From Seabirds to Sediments:

The ecological footprint of seabirds at a prominent North Atlantic breeding colony assessed using a multi-proxy paleolimnological approach

By

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ABSTRACT

Seabirds, by assimilating marine prey and subsequently redistributing nutrients through their feces and decaying remains, actively participate in the transfer of marine-derived nutrients from the sea to the land in their role as ecological intermediaries. Through analysis of lake/pond sediments influenced by these nutrients, paleolimnology has shed light on the ecological processes that shape seabird nesting habitats. However, there is a current need to develop species-specific proxies that can be used to identify the source of nutrient transfer in multispecies colonies. This thesis examines the historical shifts of the northern gannet (*Morus bassanus*) colony in Cape St. Mary's (CSM) Ecological Reserve using a paleolimnological approach, and also examines the use of sedimentary DNA (*sed*DNA) as a tool to identify relationships between the fecal bacterial communities of various seabird species breeding in Newfoundland and the bacterial communities of the sediments that they interact with. I inferred past colony sizes using a dated core collected from a pond 240 m upwind of the main breeding site (Bird Rock) and observed a significant increase across the proxy data associated with seabird presence (δ^{15}N, P, Zn, Cd, and chlorophyll *a*), accompanied by shifts in dominant diatom assemblages. The records (dating back to the 1700s) indicate no evidence of gannets nesting on Bird Rock before this period. I then characterized the fecal bacterial communities of several species of pelagic seabirds and found that gannets exhibited a significantly different bacterial community structure compared to other seabirds nesting in Newfoundland. Finally, I examined the bacterial communities from the two ponds impacted by seabird nutrient transfer, as well as a reference pond, and found that the bacterial diversity in sediments of seabird influenced ponds has significantly lower diversity. Overall, this research (1) extends the current data available for seabirds nesting in CSM, namely gannets, beyond the current scope of knowledge provided by monitoring reports, and (2) provides insights into the affect ornithogenic nutrient transfer can have on the diversity and structure of bacterial communities in sediments of coastal systems.

Gannets make daily trips out to sea to forage, where they collect marine-derived nutrients and transport them back to land

Introduced nutrients end up in a freshwater pond 240 m away from the colony due to wind transport.

A thick layer of seabird guano builds up at the bottom of the pond. As the seabird colony grows, more nutrients are deposited into the sediments.

CAPE ST MARY'S ECOLOGICAL RESERVE, NEWFOUNDLAND

ACKNOWLEDGMENTS

The territory in which this research was conducted is the ancestral homeland of the Beothuk. I recognize the island of Newfoundland as the ancestral homelands of the Mi’kmaq and Beothuk. I recognize the Inuit of Nunatsiavut and NunatuKavut and the Innu of Nitassinan, and their ancestors, as the original people of Labrador. I strive for respectful relationships with all the peoples of this province as we search for collective healing and true reconciliation and honour this beautiful land together.

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CO-AUTHORSHIP

This thesis conforms to the Manuscript Format outlined by the School of Graduate Studies. I was the lead author for all chapters presented in this thesis. My thesis supervisors are co-authors on each chapter and provided support during the field work, lab work and statistical analyses required for this project. The interpretation of results and editing of each chapter were also assisted by my co-supervisors. I was responsible for collecting and processing sediment samples and conducting DNA isolations, statistical analyses, and preparation of the manuscript, including all tables and figures. Post-doctoral fellow, Dr. Inmaculada Álvarez-Manzaneda, studying at Queen's University (Canada) and Universidad de Granada (Spain), co-authored and conducted the diatom counts of chapter 2. Dr. Neal Michelutti conducted the chlorophyll *a* analysis and lead-dating, and Dr. John Smol provided interpretations and editing for chapter 2. Dr. Sabina Wilhelm provided population monitoring data for seabirds nesting in Cape St. Mary's Ecological Reserve, as documented by the Canadian Wildlife Society, and Dr. William Montevecchi provided seabird fecal samples from the gannets nesting in the Reserve that were used in both chapter 2 and 3.

COVID-19 MITIGATION

The COVID-19 pandemic had a small impact on the execution of this research project. The timeline for data analysis and subsequent report writing was set back, and the closure of educational institutions meant that the research team had limited access to resources. To mitigate the effects of the pandemic on the research project, the research team explored alternative data collection methods and adjusted the project timeline accordingly.

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LIST OF ABBREVIATIONS

ATPU	Atlantic puffin
ASV	Amplicon sequence variant
Chl *a*	chlorophyll *a*
COMU	Common murre
CSM	Cape St. Mary's Ecological Reserve
CSM-I	Cape St. Mary's Impacted
CSM-R	Cape St. Mary's Reference
CRS	Constant rate of supply model
CWS	Canadian Wildlife Service
DL	Detection Limit
DOC	Dissolved organic carbon
ECCC	Environment and Climate Change Canada
GAM	Generalized additive model
LFI	Little Fogo Island
NOGA	Northern gannet
NGS	Next-generation sequencing
PERMANOVA	Pairwise Permutational Multivariate Analysis of variance
*sed*DNA	Sedimentary DNA
TIC	Total inorganic carbon
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus

LIST OF SYMBOLS

α	Alpha
β	Beta
k	Basis dimension
μ	Arithmetic mean

CHAPTER 1

1. Introduction

This thesis investigates the intricate and multifaceted interplay between seabird populations and their surrounding environment, specifically exploring the ecological shifts induced by the presence of seabirds and their consequential effects on nutrient transport and microbial diversity. The introductory chapter examines the ecological shifts related to marine-derived seabird nutrient transfer and the contribution of paleolimnology to seabird conservation monitoring. I highlight the need to explore questions surrounding the influence of seabird nutrients to ponds that are not directly adjacent to colonies. Furthermore, I consider how the seabird gut microbiome can help us understand the complex interactions among microorganisms in sediments affected by seabird nutrients, thereby enhancing our understanding of the intricate relationships that govern coastal ecosystems.

