autumn	9606.82	246.37	202.62	0.00	0.00	0.00	1943.87	1454.68
Las Estrellas	Spring	199.27	0.00	9.05	0.00	0.00	0.00	0.00	0.00
	Autumn	700.96	0.00	28.99	0.00	0.00	0.00	0.00	26.38
Xihu	Spring	117.97	43.38	18.28	0.00	0.00	0.97	0.00	18.77
	Autumn	579.74	51.99	37.65	0.00	0.00	7.77	0.00	94.89

Table A.3. Unknown carotenoid concentrations with the absorption maxima and retention times (ng L^{-1}). NA: not available, ND: not detectable.

Lake	Season	Unk. carotenoid 455, 480 nm 8.43 min	Unk. carotenoid 470 nm 11.80 min	Unk. carotenoid 460 nm 12.00 min	Unk. carotenoid 475 nm 15.90 min	Unk. carotenoid 475 nm 17.80 min	Unk. carotenoid 450 nm 19.30 min	Unk. carotenoid 470 nm 20.90 min	Unk. carotenoid 500 nm 27.30 min	Unk. carotenoid 440, 470 nm 28.40 min	Unk. carotenoid 416, 440, 470 nm 29.60 min	Unk. carotenoid 475 nm 30.90 min
Mondsee	Spring	0.00	0.00	9.60	0.00	12.76	0.00	0.00	0.00	1.63	0.00	0.00
	Autumn	0.00	3.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Jurasico	Spring	3.78	8.16	0.00	0.00	2.90	0.00	0.00	0.00	0.00	0.00	0.00
	Autumn	4.88	4.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Uruguay	Spring	0.00	0.00	9.57	0.00	10.00	0.00	0.00	0.00	0.97	2.55	0.00
	Autumn	0.00	4.42	0.00	0.00	0.00	1.38	0.54	0.00	0.00	0.00	0.00
Kitiesh	Spring	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Autumn	0.00	6.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hotel	Spring	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Autumn	0.00	0.00	29.69	0.00	20.46	0.00	0.00	0.00	0.00	0.00	0.00
Las Estrellas	Spring	0.00	5.90	0.00	5.55	0.00	0.00	3.16	24.56	1.34	3.02	4.92
	Autumn	0.00	4.27	0.00	3.29	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xihu	Spring	0.00	13.65	0.00	0.00	5.17	0.00	0.00	0.00	0.59	0.00	0.00
	Autumn	0.00	5.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table A.4. Carotenoid concentrations (ng L^{-1}). NA: not available, ND: not detectable.

Lake	Season	Antheraxanthin	Astaxanthin	β,β-carotene	β,Ɛ-carotene	9-cis-neoxanthin	Diadinoxanthin	Dinoxanthin	Fucoxanthin	19-hex-fucoxanthin	Lutein	Peridinin	Zeaxanthin	Violaxanthin
Mondsee	Spring	0.00	8.47	6.76	1.41	13.50	6.52	0.00	27.57	3.60	56.24	7.38	5.12	14.28
	Autumn	0.00	0.00	4.11	0.31	8.42	2.78	0.00	9.37	5.91	48.37	0.00	3.40	9.14
Jurasico	Spring	0.00	0.00	2.99	0.00	1.62	0.73	0.00	13.43	10.00	5.28	0.00	3.07	7.86
	Autumn	0.00	13.28	4.57	0.00	12.53	0.64	0.00	24.44	2.59	26.30	0.00	3.83	7.64
Uruguay	Spring	0.00	5.31	5.22	0.00	2.64	4.77	3.03	17.94	4.16	13.66	7.36	2.56	6.79
	Autumn	0.00	20.02	2.74	0.00	9.93	3.48	1.89	8.30	4.27	46.51	2.03	1.95	8.78
Kitiesh	Spring	0.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Autumn	0.00	4.43	8.66	0.00	11.76	8.94	4.41	29.73	4.36	58.61	0.00	5.39	22.96
Hotel	Spring	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Autumn	15.89	98.75	90.48	0.00	171.61	81.00	0.00	0.00	39.81	932.34	0.00	54.08	186.37
Las Estrellas	Spring	0.00	24.29	1.38	0.00	0.00	0.00	0.00	3.12	0.00	2.99	8.48	1.75	5.61
	Autumn	0.00	13.80	0.00	0.00	13.44	0.00	0.00	17.61	12.33	51.55	0.00	2.84	9.32
Xihu	Spring	0.00	1.14	1.60	0.00	2.32	1.62	0.00	2.40	0.00	10.51	4.09	1.75	15.83
	Autumn	0.00	0.00	2.80	0.00	9.00	0.60	0.00	14.47	4.11	31.98	0.00	1.98	6.78

Table A.5. LCBD: Local contribution to beta diversity. Values in bold are significant prior to Holm's correction for multiple testing ($p < 0.05$), and those in bold and with an asterisk are still significant after correction. NA: Not available, ND: Not detectable.

Lake	Season	LCBD Pigments	LCBD Bacterioplankton
Mondsee	Spring	0.072	0.102
Jurasico	Spring	**0.311**	0.129
Uruguay	Spring	0.066	0.124
Kitiesh	Spring	NA	0.095
Hotel	Spring	ND	**0.212***
Las Estrellas	Spring	**0.395***	**0.228***
Xihu	Spring	0.156	0.11
Mondsee	Autumn	0.095	0.115
Jurasico	Autumn	0.169	0.119
Uruguay	Autumn	0.117	0.143
Kitiesh	Autumn	0.085	0.081
Hotel	Autumn	**0.255**	**0.297***
Las Estrellas	Autumn	0.112	0.161
Xihu	Autumn	0.167	0.084

Table A.6. Spearman correlation matrix with the R^2 values between unknown and identified pigments. Values in bold are significant prior to Holm's correction for multiple testing ($p < 0.05$), and the one in bold and with an asterisk are still significant.

	Unk. carotenoid 455, 480 nm 8.43 min	Unk. carotenoid 470 nm 11.80 min	Unk carotenoid 460 nm 12.00 min	Unk. carotenoid 475 nm 15.90 min	Unk. carotenoid 475 nm 17.80 min	Unk. carotenoid 450 nm 19.30 min	Unk. carotenoid 470 nm 20.90 min	Unk. carotenoid 500 nm 27.30 min	Unk carotenoid 440, 470 nm 28.40 min	Unk. carotenoid 416, 440, 470 nm 29.60 min	Unk. carotenoid 475 nm 30.90 min
MgDVP	0.11	0.42	-0.46	-0.36	-0.35	-0.24	-0.36	-0.24	-0.39	-0.36	-0.24
Chl-c1	**0.74**	-0.32	-0.17	-0.13	-0.21	-0.09	-0.13	-0.09	-0.21	-0.13	-0.09
Peridinin	-0.36	0.13	0.28	0.26	0.12	0.15	0.53	0.54	**0.93***	**0.66**	**0.54**
Fucoxanthin	0.22	-0.21	0.01	-0.16	-0.32	-0.22	-0.39	-0.31	0.04	-0.10	-0.31
9-cis-neoxanthin	-0.09	-0.36	0.49	-0.18	0.26	0.04	-0.35	-0.48	-0.26	-0.53	-0.48
Violaxanthin	-0.26	0.05	0.33	-0.30	**0.58**	-0.04	-0.41	-0.48	-0.17	**-0.59**	-0.48
19-hex-fucoxanthin	-0.04	-0.36	0.19	-0.08	0.11	0.04	-0.32	-0.44	**-0.64**	-0.38	-0.44
Astaxanthin	-0.16	-0.12	0.36	0.47	0.09	0.31	0.53	0.40	0.16	0.29	0.40
Diadinoxanthin	0.12	-0.50	**0.66**	**-0.65**	0.48	-0.04	-0.38	-0.44	-0.03	-0.20	-0.44
Dinoxanthin	-0.25	0.17	0.04	-0.25	-0.39	0.40	0.13	-0.17	-0.03	0.21	-0.17
Antheraxanthin	-0.13	-0.32	**0.63**	-0.13	0.57	-0.09	-0.13	-0.09	-0.21	-0.13	-0.09
Alloxanthin	-0.37	-0.44	**0.67**	0.18	0.21	-0.22	-0.11	0.04	0.21	0.12	0.04
Zeaxanthin	0.20	-0.54	0.48	-0.34	0.30	-0.31	-0.52	-0.39	-0.30	-0.40	-0.39
Lutein	-0.37	-0.15	0.43	-0.24	0.20	0.04	-0.35	-0.48	-0.32	-0.53	-0.48
β,Ɛ-carotene	-0.20	-0.28	0.30	-0.20	0.23	-0.13	-0.20	-0.13	0.32	-0.20	-0.13
β,β-carotene	0.08	-0.50	0.66	-0.64	0.35	-0.22	-0.46	-0.39	-0.04	-0.17	-0.39
Chl-b	-0.22	-0.24	0.49	-0.40	0.22	-0.04	-0.34	-0.39	-0.21	-0.46	-0.39
Chl-a	-0.43	-0.11	0.43	-0.16	0.12	-0.04	-0.27	-0.31	-0.25	-0.39	-0.31

Table A.7. Spearman correlation matrix with the R^2 values between unknown pigments. Values in bold are significant prior to Holm's correction for multiple testing ($p < 0.05$), and those in bold and with an asterisk are still significant after correction.

	Unk. carotenoid 455, 480 nm 8.43 min	Unk. carotenoid 470 nm 11.80 min	Unk carotenoid 460 nm 12.00 min	Unk. carotenoid 475 nm 15.90 min	Unk. carotenoid 475 nm 17.80 min	Unk. carotenoid 450 nm 19.30 min	Unk. carotenoid 470 nm 20.90 min	Unk. carotenoid 500 nm 27.30 min	Unk carotenoid 440, 470 nm 28.40 min	Unk. carotenoid 416, 440, 470 nm 29.60 min	Unk. carotenoid 475 nm 30.90 min
Unk. carotenoid 455, 480 nm 8.43 min	1.00	-0.47	-0.25	-0.20	0.01	-0.13	-0.20	-0.13	-0.31	-0.20	-0.13
Unk. carotenoid 470 nm 11.80 min	-0.47	1.00	**-0.60**	0.28	**-0.30**	0.14	0.35	0.32	0.02	0.04	0.32
Unk. carotenoid 460 nm 12.00 min	-0.25	**-0.60***	1.00	-0.25	0.61	-0.17	-0.25	-0.17	0.40	0.13	-0.17
Unk. carotenoid 475 nm 15.90 min	-0.20	0.28	-0.25	1.00	-0.31	-0.13	0.50	**0.74**	0.23	0.50	**0.74**
Unk. carotenoid 475 nm 17.80 min	0.01	-0.30	**0.61***	-0.31	1.00	-0.21	-0.31	-0.21	0.25	-0.31	-0.21
Unk. carotenoid 450 nm 19.30 min	-0.13	0.14	-0.17	-0.13	-0.21	1.00	**0.60**	-0.09	-0.21	-0.13	-0.09
Unk. carotenoid 470 nm 20.90 min	-0.20	0.35	-0.25	0.50	-0.31	**0.60***	1.00	**0.74**	0.23	0.50	**0.74**
Unk. carotenoid 500 nm 27.30 min	-0.13	0.32	-0.17	**0.74***	-0.21	-0.09	**0.74***	1.00	0.47	**0.74**	1.00
Unk. carotenoid 440, 470 nm 28.40 min	-0.31	0.02	0.40	0.23	0.25	-0.21	0.23	0.47	1.00	**0.62**	0.47
Unk. carotenoid 416, 440, 470 nm 29.60 min	-0.20	0.04	0.13	0.50	-0.31	-0.13	0.50	**0.74***	**0.62***	1.00	**0.74**
Unk. carotenoid 475 nm 30.90 min	-0.13	0.32	-0.17	**0.74***	-0.21	-0.09	**0.74***	**1.00***	0.47	0.74	1.00

Table A.8. Bacteria indicator species for spring. The closest relative in GeneBank, the environment where it was found, the identity percentage and the accession number is indicated.

Class	Order	Family	Genus	Closest relative	Environmental source	% identity	GenBank Accession
Actinobacteria	Micrococcales	*Microbacteriaceae*	*Parafrigoribacterium*	Uncultured bacterium	Soil near a sulfidic pond in Greenland	99.26	MT362452
Actinobacteria	Micrococcales	*Microbacteriaceae*	*Cryobacterium*	Cryobacterium sp. L11	Permafrost of Victoria Valley (Antarctica)	99.63	KT965167
Actinobacteriota	Actinobacteria	*Micrococcales*	*Cryobacterium*	Cryobacterium sp. L11	Permafrost of Victoria Valley (Antarctica)	100	KT965167
Acidobacteriae	Paludibaculum	NA	NA	uncultured bacterium	Benthic sediments from oligotrophic Andean lake	99.63	KY691677
Alphaproteobacteria	Rhodobacterales	*Rhodobacteraceae*	*Pseudorhodobacter*	Pseudorhodobacter sp	Antarctic soils	99.63	KY476581
Alphaproteobacteria	Sphingomonadales	*Sphingomonadaceae*	*Polymorphobacter*	Polymorphobacter sp. PAMC 29334	Antarctic lichen	98.53	CP080243
Alphaproteobacteria	Sphingomonadales	*Sphingomonadaceae*	*Polymorphobacter*	Polymorphobacter sp	Antarctic lichen	98.16	MW507628
Alphaproteobacteria	Reyranellales	*Reyranellaceae*	*Reyranella*	Uncultured bacterium	Benthic sediments from oligotrophic Andean lake	100	KY691029
Alphaproteobacteria	Acetobacterales	*Acetobacteraceae*	*Rhodovastum*	Uncultured bacterium	Soil environments under mosses on Anchorage Island (Antarctica)	99.26	EF219762
Bacteroidia	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	Flavobacterium sp	Antarctic soil	99.63	KY476592
Bacteroidia	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	Uncultured bacterium	Baltic Sea thick ice	100	LM651934
Bacteroidia	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	Flavobacterium sp.	Cryoconite hole sediment	100	MT473233
Bacteroidia	Chitinophagales	NA	NA	Uncultured bacterium	Baltic Sea thick ice	100	LM651944
Bacteroidia	Chitinophagales	NA	NA	Uncultured bacterium	Lake Nahuel Huapi	100	KM127806
Bacteroidia	Cytophagales	*Cyclobacteriaceae*	*Algoriphagus*	Algoriphagus sp. R-36727	Transantarctic Mountains, Forlidas Pond (Antarctica)	100	FR691439
Gammaproteobacteria	Burkholderiales	NA	NA	Uncultured bacterium	Microbial mats from King George Island	100	KX704294
Gammaproteobacteria	Burkholderiales	NA	NA	Uncultured bacterium	Biofilm attached on the membrane surfaces	95.59	GU257829
Gammaproteobacteria	Burkholderiales	*Alcaligenaceae*	GKS98 freshwater group	Uncultured Alcaligenaceae bacterium	Lake Michigan	100	EU641811
Gammaproteobacteria	Burkholderiales	*Comamonadaceae*	*Rhodoferax*	Uncultured bacterium	Arctic lake sediment	99.63	MN788553
Gammaproteobacteria	Burkholderiales	*Comamonadaceae*	*Rhodoferax*	Rhodoferax sp.	Fresh water	100	KC759436
Gammaproteobacteria	Burkholderiales	*Comamonadaceae*	*Rhodoferax*	Rhodoferax saidenbachensis	Sea-ice melt pool	99.63	KU179847
Gammaproteobacteria	Burkholderiales	*Comamonadaceae*	*Polaromonas*	Polaromonas sp.	Cryoconite hole (Antarctica)	100	MG098813
Gammaproteobacteria	Burkholderiales	*Oxalobacteraceae*	*Aquaspirillum, arcticum group*	Uncultured Aquaspirillum sp.	Glacier ice	100	KT752942
Gammaproteobacteria	Burkholderiales	*Oxalobacteraceae*	*Aquaspirillum, arcticum group*	Uncultured Aquaspirillum sp.	Glacier ice	100	KT752934
Gammaproteobacteria	Burkholderiales	*Oxalobacteraceae*	Aquaspirillum, arcticum group	Uncultured Aquaspirillum sp.	Glacier ice	99.26	KT752942
Gammaproteobacteria	Burkholderiales	*Oxalobacteraceae*	*Undibacterium*	Undibacterium sp.	Glacier ice	98.90	MN334244
Gammaproteobacteria	Burkholderiales	*Oxalobacteraceae*	*Undibacterium*	Undibacterium sp.	Glacier ice	99.26	MN334243
Planctomycetes	Gemmatales	*Gemmataceae*	NA	Uncultured bacterium	Lake Taihu	98.53	JN868815
Planctomycetes	Gemmatales	*Gemmataceae*	NA	Uncultured Planctomycetaceae bacterium	Stratified ferrous lake	98.17	MG740937
Thermoleophilia	Gaiellales	NA	NA	Uncultured bacterium	Lake water (Ontario)	97.33	KY515625
Thermoleophilia	Gaiellales	NA	NA	Uncultured bacterium	High-altitude Pyrenean lakes	96.34	HE858024

Table A.9. Bacteria indicator species for autumn. The closest relative in GeneBank, the environment where it was found, the identity percentage and the accession number is indicated.

Class	Order	Family	Genera	Closest relative	Environmental source	% identity	GenBank Accession
Actinobacteria	Frankiales	Sporichthyaceae	NA	Uncultured bacterium	Argentina: Lake Espejo	100	KM169310
Actinobacteria	Frankiales	Sporichthyaceae	NA	Uncultured bacterium	Argentina: Lake Espejo	99.63	KM169127
Actinobacteria	Frankiales	Sporichthyaceae	Candidatus Planktophila	Uncultured bacterium	Stratified freshwater lake	98.90	AB753916
Actinobacteria	Micrococcales	Intrasporangiaceae	*Oryzihumus*	Uncultured Oryzihumus sp.	Roopkund Glacier Himalaya (India)	99.63	GQ421037
Bacteroidia	Cytophagales	Spirosomaceae	*Pseudarcicella*	Uncultured Arcicella sp.	High-altitude lakes (Tibetan plateau)	99.63	EU703221
Bacteroidia	Cytophagales	Spirosomaceae	*Pseudarcicella*	Uncultured bacterium	Argentina: Lake Espejo	100	KM169218
Bacteroidia	Cytophagales	Spirosomaceae	*Pseudarcicella*	Uncultured bacterium	Argentina: Lake Espejo	99.63	KM155089
Bacteroidia	Cytophagales	Spirosomaceae	*Pseudarcicella*	Uncultured bacterium	Antarctica: Lake Limnopolar	100	KF928869
Bacteroidia	Chitinophagales	Chitinophagaceae	*Sediminibacterium*	Uncultured Chitinophagaceae bacterium	Lake Constancelake (Germany)	100	MG740845
Bacteroidia	Chitinophagales	Chitinophagaceae	*Sediminibacterium*	Uncultured bacterium	High-altitude Pyrenean lakes	99.63	HE857031
Bacteroidia	Sphingobacteriales	env.OPS 17	NA	Uncultured bacterium	Argentina: Lake Espejo	98.9	KM185189
Bacteroidia	Sphingobacteriales	env.OPS 17	NA	Uncultured bacterium	Argentina: Lake Espejo	99.26	KM185593
Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	FukuN18 freshwater group	Uncultured bacterium	Acidic high-Arctic wetland active layer soil	98.53	GU047442
Verrucomicrobiae	NA	NA	NA	Uncultured bacterium	Argentina: Lake Nahuel Huapi	100	KM156398
Verrucomicrobiae	NA	NA	NA	Uncultured bacterium	Permafrost thaw pond (Nunavik)	98.16	JN656765
Verrucomicrobiae	NA	NA	NA	Uncultured bacterium	Permafrost thaw pond (Nunavik)	98.53	JN656911
Verrucomicrobiae	NA	NA	NA	Uncultured bacterium	Limnopolar Lake (Antarctic)	99.63	FR848702