The following research chapters provide valuable insights into the long-term impacts the seabird colony of Bird Rock has had on the terrestrial ecosystem of Cape St. Mary's (CSM) Ecological Reserve. Chapter 2 validates the effectiveness of using a multiproxy approach in paleolimnology, demonstrating that the northern gannet serves as a vector for the transfer of marine nutrients to the coastal, barren habitat of the Ecological Reserve. Based on the sedimentary profiles taken from CSM, I found no indication that seabirds, mainly gannets, occurred in the study area prior to the early 1880's, thus expanding the scope of existing historical records. Chapter 3 investigates the bacterial diversity in sediments at Cape St. Mary's that are influenced by seabird nutrients, comparing it to an unaffected site and a single-species colony, and examines the gut microbiome of different seabirds to better understand the effect of their feces on nearby ponds. A key finding highlighted in this chapter is that the impact of seabird nutrients on ponds is associated with a lower bacterial diversity in sediments when compared to ponds that lack any seabird influence.

The final chapter provides a discussion of the two manuscripts, highlighting the implications my findings have for the management and conservation of the seabird colony in CSM. Overall, this research contributes to our understanding of the complex interplay between seabird populations, their ability to transport nutrients, and the terrestrial habitats surrounding their colonies.

a) Ecological shifts related to seabird nutrient transfer

The transfer of nutrients from the sea to land, and its implications for terrestrial ecosystems, remains a fundamental area in ecology that necessitates ongoing and thorough research. Many marine vertebrates play roles in distributing ingested nutrients to their habitats through the assimilation of marine prey and the subsequent redistribution of nutrients in the form of feces and decaying remains. Species such as seabirds [1], seals [2], fish [3], and sea turtles [4] have been investigated as trophic intermediaries in this nutrient cycle. Of particular interest are the mechanisms and ecological consequences of ornithogenic nutrient transfer expanding beyond the traditional ecosystem boundaries.

Since the late 1950's, ecologists have recognized that seabirds have important influences on community-level structure and ecosystem dynamics [5]. For example, seabird breeding colonies at St. Paul Island, in the Bering Sea (Alaska), directly contributed to δ^{15}N-enrichment in both terrestrial plants and phytoplankton in the nearshore zone [6]. A recent study by Estupiñán-Montaño et al. [7] underscores the vital role of the Nazca booby (*Sula granti*) in transferring nutrients, primarily guano, chick carcasses, and eggs, from the sea to land. Through isotopic analysis of 403 samples collected on Malpelo Island (Colombia) between 2017 and 2021, it was revealed that the abundance of Nazca booby guano positively impacted other communities, fostering thriving populations of crabs, lizards, and cichlids [7]. Phosphorus, Cd, K, Zn, and As are among the elements enriched in concentration due to the presence of seabirds. Notably, in ponds adjacent to a northern fulmar (Fulmarus glacialis) colony at Cape Vera (Canada), high levels of Zn and Cd exceeded thresholds for adverse biological effects set by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life and posed a significant risk to the local ecosystem [8].

The influence of seabird nutrient inputs on terrestrial habitats extends beyond direct shifts in geochemical composition; these inputs can also induce shifts in the biological structure of the habitats [9, 10]. For example, the unusually high abundance of insectivorous House martins (*Delichon urbicum*) on Stora Karlsö Island (Sweden) was due to larval chironomids (enriched in $\delta^{15}N$) that were feeding on seabird residues from common murres (*Uria aalge*) and razorbills (*Alca torda*) [9]. Seabirds on the island released nutrients at levels similar to the largest waste-water treatment plants in the region [9]. Finally, nutrient transfer from high-arctic seabirds resulted in plant species niche segregation with high diversity in areas of niche overlap, described by the $\delta^{15}N$ signature in the soils [10]. These studies provide important insights into the trophic connectivity between marine and terrestrial ecosystems, and illustrate that seabirds play a role in shaping their ecological niches.

b) The paleoecological footprint of seabird colonies

Understanding the effects of seabird nutrient inputs on an ecosystem requires knowledge of the site's pre-existing composition. Paleolimnologists use sediment cores as a primary research tool to investigate historical changes in ecosystems, since the process of sedimentation in lacustrine water bodies preserves a well-kept record of the geochemical and biological shifts occurring in an area over time. Current seabird monitoring programs stand to benefit from the long-term data paleolimnology can provide because it offers a glimpse into the past population dynamics of a colony and its effects on the surrounding terrestrial habitat [11]. This sort of chronological data is needed to make inferences on the dynamics occurring prior to historical events such as rapid population declines caused by anthropogenic presence [12].

Traditional paleolimnological methods for reconstructing historical seabird colony sizes entail assessing seabird-related nutrients through δ15N values [13, 14] and metal(loid)s [15, 16] within sedimentary records taken from ponds located nearby a colony of interest. Furthermore, biological proxies can then be used to further validate the ecological shifts left behind by shifts in a seabird colony. A frequently employed biological proxy in paleolimnology is the analysis of diatom assemblages, as certain diatom species have been linked to the presence of seabirds [17, 18]. Diatoms, characterized by their siliceous cell walls that endure well in pond sediments, possess dual advantages as a proxy: their abundance in aquatic habitats and the ease of taxonomic identification. Another frequently employed biological proxy is the concentration of chlorophyll *a* (chl *a*) [19, 20]. This pigment, found in the chloroplasts of photosynthetic organisms, is effectively preserved in sediments, and exhibits a notable increase in response to eutrophication-induced enhancements in primary production within bodies of water [21].