Appendix B: Supplementary material of chapter 2

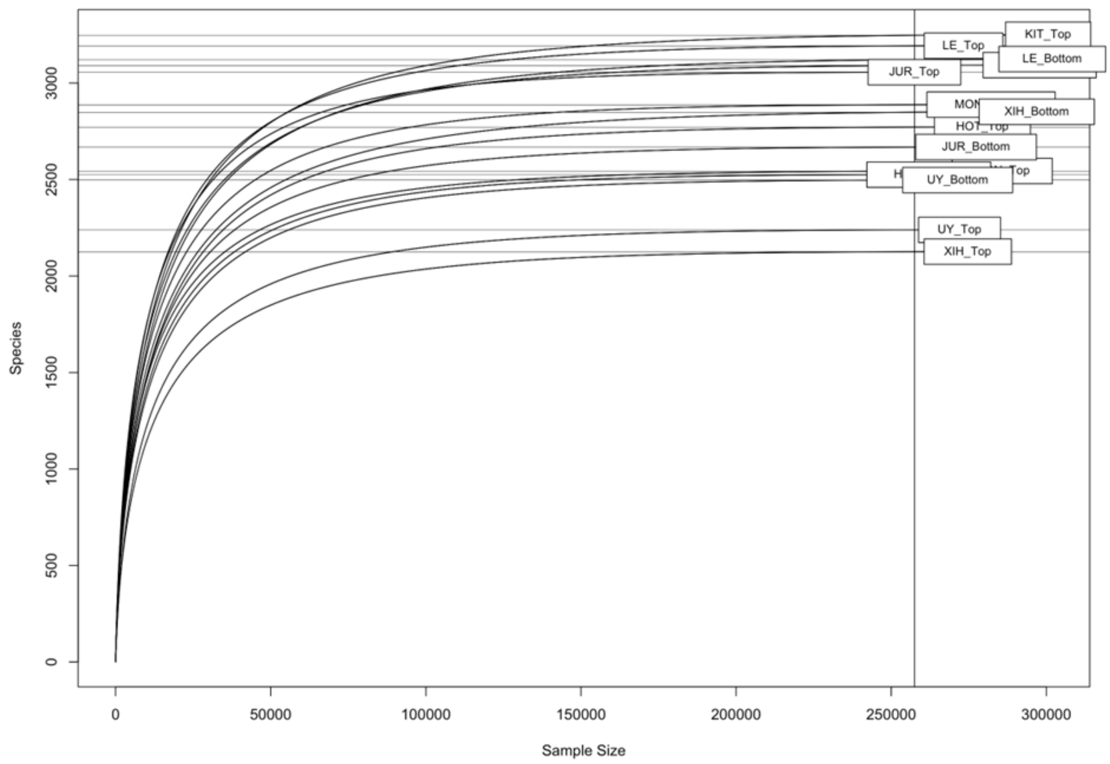


Figure B.1. Rarefaction curves. Lake names are abbreviated as MON: Mondsee; UY: Uruguay; KIT: Kitiesh; HOT: Hotel; LE: Las Estrellas; XIH: XIhu; JUR: Jurasico.

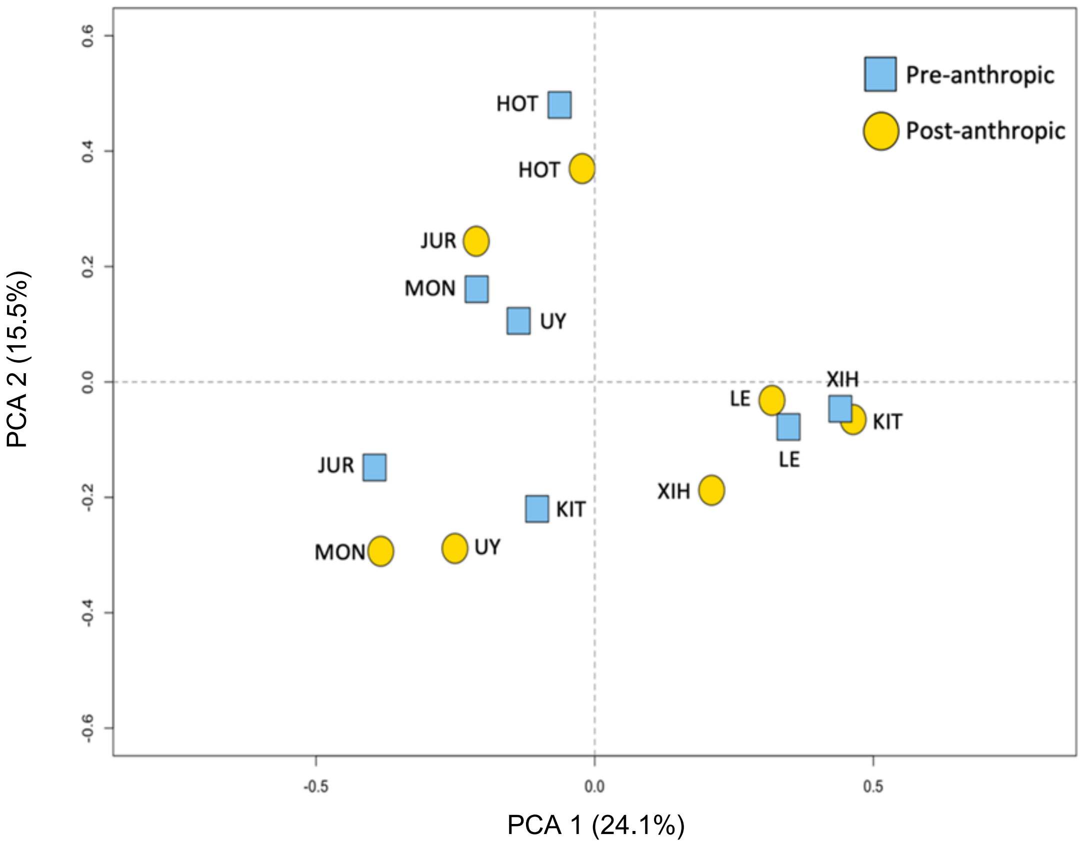


Figure B.2. First two axes of the PCA for the diversity matrix of ASVs for the top-bottom sediment pairs. The first axis explained 24.1% of the variance while the second explained 15.5%. Lake names are abbreviated as MON: Mondsee; UY: Uruguay; KIT: Kitiesh; HOT: Hotel; LE: Las Estrellas; XIH: XIhu; JUR: Jurasico.

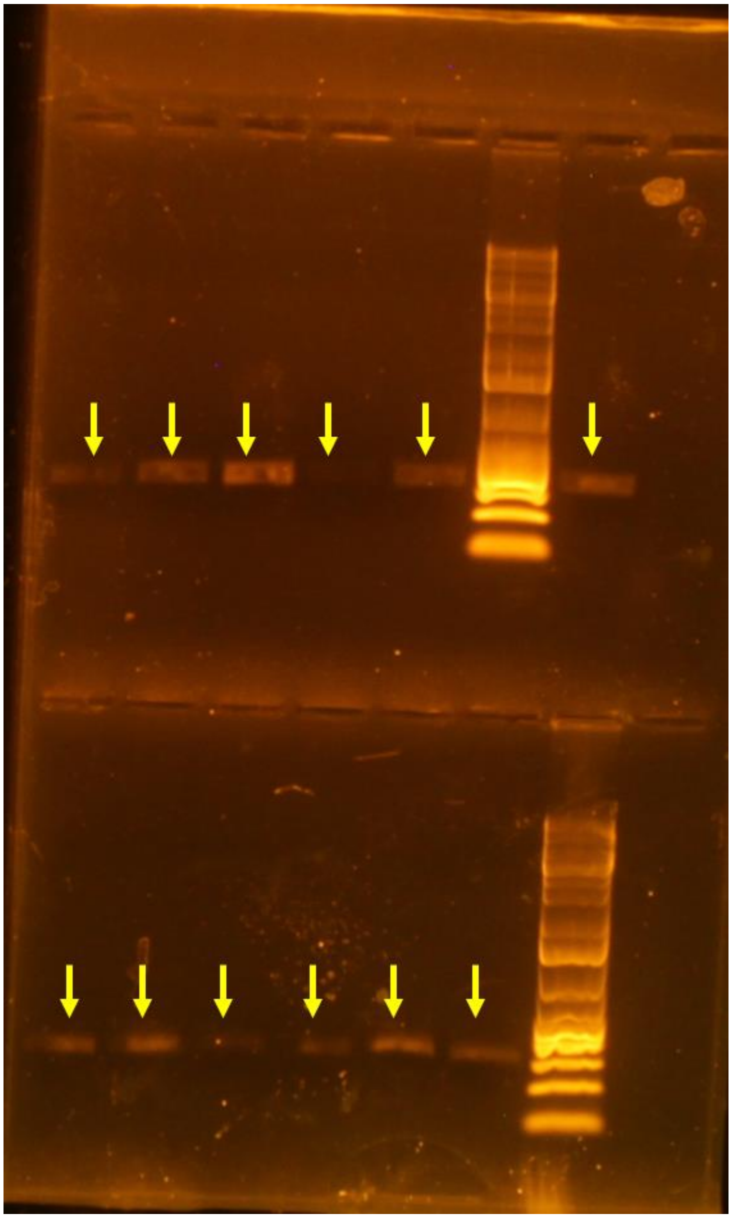
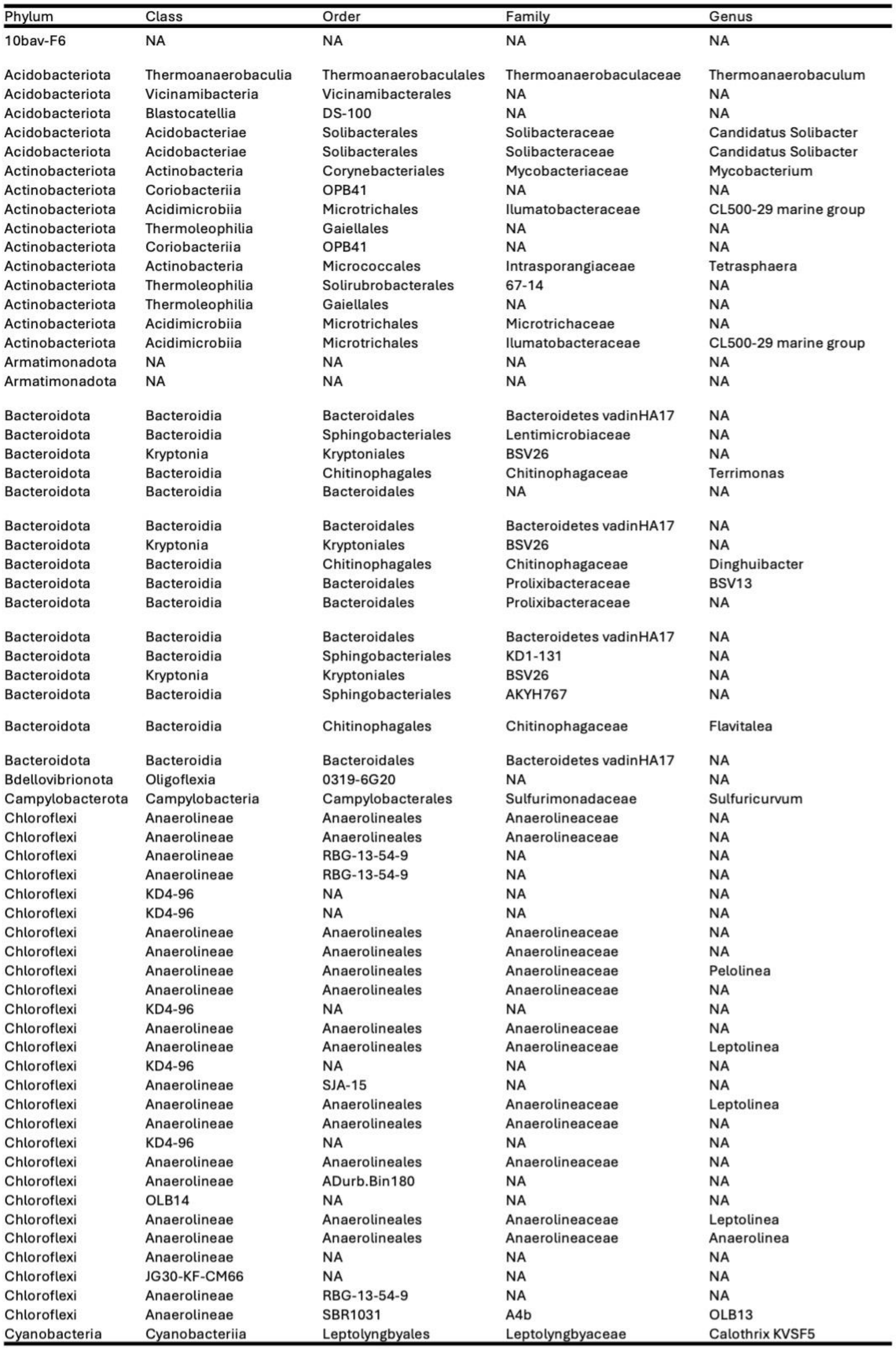


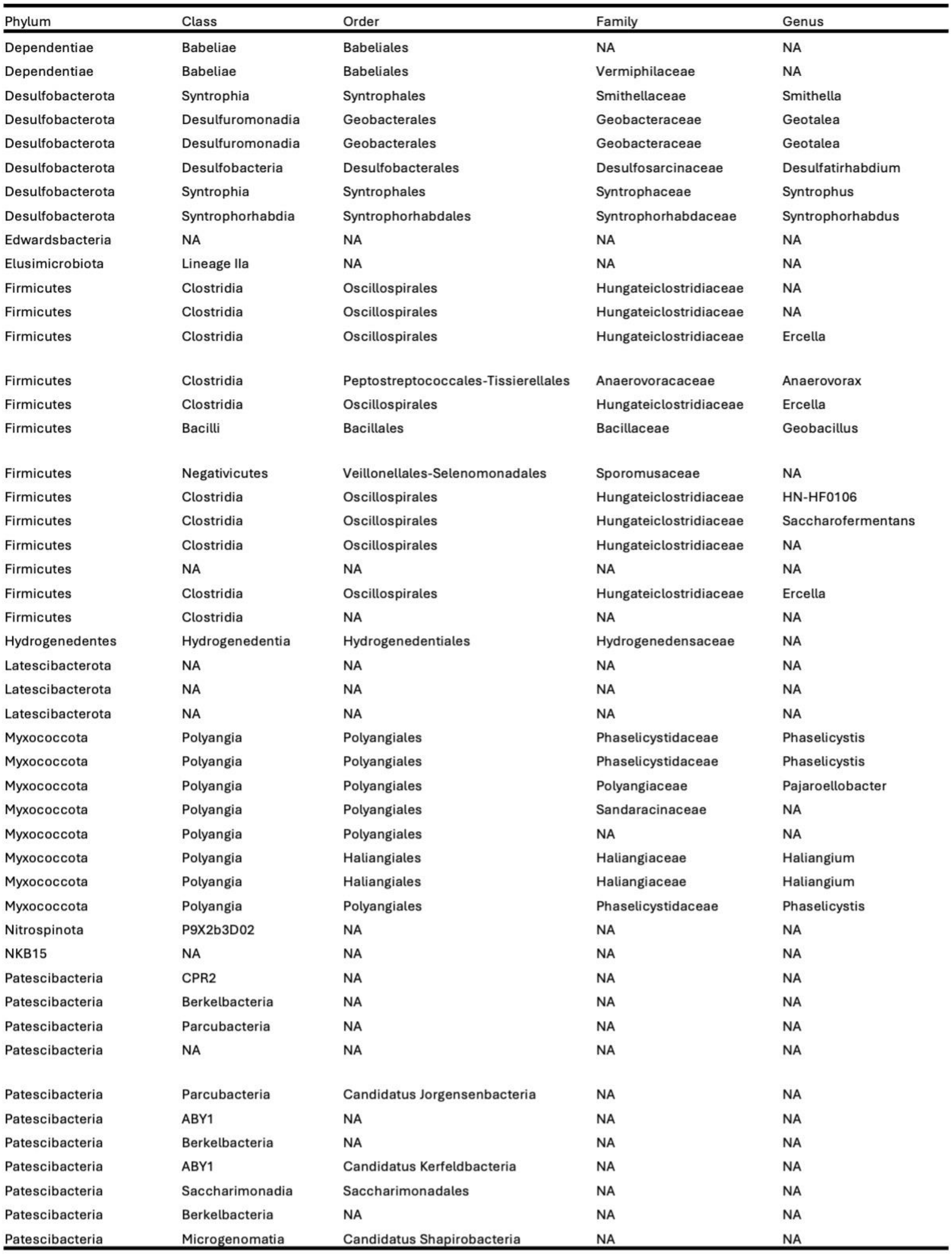
Figure B.3. Gel electrophoresis of PCR products from both top and bottom samples of different lakes. The arrow indicates the amplification product corresponding to a variable region (V4) of the ribosomal 16S gene.

Table B.1. Bacteria indicator species for Hotel and Las Estrellas ‘top’ samples with significant indicator values ($p < 0.05$). NA: not assigned.



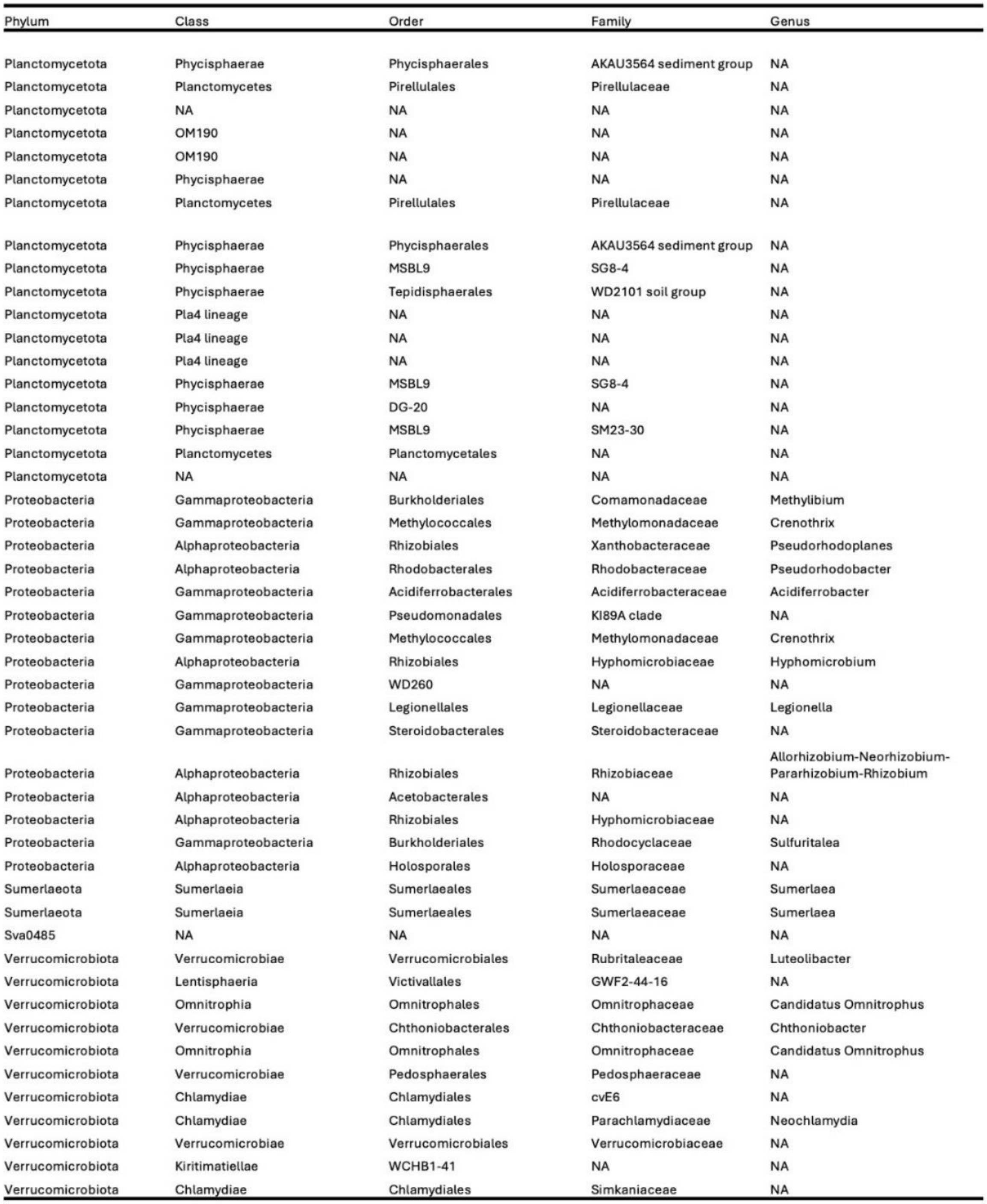
Phylum	Class	Order	Family	Genus
10bav-F6	NA	NA	NA	NA
Acidobacteriota	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Thermoanaerobaculum
Acidobacteriota	Vicinamibacteria	Vicinamibacterales	NA	NA
Acidobacteriota	Blastocatellia	DS-100	NA	NA
Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus Solibacter
Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	Candidatus Solibacter
Actinobacteriota	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium
Actinobacteriota	Coriobacteriia	OPB41	NA	NA
Actinobacteriota	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group
Actinobacteriota	Thermoleophilia	Gaiellales	NA	NA
Actinobacteriota	Coriobacteriia	OPB41	NA	NA
Actinobacteriota	Actinobacteria	Micrococcales	Intrasporangiaceae	Tetrasphaera
Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	NA
Actinobacteriota	Thermoleophilia	Gaiellales	NA	NA
Actinobacteriota	Acidimicrobiia	Microtrichales	Microtrichaceae	NA
Actinobacteriota	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group
Armatimonadota	NA	NA	NA	NA
Armatimonadota	NA	NA	NA	NA
Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	NA
Bacteroidota	Bacteroidia	Sphingobacteriales	Lentimicrobiaceae	NA
Bacteroidota	Kryptonia	Kryptoniales	BSV26	NA
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Terrimonas
Bacteroidota	Bacteroidia	Bacteroidales	NA	NA
Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	NA
Bacteroidota	Kryptonia	Kryptoniales	BSV26	NA
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter
Bacteroidota	Bacteroidia	Bacteroidales	Prolixibacteraceae	BSV13
Bacteroidota	Bacteroidia	Bacteroidales	Prolixibacteraceae	NA
Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	NA
Bacteroidota	Bacteroidia	Sphingobacteriales	KD1-131	NA
Bacteroidota	Kryptonia	Kryptoniales	BSV26	NA
Bacteroidota	Bacteroidia	Sphingobacteriales	AKYH767	NA
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	Flavitalea
Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	NA
Bdellovibrionota	Oligoflexia	0319-6G20	NA	NA
Campylobacterota	Campylobacteria	Campylobacterales	Sulfurimonadaceae	Sulfuricurvum
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	RBG-13-54-9	NA	NA
Chloroflexi	Anaerolineae	RBG-13-54-9	NA	NA
Chloroflexi	KD4-96	NA	NA	NA
Chloroflexi	KD4-96	NA	NA	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Pelolinea
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	KD4-96	NA	NA	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Leptolinea
Chloroflexi	KD4-96	NA	NA	NA
Chloroflexi	Anaerolineae	SJA-15	NA	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Leptolinea
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	KD4-96	NA	NA	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	NA
Chloroflexi	Anaerolineae	ADurb.Bin180	NA	NA
Chloroflexi	OLB14	NA	NA	NA
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Leptolinea
Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	Anaerolinea
Chloroflexi	Anaerolineae	NA	NA	NA
Chloroflexi	JG30-KF-CM66	NA	NA	NA
Chloroflexi	Anaerolineae	RBG-13-54-9	NA	NA
Chloroflexi	Anaerolineae	SBR1031	A4b	OLB13
Cyanobacteria	Cyanobacteriia	Leptolyngbyales	Leptolyngbyaceae	Calothrix KVSF5