Long-term ecological and environmental studies are essential for understanding the range of population variability, distinct from recent declines triggered by climate change or human-induced pressures. Duda et al. [22] employed a paleolimnological approach, utilizing multiple proxies to detect distinct population peaks in the world's largest Leach's storm petrel (*Hydrobates leucorhous*) colony on Baccalieu Island (Newfoundland) around 500 and 1980 CE. Their analysis, including aerial imagery and palynological assessments, indicated a connection between vegetation cover and colony growth until around 1980, with corresponding shifts in surrounding habitats [22, 23]. Hargan et al. [24] investigated nutrient transfer to ponds at different distances from a large arctic thick-billed murre (*Uria lomvia*) colony of approximately 400,000 nesting pairs, on Digges Island (Nunavut), and found that the pond located above and >100 m upwind of the colony had enriched δ^{15}N values, chl *a*, and phosphorus concentrations. They also detected a warming response in the pond nearest the colony, as evidenced by a change in diatom assemblages and chl *a* content, which they linked to a period of rising air temperatures in the Hudson Bay region from the 1940s to the mid-1950s [24].

c) Assessing paleolimnological impacts for a multi-species colony

The capacity to evaluate shifts in sedimentary records that can be linked to a specific species has offered a lot of potential to both the research area of paleolimnology and conservation biology. For example, Michelutti et al. [25] found that pelagic piscivorous seabirds (Arctic tern, *Sterna paradisaea*) had enriched $\delta^{15}N$ values and more bioaccumulated metals (Hg and Cd), while molluscivorous seabirds (common eider, *Somateria mollissima*) had lower $\delta^{15}N$ values and higher concentrations of Pb, Al, and Mn [25]. This insight into species-specific metal transport to nesting sites sheds light on the potential use of distinct metal abundance for tracking species-specific population dynamics, enhancing future conservation decisions. Despite this understanding, a species-specific identifier has not yet been employed to identify the nutrient contributions of multiple species to the same pond catchment. This is particularly relevant for catchments that receive inputs from a mixed seabird colony where multiple species contribute to the nutrient load. The challenge of distinguishing species-specific nutrient inputs from multiple species interferes with our current capacity to use a paleolimnological approach for reconstructing historical seabird populations where multiple species contribute to nutrient flow into water bodies.

Next-generation sequencing (NGS) has opened up new areas of inquiry in paleoecology, such as the study of taxa that lack discernible morphological fossils within sediments [26]. The DNA from seabird guano, including the gut microbiome's bacterial and fungal assemblages, along with tissue DNA, may preserve in seabird-affected sediments. Although DNA from seabird-related inputs in ponds affected by ornithogenic inputs remains unstudied, creating a *sed*DNA proxy for paleolimnological use as a species-specific indicator would significantly aid in identifying the presence of species of conservation concern.

While the field paleogenetic research is still relatively new, several studies have used *sed*DNA to reconstruct historical species' distributions [27] or examine how changing environments impact microbial composition of a habitat [28, 29]. Nelson-Chorney et al. [30] used *sed*DNA to trace historical species distributions of freshwater fishes in a lake in Banff National Park, and identified human-mediated introductions of non-native trout, highlighting the need to protect native populations. Tse et al. [31] employed paleolimnological proxies and NGS to examine cyanobacterial communities in a Canadian Great Plains reservoir, and identified changes in community composition (increased levels of toxic cyanobacteria like *Dolichospermum*) that aligned with shifts in the additional proxies used, including lipids, pigments and diatom assemblages. These studies highlight the importance of employing *sed*DNA as a tool to unravel historical ecological dynamics, track species introductions, and assess environmental changes over long time periods.

In a comprehensive literature review, Capo et al. [32] provided methodological recommendations for using *sed*DNA in paleolimnological research geared towards reconstructing shifts in biota over long time periods. One of their primary recommendations is to select a lake based on ecological questions and adapt protocols to improve DNA extraction and recovery from the sediments. They suggested conducting a pilot study with surface sediments to test the amenability of the sedimentary record for *sed*DNA analysis, and highlight that the ideal number of sediment cores and analytical replicates to sample depends on the target taxon and question. To provide a more reliable reconstruction, they also recommended monitoring microbial composition using negative controls and performing a multi-proxy approach to cross-validate the *sed*DNA approach [32].

d) Colony dynamics of Bird Rock, a multispecies nesting site

This project aimed to investigate seabird nutrient transfer from a multispecies seabird colony in Cape St. Mary's (CSM) Ecological Reserve (Newfoundland), known as Bird Rock (46.83°N 54.17°W), to a freshwater pond ('impacted pond') 240 m upwind of the colony (Figure 1.1). The colony on Bird

Rock is mainly made up of northern gannets (*Morus bassanus*), common murres, and black-legged kittiwakes (*Rissa tridactyla*), as well as razorbills and black guillemots (*Cepphus grylle*). The first report of northern gannets nesting on Bird Rock was in 1883 [33], when 10 nesting pairs were recorded. The gannet colony has been monitored regularly since around the time the Ecological Reserve was protected in 1983 [34], with most recent reports suggesting the population has begun to plateau around 14,600 nesting pairs over the last ten years [35]. The Canadian Wildlife Service (CWS) has also conducted monitoring surveys and provided reports for the black-legged kittiwakes in CSM, which record a population decrease in kittiwakes from 10,000 nesting pairs in 1979, to 4391 nesting pairs in 2019 [35]. The data available for murres in CSM are also sparse, with the last monitoring report in 2007 recording a total of 15,484 nesting pairs [36].