Table B.1 cont'd. Bacteria indicator species for Hotel and Las Estrellas 'top' samples.



Phylum	Class	Order	Family	Genus
Dependentiae	Babeliae	Babeliales	NA	NA
Dependentiae	Babeliae	Babeliales	Vermiphilaceae	NA
Desulfobacterota	Syntrophia	Syntrophales	Smithellaceae	Smithella
Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Geotalea
Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	Geotalea
Desulfobacterota	Desulfobacteria	Desulfobacterales	Desulfosarcinaceae	Desulfatirhabdium
Desulfobacterota	Syntrophia	Syntrophales	Syntrophaceae	Syntrophus
Desulfobacterota	Syntrophorhabdia	Syntrophorhabdales	Syntrophorhabdaceae	Syntrophorhabdus
Edwardsbacteria	NA	NA	NA	NA
Elusimicrobiota	Lineage IIa	NA	NA	NA
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	NA
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	NA
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	Ercella
Firmicutes	Clostridia	Peptostreptococcales-Tissierellales	Anaerovoracaceae	Anaerovorax
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	Ercella
Firmicutes	Bacilli	Bacillales	Bacillaceae	Geobacillus
Firmicutes	Negativicutes	Veillonellales-Selenomonadales	Sporomusaceae	NA
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	HN-HF0106
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	Saccharofermentans
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	NA
Firmicutes	NA	NA	NA	NA
Firmicutes	Clostridia	Oscillospirales	Hungateiclostridiaceae	Ercella
Firmicutes	Clostridia	NA	NA	NA
Hydrogenedentes	Hydrogenedentia	Hydrogenedentiales	Hydrogenedensaceae	NA
Latescibacterota	NA	NA	NA	NA
Latescibacterota	NA	NA	NA	NA
Latescibacterota	NA	NA	NA	NA
Myxococcota	Polyangia	Polyangiales	Phaselicystidaceae	Phaselicystis
Myxococcota	Polyangia	Polyangiales	Phaselicystidaceae	Phaselicystis
Myxococcota	Polyangia	Polyangiales	Polyangiaceae	Pajaroellobacter
Myxococcota	Polyangia	Polyangiales	Sandaracinaceae	NA
Myxococcota	Polyangia	Polyangiales	NA	NA
Myxococcota	Polyangia	Haliangiales	Haliangiaceae	Haliangium
Myxococcota	Polyangia	Haliangiales	Haliangiaceae	Haliangium
Myxococcota	Polyangia	Polyangiales	Phaselicystidaceae	Phaselicystis
Nitrospinota	P9X2b3D02	NA	NA	NA
NKB15	NA	NA	NA	NA
Patescibacteria	CPR2	NA	NA	NA
Patescibacteria	Berkelbacteria	NA	NA	NA
Patescibacteria	Parcubacteria	NA	NA	NA
Patescibacteria	NA	NA	NA	NA
Patescibacteria	Parcubacteria	Candidatus Jorgensenbacteria	NA	NA
Patescibacteria	ABY1	NA	NA	NA
Patescibacteria	Berkelbacteria	NA	NA	NA
Patescibacteria	ABY1	Candidatus Kerfeldbacteria	NA	NA
Patescibacteria	Saccharimonadia	Saccharimonadales	NA	NA
Patescibacteria	Berkelbacteria	NA	NA	NA
Patescibacteria	Microgenomatia	Candidatus Shapirobacteria	NA	NA

Table B.1 cont'd. Bacteria indicator species for Hotel and Las Estrellas 'top' samples.



Phylum	Class	Order	Family	Genus
Planctomycetota	Phycisphaerae	Phycisphaerales	AKAU3564 sediment group	NA
Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	NA
Planctomycetota	NA	NA	NA	NA
Planctomycetota	OM190	NA	NA	NA
Planctomycetota	OM190	NA	NA	NA
Planctomycetota	Phycisphaerae	NA	NA	NA
Planctomycetota	Planctomycetes	Pirellulales	Pirellulaceae	NA
Planctomycetota	Phycisphaerae	Phycisphaerales	AKAU3564 sediment group	NA
Planctomycetota	Phycisphaerae	MSBL9	SG8-4	NA
Planctomycetota	Phycisphaerae	Tepidisphaerales	WD2101 soil group	NA
Planctomycetota	Pla4 lineage	NA	NA	NA
Planctomycetota	Pla4 lineage	NA	NA	NA
Planctomycetota	Pla4 lineage	NA	NA	NA
Planctomycetota	Phycisphaerae	MSBL9	SG8-4	NA
Planctomycetota	Phycisphaerae	DG-20	NA	NA
Planctomycetota	Phycisphaerae	MSBL9	SM23-30	NA
Planctomycetota	Planctomycetes	Planctomycetales	NA	NA
Planctomycetota	NA	NA	NA	NA
Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	Methylibium
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonadaceae	Crenothrix
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	Pseudorhodoplanes
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Pseudorhodobacter
Proteobacteria	Gammaproteobacteria	Acidiferrobacterales	Acidiferrobacteraceae	Acidiferrobacter
Proteobacteria	Gammaproteobacteria	Pseudomonadales	KI89A clade	NA
Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonadaceae	Crenothrix
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Hyphomicrobium
Proteobacteria	Gammaproteobacteria	WD260	NA	NA
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella
Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
Proteobacteria	Alphaproteobacteria	Acetobacterales	NA	NA
Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	NA
Proteobacteria	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Sulfuritalea
Proteobacteria	Alphaproteobacteria	Holosporales	Holosporaceae	NA
Sumerlaeota	Sumerlaeia	Sumerlaeales	Sumerlaeaceae	Sumerlaea
Sumerlaeota	Sumerlaeia	Sumerlaeales	Sumerlaeaceae	Sumerlaea
Sva0485	NA	NA	NA	NA
Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Rubritaleaceae	Luteolibacter
Verrucomicrobiota	Lentisphaeria	Victivallales	GWF2-44-16	NA
Verrucomicrobiota	Omnitrophia	Omnitrophales	Omnitrophaceae	Candidatus Omnitrophus
Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Chthoniobacter
Verrucomicrobiota	Omnitrophia	Omnitrophales	Omnitrophaceae	Candidatus Omnitrophus
Verrucomicrobiota	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	NA
Verrucomicrobiota	Chlamydiae	Chlamydiales	cvE6	NA
Verrucomicrobiota	Chlamydiae	Chlamydiales	Parachlamydiaceae	Neochlamydia
Verrucomicrobiota	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	NA
Verrucomicrobiota	Kiritimatiellae	WCHB1-41	NA	NA
Verrucomicrobiota	Chlamydiae	Chlamydiales	Simkaniaceae	NA

Table B.2. Chlorophyll *a* and chlorophyll degradation product concentrations (ng g OM-1). Chl-*a*: Chlorophyll *a*. Chl-*a*:*a*-phorbin ratio where *a*-phorbin is the sum of Chl-*a* and derivative products of Chl-*a*. ND: not detected.

Lake	Sample	Chl-a	Chlorophyllide a	Pheophorbide a	Pheophytin a	Chl-a: a-phorbins
Mondsee	Top	245	10	212	2111	0.10
	Bottom	5	ND	ND	900	0.01
Uruguay	Top	938	ND	ND	4875	0.19
	Bottom	ND	ND	ND	186	0.00
Kitiesh	Top	187	ND	38	5154	0.04
	Bottom	1014	91	567	13927	0.07
Hotel	Top	1251	ND	ND	312	4.01
	Bottom	46	ND	86	44	0.35
Las Estrellas	Top	1378	94	ND	1931	0.68
	Bottom	ND	ND	ND	692	0.00
Xihu	Top	56	7	ND	982	0.06
	Bottom	13	ND	86	295	0.05
Jurasico	Top	144	ND	34	127	0.89
	Bottom	ND	ND	ND	33	0.01

Table B.3. Taxonomic pigment concentrations (ng g OM-1). ND: not detected.

Lake	Sample	Asta xanthin	β.β-caroten e	Fuco xanthin	19-but-fucoxanthin	19-hex-fucoxanthin	Cantha xanthin	Chl c1	Chl c2	Chl b	Pheophytin b	9-cis-neoxanthin	Diadino xanthin	Diato xanthin	Dino xanthin	Echinone	Lutein	Prasino xanthin	MgDV P	Zea xanthin	Viola xanthin
Mondsee	Top	12	14	70	ND	9	ND	8	9	31	25	ND	1	ND	ND	ND	23	4	5	ND	ND
	Bottom	ND	ND	ND	ND	ND	3	ND	ND	ND	9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Uruguay	Top	32	146	203	171	ND	12	22	43	61	212	ND	ND	93	ND	46	6	ND	ND	24	ND
	Bottom	ND	ND	ND	ND	ND	ND	ND	ND	ND	5	ND	ND	1	ND	ND	ND	ND	ND	ND	ND
Kitiesh	Top	47	26	46	ND	ND	9	ND	6	22	62	ND	2	22	19	18	42	ND	4	ND	ND
	Bottom	37	119	13	ND	ND	6	ND	ND	282	265	ND	9	124	ND	52	126	ND	ND	ND	ND
Hotel	Top	61	144	69	ND	10	22	9	5	176	271	2	15	87	ND	ND	256	ND	ND	200	11
	Bottom	3	1	0.53	ND	ND	2	ND	ND	ND	33	ND	1	2	ND	ND	2	ND	ND	ND	ND
Las Estrellas	Top	2	43	221	ND	1	ND	28	26	106	36	1	22	65	ND	ND	27	ND	ND	4	ND
	Bottom	1	ND	ND	ND	ND	1	ND	ND	ND	8	ND	ND	1	ND	ND	ND	ND	ND	ND	ND
Xihu	Top	ND	ND	10	9	ND	1	3	3	4	11	ND	ND	3	ND	ND	1	ND	ND	ND	ND
	Bottom	ND	ND	ND	ND	ND	ND	ND	ND	3	ND	ND	ND	ND	ND	ND	2	ND	ND	ND	ND

Table B.4. Fe and Mn from XRF data.

Lake	Sample	Fe/Mn	$(Fe_{top}/Mn_{top})/(Fe_{bottom}/Mn_{bottom})$
Mondsee	Top	59.41	0.74
	Bottom	80.05	
Uruguay	Top	144.91	2.75
	Bottom	52.64	
Kitiesh	Top	37.17	0.34
	Bottom	108.23	
Hotel	Top	97.62	1.37
	Bottom	71.17	
Las Estrellas	Top	103.77	1.23
	Bottom	84.20	
Xihu	Top	25.59	0.40
	Bottom	63.97	
Jurasico	Top	105.42	1.62
	Bottom	64.89	

Table B.5. XRF data for the seven sediment cores analyzed. Unit in cps (counts per second). ND: not data.

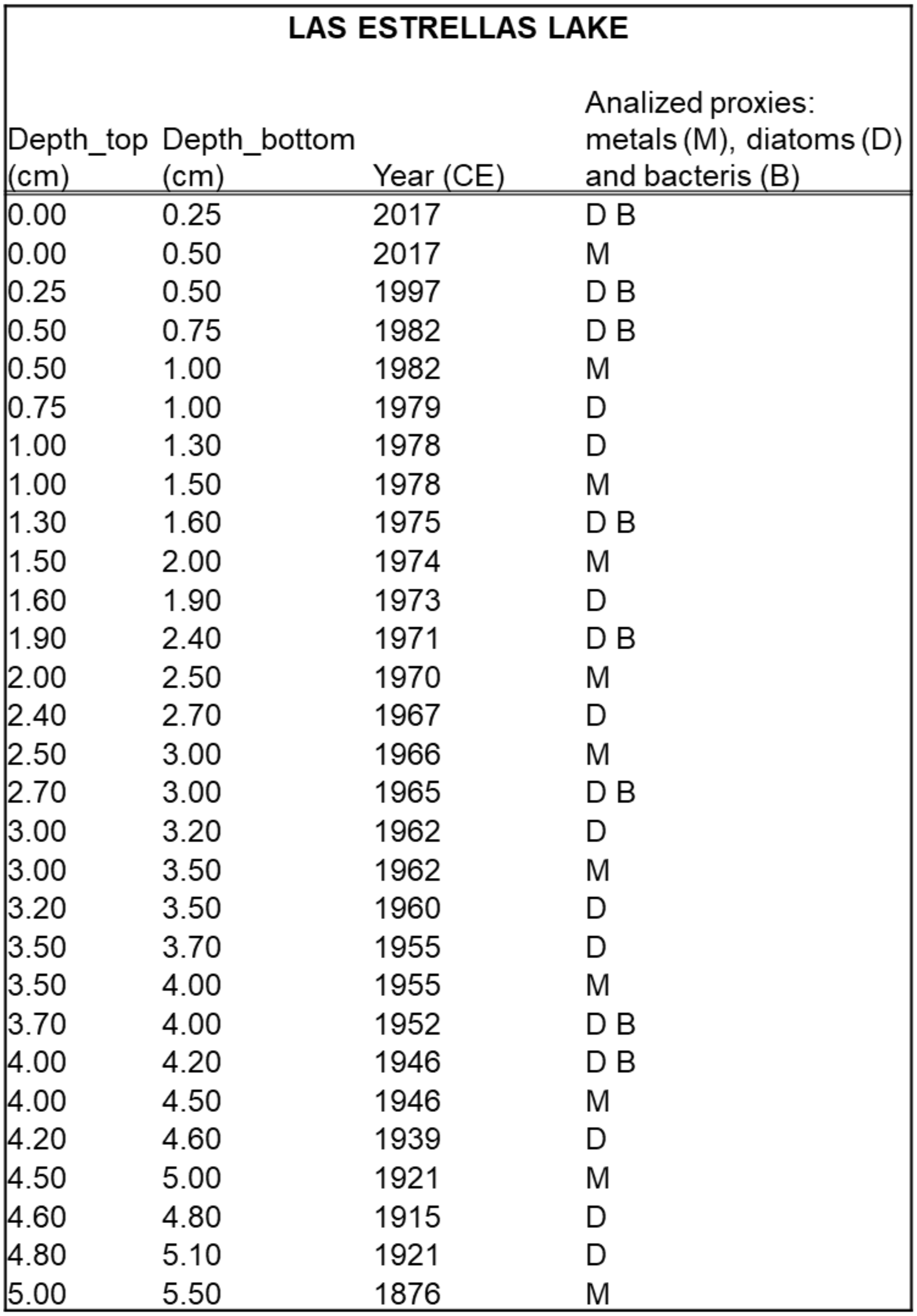
Lake	Sample	Al	Si	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	As	Br	Rb	Sr	Zr	Th	U
Mondsee	Top	22.33	194.33	643.33	5064.33	2732.67	184.67	45.00	1553.67	92297.67	201.00	329.00	217.00	ND	300.00	182.33	2656.33	201.67	5.33	123.00
	Bottom	37.80	105.00	329.00	2800.60	1695.00	149.20	42.20	762.80	61059.00	302.20	298.20	177.00	ND	211.00	181.40	2720.20	63.60	82.80	78.60
Uruguay	Top	21.82	25.64	140.55	1370.09	1086.82	87.64	67.55	1036.82	150245.73	191.18	69.64	37.55	43.36	202.82	201.82	1269.45	91.73	43.64	132.64
	Bottom	29.67	68.33	231.00	1900.67	1501.33	97.67	51.33	1254.67	66046.00	279.67	151.33	125.67	35.67	269.33	107.00	2557.33	162.00	28.00	81.00
Kitiesh	Top	34.00	46.56	79.67	1923.78	843.44	80.56	31.00	1554.89	57793.44	260.11	170.67	82.89	39.56	111.00	97.00	1797.11	145.00	50.56	103.44
	Bottom	22.14	61.14	106.29	1874.14	948.71	160.43	28.71	548.14	59325.57	307.29	260.00	89.71	18.43	324.43	76.86	1470.29	70.86	22.86	57.86
Hotel	Top	45.00	148.33	338.00	2307.00	1530.67	116.33	66.33	596.33	58216.00	283.67	644.00	1791.33	169.67	128.67	97.33	1299.33	28.33	104.33	89.67
	Bottom	28.71	146.86	351.86	8339.57	4199.14	191.86	68.86	1170.43	83298.14	209.29	170.14	231.71	9.50	25.86	150.14	2747.43	509.57	51.71	162.29
Las Estrellas	Top	20.14	63.29	158.29	2804.86	1471.57	139.86	51.71	557.43	57844.29	235.43	157.71	552.86	20.00	1206.42	1294.39	1397.62	1208.22	1317.25	1498.72
	Bottom	33.67	95.00	250.67	4477.33	3158.67	199.33	47.00	1277.00	107517.33	168.67	159.33	206.67	28.60	27.67	83.67	2184.67	518.00	53.67	168.33
Xihu	Top	33.67	105.14	496.33	3637.52	2492.43	168.57	61.95	4170.76	106711.43	201.62	258.76	188.62	147.48	56.95	199.33	1939.95	291.43	71.90	94.62
	Bottom	33.81	191.00	603.68	3130.42	2449.16	148.81	53.29	1090.03	69729.45	249.97	336.68	199.10	73.61	136.39	212.26	1939.84	305.71	69.94	71.48
Jurasico	Top	29.76	56.41	190.35	1676.88	1214.00	107.53	36.94	489.65	51617.18	278.53	215.12	180.65	96.88	81.94	148.94	2120.06	108.88	101.18	89.59
	Bottom	30.20	63.96	177.96	2874.09	2368.11	134.15	59.31	1389.62	90170.96	250.31	146.38	158.75	132.84	54.56	142.65	1495.62	264.49	75.56	91.84

Appendix C: Supplementary material of chapter 3

Table C.1. Samples analyzed in Mondsee Lake. The right-hand column indicates which proxy was analyzed in each sample. M: metals; D: diatoms; B: bacteria.

MONDSEE LAKE			
Depth top (cm)	Depth bottom (cm)	Year (CE)	Analyzed proxies: metals (M), diatoms (D) and bacteria (B)
0.0	0.2	2017	M D B
0.2	0.5	2015	M D B
0.5	0.8	2012	M D
0.8	1.0	2007	D B
1.0	1.2	2004	D B
1.2	1.5	1999	M D B
1.5	1.8	1991	M D B
1.8	2.0	1981	M D B
2.0	2.3	1973	M D
2.3	2.5	1956	MD B
2.5	2.8	1948	M D B
2.8	3.1	1936	M D
3.1	3.4	1927	M D B
3.4	3.7	1918	M
3.7	4.1	1908	M
4.1	4.4	1890	M
4.8	5.1	1854	D

Table C.1 cont'd. Samples analyzed in Las Estrellas Lake. The right-hand column indicates which proxy was analyzed in each sample. M: metals; D: diatoms; B: bacteria.