In terms of species distribution along Bird Rock, northern gannets dominate the top of Bird Rock. The black-legged kittiwakes and common murres compete with the gannets for space on both the main stack and mainland areas to the east and west of the stack. The kittiwakes tend to nest and spend most time on the steeper cliff sides, while the murres mainly occupy the bottom regions where the terrain is flatter (Figure 1.1). Since northern gannets are the main nesters at the top of the stack, we believe they contribute to most of the marine-derived nutrients that are transferred via wind to the impacted pond. However, black-legged kittiwakes also use the pond during the day for bathing and resting, and they defecate there, directly contributing nutrients into the pond. I consider these dynamics within the next two chapters of this thesis by first assessing shifts in the nutrient content of a sedimentary record taken from the pond nearby the colony, and aligning it to the known population dynamics of three main seabirds on Bird Rock. I then examine the relationship between the bacterial composition of these species' fecal microbiomes and the bacterial community structure of the most recently impacted sedimentary layers from the cores (0 cm to 8 cm) taken in CSM to better understand if we can use *sed*DNA to reconstruct multispecies seabird colonies.

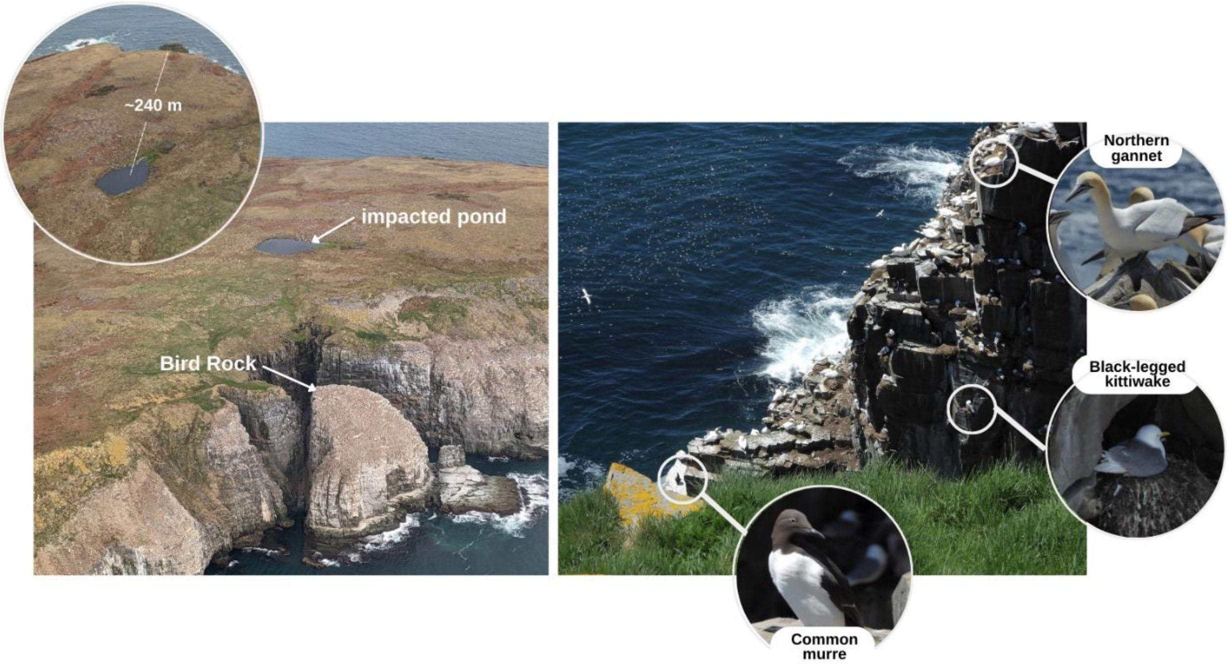

Figure 1.1. Aerial photographs of Bird Rock relative to the 'impacted pond' [LEFT]. [Photos: Kathryn Hargan] Seabirds nesting on the mainland adjacent to Bird Rock [RIGHT], including northern gannets (*Morus bassanus*), black-legged kittiwakes (*Rissa tridactyla*) and common murres (*Uria lomvia*). [Photos: Don Littner]

e) Thesis objectives and approach

An important aim of this project was to verify methods for reconstructing historical seabird colony dynamics and expand our knowledge of Bird Rock's seabird presence beyond the existing historical monitoring data. Overall, the research methods I use here incorporate the use of classic paleolimnological proxies associated to seabird presence with an analysis of sedimentary bacterial composition and diversity. I chose to focus on the chl *a*, $\delta^{15}N$ values, P, Cd and Zn concentrations, and diatom assemblages in sediment records taken from the impacted pond in CSM, and a reference core from a pond 2.56 km away from the colony not experiencing any seabird nutrient inputs. Given that CSM is a multispecies colony, I wanted to examine how bacterial diversity and community structure differed across ponds influenced by nutrient input from a single species, multiple species, or no species at all. I first examined bacterial composition of three sediment cores collected from two ponds in CSM, the impacted pond nearby the colony and the reference pond 2.56 km away from Bird Rock (0 cm to 8 cm of the core), as well as one pond on Little Fogo Island that is directly influenced by Atlantic puffins, who burrow along the pond's perimeter (0 cm to 6 cm of the core) (Figure 1.2). Finally, I examined the fecal microbiome of the four seabird species that we studied, including the northern gannet, black-legged kittiwake, common murre and Atlantic puffin.

Overall, this research aimed to broaden our understanding of the seabird colony dynamics in CSM, provide insight into the influence marine-derived seabird nutrients have on the bacterial diversity and community structure in impacted ponds, and validate methods for reconstructing past seabird colony dynamics using sedimentary records.