LAS ESTRELLAS LAKE

Depth_top (cm)	Depth_bottom (cm)	Year (CE)	Analized proxies: metals (M), diatoms (D) and bacteris (B)
0.00	0.25	2017	D B
0.00	0.50	2017	M
0.25	0.50	1997	D B
0.50	0.75	1982	D B
0.50	1.00	1982	M
0.75	1.00	1979	D
1.00	1.30	1978	D
1.00	1.50	1978	M
1.30	1.60	1975	D B
1.50	2.00	1974	M
1.60	1.90	1973	D
1.90	2.40	1971	D B
2.00	2.50	1970	M
2.40	2.70	1967	D
2.50	3.00	1966	M
2.70	3.00	1965	D B
3.00	3.20	1962	D
3.00	3.50	1962	M
3.20	3.50	1960	D
3.50	3.70	1955	D
3.50	4.00	1955	M
3.70	4.00	1952	D B
4.00	4.20	1946	D B
4.00	4.50	1946	M
4.20	4.60	1939	D
4.50	5.00	1921	M
4.60	4.80	1915	D
4.80	5.10	1921	D
5.00	5.50	1876	M

Table C.1 cont'd. Samples analyzed in Hotel Lake. The right-hand column indicates which proxy was analyzed in each sample. M: metals; D: diatoms; B: bacteria.

HOTEL LAKE			
Depth_top (cm)	Depth_bottom (cm)	Year (CE)	Analized proxies: metals (M), diatoms (D) and bacteria (B)
0.0	0.2	2014	M D B
0.2	0.5	1998	M D B
0.5	0.8	1976	M D B
0.8	1.1	1940	M D B
1.1	1.4	1907	M D
1.4	1.7	1895	M D
1.7	2.1	1885	D
2.1	2.5	1867	D B

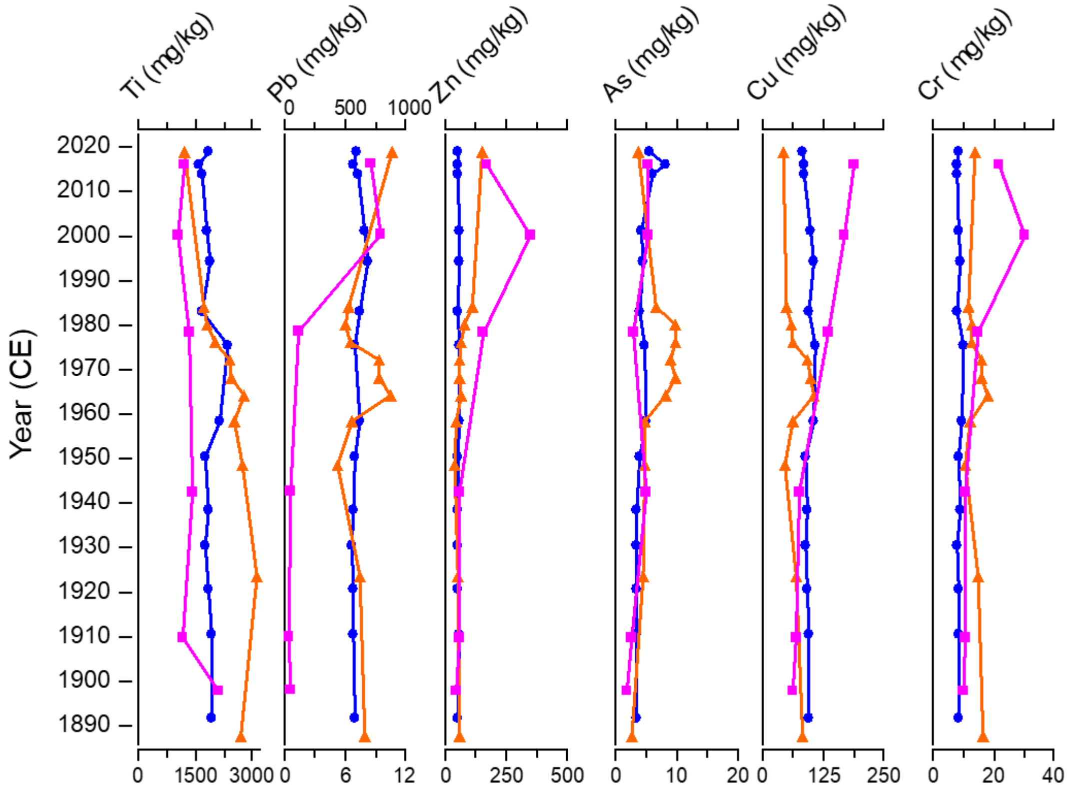


Figure C.1. Metal concentrations (mg kg^{-1}) in the three study lakes. Blue lines: Mondsee Lake, orange lines: Las Estrellas Lake and pink lines Hotel lake. Note the different x-axis for Pb in Hotel Lake (top).

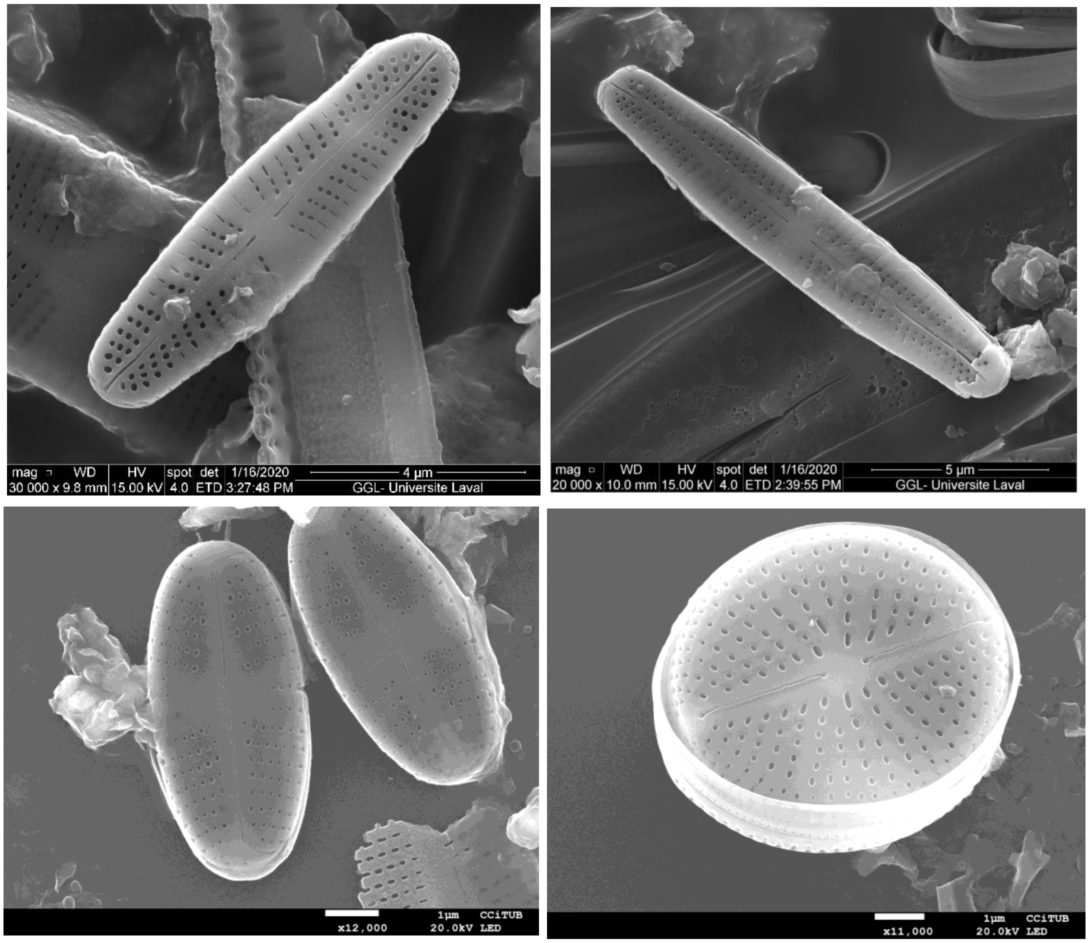


Figure C.2. Selected diatom species by scanning electron microscope (SEM). A) *Achnanthidium indistinctum*, B) *Achnanthidium maritimo-antarcticum*, C) *Sellaphora nigri*, D) *Cavinula pseudoscutiformis*. Photos by Samuel Yergeau.

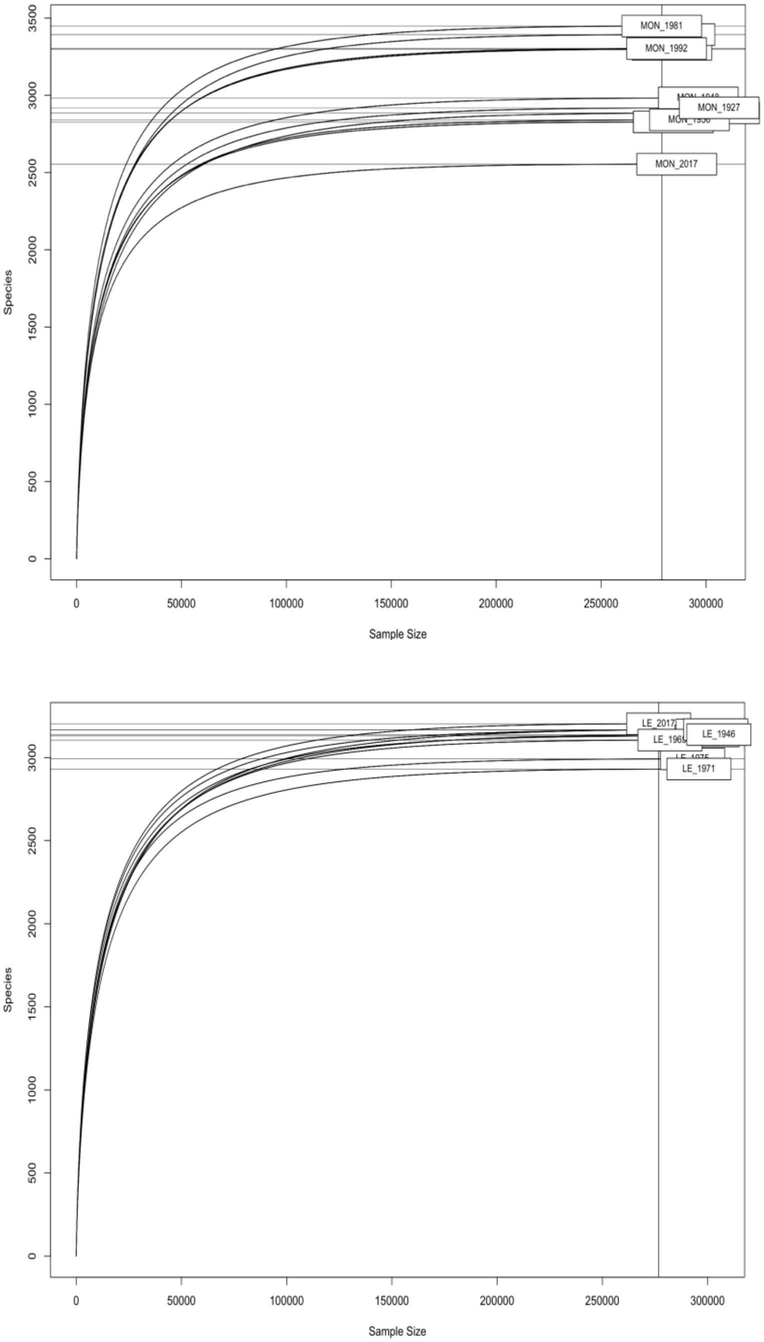


Figure C.3. Rarefaction curves. Top: Mondsee Lake; Bottom: Las Estrellas Lake.