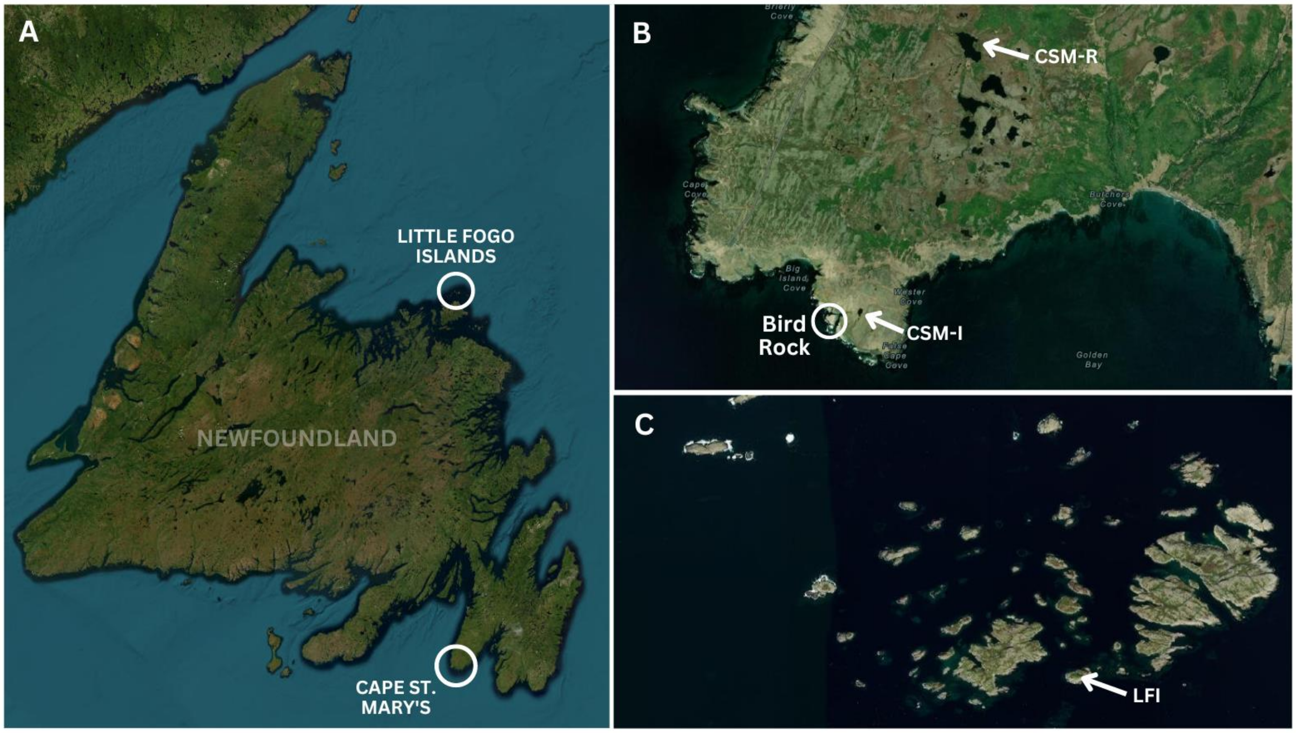

Figure 1.2. Map of the two coring sites for this study in Newfoundland (Canada) circled white [A]. The main nesting site in CSM, Bird Rock, is also circled [B]. The two ponds located in the region of

Cape St. Mary's (CSM) Ecological Reserve, including the impacted pond (CSM-I) and the reference pond (CSM-R) [B] are labelled, and the pond on Puffin Island in the Little Fogo Islands (LFI) is also labelled [C].

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CHAPTER 2

Paleolimnology successfully tracks the establishment and growth of a seabird colony in Cape St. Mary's Ecological Reserve, Newfoundland

Authors Note:

This chapter has been prepared and structured in accordance with the guidelines set forth by the intended publication venue, Proceedings of the Royal Society B.

Abstract

Marine-derived nutrients transferred by seabirds from their feeding grounds to their nesting colonies can cause shifts in nearby pond chemistry and biology. Using sediment cores, the accumulation of marine-derived nutrients can reveal the historical patterns of ecosystem changes caused by seabirds. Here, our goal was to extend current knowledge concerning the long-term colony sizes of northern gannets (*Morus bassanus*) within the Cape St. Mary's Ecological Reserve in Newfoundland, Canada over the last few hundred years. While paleolimnology had never been conducted in Cape St. Mary's region, we have a record of colony size for the gannets nesting there. Most paleolimnological approaches that track colony establishment are conducted on seabird colonies that are adjacent to ponds. This study explored whether we could use sediment cores taken from a pond located upwind of a seabird colony to reconstruct colony establishment and size. We used a multi-proxy paleolimnological approach, inferring historical changes in nesting colony size from sedimentary chlorophyll *a*, diatom assemblages, stable-nitrogen isotopes, and metal(loid)s in a dated core collected from the pond above the colony (the ‘impact pond’). Using time-series developed from sediment core analyses, we confirm seabird nutrients are entering the impact pond and are absent in a more distant reference pond. Across all of our proxies, we observed a significant increase in sedimentary nutrients ($\delta^{15}N$ values, P), metalloids (Zn, Cd), and chlorophyll *a* in the pond nearby the colony starting in ~1868, and we found no signs of seabird colony presence on Bird Rock before that time, with records extending back to the 1700s (depth of ~13 cm). We also observed a distinct change in the dominant diatom species and related this to a shift in pond water chemistry. The striking shift in ornithogenic-related proxies matches the population data available for northern gannets nesting at Cape St. Mary’s and indicates that the gannet colony has reached a historical maximum in present day. Overall, our study a) provides valuable insights into the long-term ecological effects of this nesting colony, b) contributes to a deeper understanding of the interplay between seabirds and their environment and c) shows that our paleolimnological reconstruction of seabird colony sizes align well with the historical population counts, even in circumstances when the colony is not located directly adjacent to a watershed.

Keywords: nutrient transfer, paleolimnology, northern gannets, chlorophyll *a*, isotopes, diatoms

1. Introduction

Seabirds are often apex predators in the marine food-chain, and are under severe threat as the structure and function of oceans continue to degrade due to human activities. Current research suggests nearly half (47%) of all seabird species are suspected to be experiencing population declines, making them one of the currently most threatened vertebrate groups [1]. Loss of seabird biodiversity could have far-reaching ecological effects, given their crucial role in nutrient cycling across ecosystem boundaries [2] and supplying energy to food webs on islands [3]. Seabirds serve as indicators of marine ecosystem health, therefore monitoring their populations is increasingly important.

Establishing a population baseline for any threatened group is a necessary component to assessing future population shifts [4]. Oftentimes, data on colony size do not extend far enough back in time to make conclusions about the long-term trends of a population prior to anthropogenic or climate-related impacts. Understanding the current population status is a challenging task if we cannot pinpoint significant changes in a colony over time. Paleolimnology gives us an opportunity to track ecosystem changes related to ornithogenic nutrient transfer over long time scales and can help establish a baseline for long-term monitoring efforts.

Colonial seabirds can subsidize terrestrial ecosystems with marine-derived nutrients [5, 6, 7]. The activities of many individuals at a large breeding colony can strongly influence the composition and structure of the surrounding environment and biological communities [8, 9]. Signals of chemical, physical, and biological shifts caused by seabird nutrient transfer are well-preserved within sediment layers of ponds adjacent to nesting colonies, and modern paleolimnological techniques can offer insight into the ecological shifts occurring in the ornithogenic sediments over long time periods [10].

Northern gannets (*Morus bassanus*) breed within dense colonies that are usually remote and located on inaccessible cliffs, stacks, and islands. Of the six colonies in North America, Cape St. Mary's (46.83°N 54.17°W) is the only one easily viewed from land. The population data available for gannets nesting at Cape St. Mary's are sparse (Table 2.1), but span over 130 years with the first systematic count taking place in 1934 [11].

Table 2.1 Historical population reports collected for northern gannet (*Morus bassanus*), black-legged kittiwake (*Rissa tridactyla*), and common murre (*Uria aalge*) nesting in Cape St. Mary's Ecological Reserve, 1883-2018. A blank cell indicates that population counts were not collected for that year.

Year	Population counts (pairs) [reference]		
	Gannet	Kittiwake	Common murre
1883	10 [11]		
1918	2000 [13]		
1934	4500 [11]		
1939	4349 [14]		
1942	5000 [15]	7500 [15]	2500 [15]
1959			2500 [21]*
1960	colony collapse [12]		
1969	3000 [13]		
1972	5260 [16]	10 000 [13]	
1977	4866 [16]		
1978	5050 [16]		
1979		10 000 [20]*	10 000 [13]
1980	5000 [12]		
1984	5515 [17]		
1988	400 (mainland) [13]		
1994	7179 [18]		
1999	12 986 [16]		
2004	12 432 [18]		
2007			15 484 [16]
2009	14 789 [18]		
2010	14 741 [16]		
2011	14 696 [16]		
2012	12 970 [16]		
2013	13 515 [16]		
2018	14 598 [19]		
2019		4391 [6]	

* Nettleship [20] and Tuck [21] are primary sources mentioned in Wilhelm [16]

Cape St. Mary's was established in 1983 as an Ecological Reserve to protect the rich diversity of breeding and overwintering seabirds in the region [22]. Cape St. Mary's is occupied by several seabird species throughout the breeding season, including 15 500 pairs of common murres (*Uria aalge*) [16], 14 598 pairs of northern gannets [19], 100 pairs of razorbills (*Alca torda*), which were only censused in 1959 [21] and 1979 [20], 4400 pairs of black-legged kittiwakes (*Rissa tridactyla*) [23]. The main nesting site for these seabirds is known as Bird Rock, a 100 m high sea stack separated from shore by a deep gorge, but only 10 m from the cliff edge. Bird Rock has historically been dominated by northern gannets. A colony collapse occurred in the 1960s, and is attributed to human disturbance [12], but according to the most recent surveys the colony has grown steadily over the years and reached a stable plateau which began in 2009.

Here, we examine the ecological process of seabird nutrient transfer from sea to land at the seabird colony of Cape St. Mary's. We use a multi-proxy paleolimnological approach to infer trends in the past dynamics of the northern gannet nesting colony beyond the scope of historical monitoring data, as well as confirm historical population counts and determine when this current colony was established. Sedimentary records from two different freshwater ponds were obtained. One sampling site was a pond affected by seabird nutrient inputs, referred to here as the impact pond. The second site was a pond 2.56 km away from the colony, and not influenced by seabird nutrient inputs, referred to here as the reference pond (Appendix I - Figure AI1). We expected the sediment core sampled from the impact pond to contain an enrichment of metal(loid)s, phosphorus, and stable isotopes (δ^{15}N and δ^{13}C) caused by bioaccumulation of gannet nutrients as the colony grew over time. Along with a shift in primary production within the impact pond sediments (recorded using measurements of sedimentary chlorophyll *a*), we also expected to record a change in the diatom assemblages from the early 1900's to present as the gannet population grew [12] and resulted in water chemistry changes within the pond. This investigation focuses on the Bird Rock colony, rather than the mainland nesting areas of Cape St.

Mary's, as we have higher confidence in the nutrient transfer occurring via wind from this area to the pond, which would not extend to the mainland nesting sites even as the colony expanded.