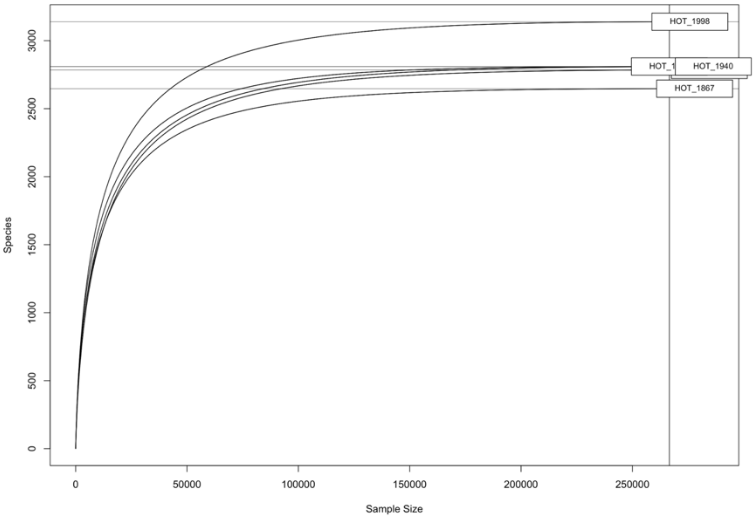


Figure C.3 cont’d. Rarefaction curve for Hotel Lake.

Table C.2. Metal enrichment factors (EFs) in the three evaluated lakes.

MONDSEE LAKE

Depth top (cm)	Depth bottom (cm)	Year (CE)	Cr EF	Mn EF	Fe EF	Co EF	Ni EF	Cu EF	Zn EF	As EF	Se EF	Cd EF	Pb EF	Ti EF
0.0	0.2	2017	1.0	1.7	1.2	1.0	1.0	0.9	1.0	1.7	1.3	1.0	1.1	0.9
0.2	0.5	2015	1.1	2.9	1.7	1.3	1.2	1.1	1.2	3.0	2.9	1.2	1.2	0.8
0.5	0.8	2012	1.1	2.7	1.6	1.2	1.2	1.1	1.1	2.1	2.8	1.2	1.2	0.8
1.2	1.5	1999	1.1	1.6	1.2	1.2	1.2	1.1	1.1	1.4	2.2	1.4	1.2	0.9
1.5	1.8	1991	1.1	1.1	1.1	1.2	1.2	1.1	1.0	1.3	2.2	1.3	1.2	0.9
1.8	2	1981	1.1	1.0	1.1	1.3	1.3	1.1	1.1	1.4	1.8	1.3	1.2	0.8
2.0	2.3	1973	1.0	1.0	1.0	1.3	1.1	1.0	0.9	1.2	1.5	1.0	0.8	1.1
2.3	2.5	1956	1.0	1.0	1.1	1.4	1.2	1.0	0.9	1.4	1.3	1.0	1.0	1.1
2.5	2.8	1948	1.1	1.1	1.2	1.4	1.2	1.0	1.1	1.3	1.3	1.2	1.1	1.0
2.8	3.1	1936	1.1	1.1	1.1	1.3	1.2	1.0	1.0	1.1	1.0	0.9	1.0	1.0
3.1	3.4	1927	1.0	0.9	1.1	1.3	1.2	1.1	1.0	1.2	1.0	1.0	1.1	0.8
3.4	3.7	1918	1.0	1.0	1.1	1.2	1.1	1.0	1.0	1.1	1.1	0.9	1.0	0.9
3.7	4.1	1908	1.0	1.1	1.1	1.1	1.1	1.0	1.0	1.0	0.8	1.0	1.0	0.8
4.1	4.4	1890	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9

LAS ESTRELLAS LAKE

Depth_top (cm)	Depth_bottom (cm)	Year (CE)	Cr EF	Mn EF	Fe EF	Co EF	Ni EF	Cu EF	Zn EF	As EF	Se EF	Cd EF	Pb EF	Ti EF
0.0	0.5	2017	1.9	1.9	1.7	1.8	2.3	1.1	5.7	3.0	1.3	1.8	2.9	0.5
0.5	1.0	1982	1.1	1.9	2.2	1.6	1.1	0.9	3.1	3.9	1.7	1.0	1.2	0.7
1.0	1.5	1978	1.2	2.8	2.9	2.1	1.3	1.1	2.2	5.6	1.5	0.8	1.1	0.7
1.5	2.0	1974	1.0	3.0	2.5	1.9	1.1	1.0	1.5	4.9	1.4	0.9	1.1	0.8
2.0	2.5	1970	1.1	3.8	2.1	2.0	1.2	1.2	1.1	3.8	2.0	1.2	1.3	0.9
2.5	3.0	1966	1.1	3.8	2.2	2.0	1.2	1.3	1.1	4.1	2.0	1.2	1.3	0.9
3.0	3.5	1962	1.1	3.0	1.9	1.8	1.2	1.2	1.2	3.0	1.5	1.0	1.3	1.0
3.5	4.0	1955	0.8	1.7	1.4	1.3	0.9	0.8	0.9	2.0	0.8	0.8	0.9	0.9
4.0	4.5	1946	0.6	1.4	1.4	1.1	0.8	0.6	0.7	1.8	0.6	0.7	0.6	1.0
4.5	5.0	1921	0.8	1.4	1.2	1.1	0.9	0.7	0.8	1.4	0.4	0.5	0.8	1.2
5.0	5.5	1876	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

HOTEL LAKE

Depth_top (cm)	Depth_bottom (cm)	Year (CE)	Cr EF	Mn EF	Fe EF	Co EF	Ni EF	Cu EF	Zn EF	As EF	Se EF	Cd EF	Pb EF	Ti EF
0.0	0.2	2014	3.9	2.4	2.3	2.7	2.5	5.4	6.4	5.1	1.7	3.7	114.7	0.7
0.2	0.5	1998	6.2	2.6	2.5	2.9	2.7	5.5	14.9	6.1	2.2	10.6	146.2	0.6
0.5	0.8	1976	2.4	2.2	2.1	2.7	2.2	3.5	5.3	2.6	1.4	3.8	12.2	0.7
0.8	1.1	1940	1.6	1.5	1.4	2.4	1.7	1.8	1.9	4.1	2.0	3.7	1.2	0.8
1.1	1.4	1907	1.9	2.5	2.1	3.3	2.1	2.1	2.2	2.7	1.4	2.9	0.9	0.6
1.4	1.7	1895	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.1

Table C.3. Diatom species identified in the three study lakes.

Achnanthidium australexiguum	*Microcostatus naumanii*
Achnanthidium indistinctum	*Muelleria spuldingiana*
Achnanthidium maritimo-antarcticum	*Navicula cremeri*
Adlafia submuscora	*Navicula gregaria*
Aulacoseira lirata	*Navicula* sp.5
Caloneis australis	*Nitzschia annewillemsiana*
Cavinula pseudoscutiformis	*Nitzschia archibaldii*
Chamaepinnularia antarctica	*Nitzschia gracilis*
Chamaepinnularia australomediocris	*Nitzschia homburgiensis*
Chamaepinnularia elliptica	*Nitzschia kleinteichiana*
Chamaepinnularia evanida	*Nitzschia perminuta*
Chamaepinnularia gerlachei	*Nitzschia soratensis*
Chamaepinnularia krookiformis	*Nitzschia* sp.1
Chamaepinnularia krookii	*Nitzschia velazqueziana*
Craticula subpompeana	*Orthoseira roseana*
Diatomella balfouriana	*Pinnularia borealis*
Fragilaria capucina var. *Vaucheriae*	*Pinnularia gemella*
Fragilaria parva	*Pinnularia livingstonensis*
Gomphonema cf. *angustatum*	*Pinnularia microstauroides*
Gomphonema jamesrossense	*Pinnularia sergiplaiana*
Gomphonema maritimo-antarcticum	*Pinnularia* sp.11
Gomphonema sp.1	*Pinnularia* sp.9
Halamphora ausloosiana	*Pinnularia subantarctica*
Halamphora oligotraphenta	*Placoneis australis*
Hippodonta hungarica	*Planothidium australe*
Humidophila keiliorum	*Planothidium capitatum*
Humidophila komarekiana	*Planothidium* cf. *densistriatum*
Humidophila sceppacuerciae	*Planothidium frequentissimum*
Humidophila tabellariaeformis	*Planothidium lanceolatum*
Humidophila vojtajarosikii	*Planothidium renei*
Luticola muticopsis	*Planothidium rostrolanceolatum*
Luticola truncata	*Planothidium* sp.2

Table C.3 cont'd. Diatom species identified in the three study lakes.

Planothidium wetzelectorianum
Psammothidium abundans
Psammothidium aretasii
Psammothidium confusoneglectum
Psammothidium incognitum
Psammothidium manguinii
Psammothidium papilio
Psammothidium rostrogermainii
Psammothidium sp.1
Psammothidium subatomoides
Psammothidium superpapilio
Sellaphora antarctica
Sellaphora cf. *seminulum*
Sellaphora nana
Sellaphora nigri
Sellaphora sp
Stauroforma inermis
Stauroneis bryocola
Stauroneis delicata
Stauroneis latistauros
Stauroneis sofia
Stauroneis subgracilis
Staurosira pottiezii
Staurosira sp.1
Staurosirella antarctica
Staurosirella sp.2
Surirella sp.1
Tryblionella debilis