2. Materials and methods

a) Study site description

Cape St Mary's Ecological Reserve protects a small portion of Newfoundland's fragmented Eastern Hyper-Oceanic Barren ecoregion [22], spanning a total of 64 km^2, of which only 10 km^2 is land mass. The reserve has a cool summer climate (July average = 14°C) and because of the onshore winds, experiences an average of 200 days of fog throughout the year [24]. The topography of the ecological reserve is generally flat, and consists of exposed rocky terrain, patches of alpine mosses and small, windswept stands of black spruce, white spruce, and balsam fir [25]. Soils in this ecoregion are composed mostly of organic matter that occur in peatlands dominated by alpine mosses (*Diapensia* spp. and *Racomitrium lanuginosum*) [22]. The main nesting site is surrounded by sheer cliffs that tower 100 m above the rugged shoreline, and the clifftops are covered with a variety of subarctic flowering species.

Today, approximately half the nesting gannets at Cape St. Mary's are on Bird Rock, while the other half takes up the top portion of the mainland cliffs surrounding the stack to the east and west [26]. The impact pond we sampled was ~240 m away from Bird Rock (46.81827, -54.179791), and the reference pond was 2.56 km away from the colony (46.838331, -54.167806). Sediment cores collected from the ponds were named CSM-IMP-2 and CSM-REF-3, respectively (Appendix I - Table AI1).

Before collecting a sediment core from each site, pond water pH and conductivity (µS cm^{-1}) were measured using a Hanna portable pH/conductivity meter. We took water samples from the impact pond on three separate occasions to ensure nutrient content remained elevated over time, compared to the reference pond, as a result of seabird nutrient inputs. We sampled surface water from the impact pond using 1 L Nalgene bottles, that were first pre-rinsed with lake water, on 18 July 2020, 18 July

2021, and 12 September 2021. Surface water samples for the reference pond were also taken on 18 September 2021 using the same method. The water samples collected in July 2020 were sent to The National Lab for Environmental Testing (Burlington, ON) for analysis of calcium (Ca^{2+}), fluoride (F^-), chloride (Cl^-), magnesium (Mg^{2+}), sodium (Na^+), sulfate (SO_4^{2-}), potassium (K^+), silica (SiO_2), and unfiltered total phosphorus (U-TP). The other water samples were sent to Avalon Laboratories (St. John's, NL) for analysis of dissolved organic carbon (DOC), total inorganic carbon (TIC), total Kjeldahl nitrogen (TKN), Ca^{2+}, Mg^{2+}, Na^+, and K^+.

b) Obtaining a sedimentary record

Sediment cores were collected using a polypropylene tube (88 mm) mounted on a Glew and Smol [27] push corer. A vertical extruder [28] and plastic scraper tool were used to section the sediment cores, with each piece thoroughly between sections. The impact pond's sediment core was taken at the pond depth of 1.12 m, sectioned every 0.25 cm to a core depth of 16 cm, and then sectioned every 0.5 cm to the bottom of the core (26 cm total). The reference core was taken at the pond at a depth of 0.79 m, and the entire core sectioned every 0.5 cm (30 cm total). Samples were placed into Whirl-Pak® bags (2 oz.) that were then transported back to the lab and placed into a -80°C freezer. The samples were subsequently freeze-dried for 48 hours in a Labconco FreeZone 12 L Console apparatus. After freeze-drying, the dried sediment was weighed (g), and the percentage of water in each sample was calculated. The freeze-dried sediment of each core was sub-sampled for ^{210}Pb dating, chlorophyll *a* (chl *a*), stable isotopes, diatom assemblages, and metal(loid)s.

Preparation of the sediment samples for ^{210}Pb-dating followed comprehensive methods designed for low-background gamma counting [29, 30]. Approximately 0.5 g of freeze-dried sediment from every centimeter of the impact and reference cores were placed into a gamma tube with a cap. The top of each sediment sample within the gamma tube was sealed with a 50:50 epoxy resin and polyamine hardener (2-Ton Epoxy). The Paleoecological Environmental Assessment and Research Lab (PEARL) of Queen's University assigned sediment chronologies for each core with the constant rate of supply (CRS) model [29] using the software ScienTissiME (Barry's Bay, Ontario, Canada).

c) Multiproxy analyses

We collected fecal samples from two gannets to obtain reference $\delta^{15}N$ and $\delta^{13}C$ values for their feces. The gannets were captured by an animal care expert following methods established by the Canadian Council on Animal Care under an Environment and Climate Change Canada (ECCC) Scientific Permit to Capture and Band (Permit No.:10332K). Fecal samples were kept in a -80°C freezer until being transferred to a Labconco FreeZone 12 L Console freeze dryer for 24 hours. Approximately 1 mg of the fecal sample was used for the analysis. We also sub-sampled approximately 5 mg of freeze-dried sediment from every centimeter of the sediment cores after removing any debris. The fecal and sediment samples were weighed on an analytical scale and placed into tin capsules. The tins containing sediment and fecal material were folded with metal spatulas and placed into a 96-well sample tray. Samples were analyzed using a Carlo Erba elemental analyzer coupled to a Delta V Plus isotope ratio mass spectrometer (Thermo Scientific) via a ConFlo III interface at The Earth Resources Research and Analysis Facility (Memorial University of Newfoundland, NL). The temperature of the combustion reactor (chromium oxide and silvered cobaltous oxide) was set to 1050 °C, while the reduction reactor (Cu) temperature was set to 600 °C. For $\delta^{13}C$ isotope analysis, $CaCO^3$ ($\delta^{13}C = -40.11 \pm 0.15$) and D-fructose ($\delta^{13}C = -10.53 \pm 0.11‰$) were used to calibrate the scale; while IAEA-N-2 ($(NH_4)_2SO_4$; $^{15}N = +20.32 \pm 0.09‰$) and USGS25 ($(NH_4)_2SO_4$; $\delta^{15}N = -30.25 \pm 0.38‰$) were used to calibrate the scale for $\delta^{15}N$ air/‰ of peak. Bulk $\delta^{13}C$ values were corrected to account for the *Suess* effect [31]. For the purpose of replication and due to the high organic content of the sediment, we also sent several of the same samples from the cores to be analyzed by the Ján Veizer Stable Isotope Lab (University of Ottawa, ON).

For metal(loid) analysis, samples from both cores were prepared by weighing approximately 0.5 g of freeze-dried sediment from every centimeter interval. The sediment sub-samples were ground using a pestle and mortar and placed into plastic scintillation vials. The samples were geochemically analyzed at SGS Inc. (Lakefield, Ontario) for thirty-five different metal(loid)s using inductively coupled plasma mass spectrometry (Appendix I - Table AI2) and certified standards and blanks were used to ensure quality of the metals analysis was maintained [32]. Subsequent data analysis focused on the concentrations of metal(loid)s associated with seabird nutrient input, such as phosphorus and the concentrations of cadmium (Cd) and zinc (Zn), which were normalized against levels of aluminum (Al) to reduce signals from any long-term changes in erosion [33].

For chl *a* analysis, freeze-dried sediment was sieved (125 µm mesh) and analyzed in glass vials using visible range spectroscopy with a Model 6500 series Rapid Content Analyzer (FOSS NIRSystems Inc.). Chl *a* concentration was inferred from spectral data by measuring the area under the peak between 650 and 700 nm that is associated with chl *a* and its diagenetic products, and then applying a linear equation to convert the area measurement to an estimate of concentration [34].

For diatom isolation, ~0.1 g of freeze-dried sediment was digested using a 1:1 molecular weight ratio of concentrated H_2SO_4 and HNO_3 and heated at 80 °C for 2 hours. The resultant slurry was rinsed with deionized water until reaching neutral pH. Aliquots of these slurries were pipetted onto coverslips and dried with a slide warmer before being mounted onto slides using Naphrax®. At least 400 diatom valves per sample were counted and identified to species using a Leica DMRB microscope under oil immersion at 1000× magnification and the Krammer and Lange-Bertalot [35] guides. Subsamples from the impact core were analyzed for diatoms every 1 cm from 0 to 10 cm and every 2 cm from 10 to 16 cm. For the reference core, sub-samples were analyzed every 0.5 cm from 0 to 2.5 cm to obtain a higher resolution over the period of the gannet record since sedimentation rate of the pond was slow relative to the impact pond, and every 1 cm from 3 to 20 cm.

d) Statistical analyses and visualization

We used the R/v4.2.2 packages *tidyverse*/v1.3.2 [36] and *tidypaleo*/v0.1.2 [37] to plot stratigraphic diagrams of our geochemical data from each site, including chl *a*, $\delta^{15}N$, P, Cd/Al, Zn/Al and dominant diatom relative abundance data for species of *Stauroforma exigiformis* and *Staurosira construens*, from each site. The package *patchwork*/v1.1.2 was used to plot the geochemical and relative abundance data together [38]. To conduct a breakpoint analysis on the metal(loid)s data and find periods of significant change based on the data collected from 30 metal(loid)s, we ran a stratigraphically constrained cluster analysis using *tidypaleo* [39]. For the core from the impact pond, we used the CRS-^{210}Pb dates from 0.125 cm to 7.875 cm, and then extrapolated the dates to the bottom of the core to provide the best possible time period estimate for our analysis. The proxy data from chl *a,* $\delta^{15}N$, corrected $\delta^{13}C$, Cd/Al, Zn/Al, P, and the two dominant diatom species were normalized by transforming the data to a Z-score value and then fitted to a Generalized Additive Model (GAM) using a restricted maximum likelihood function available through the R/v4.2.1 package *mgcv*/v1.8-40 [40]. To find significant periods of change in the proxy data, we identified periods where 95% confidence intervals in the first derivative of the GAM did not bound 0 [41, 42]. Finally, we combined the z-score values from selected ornithogenic-related proxies aligned against ^{210}Pb-dates to infer colony trends over approximately 200 years. The relative abundance for *S. exigiformis* (%) was not included in the final z-score figure because it is not a recognized “ornithogenic diatom”.

3. Results

a) Water chemistry

Concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} were highest in the July 2021 samples, when the CSM-IMP-2 sediment core was taken, and remained elevated until we sampled again in September 2021 (Table 2.2). The concentrations of major ions (TN and TP) were overall higher in the impact pond than in the reference pond (Table 2.2).

Table 2.2 Water chemistry of surface water in unfiltered samples taken from the two ponds, with detection limits (DL) for nutrients and inorganic compounds, including total nitrogen (TN) and unfiltered total phosphorus (TP), Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}. The impact pond was sampled on three separate dates for water chemistry, while the reference pond was only sampled once.

Variable	**Impact Pond**			**Reference Pond**
Sampling Date	July 2020	July 2021	Sept 2021	Sept 2021
pH			7.4	6.7
Conductivity (µS cm^{-1})			1500	120
TN (mg L-1) [DL]			2.64 [<0.02]	0.451 [<0.02]
TP (µg L-1) [DL]	960 [<0.5]		414 [<6]	50 [<6]
Ca^{2+} (mg L-1) [DL]	3.29 [<0.01]	12.9 [<0.04]	7.7 [<0.1]	3.7 [<0.1]
Mg^{2+} (mg L-1) [DL]	6.81 [<0.01]	23.7 [<0.02]	18.9 [<0.1]	2.4 [<0.1]
Na^{+} (mg L-1) [DL]	87.7 [<0.01]	236 [<0.1]	206 [<0.1]	20.1 [<0.1]
K^{+} (mg L-1) [DL]	3.35 [<0.03]	9.1 [<0.08]	6.3 [<0.1]	0.6 [<0.1]

b) ^{210}Pb dating profiles

^{210}Pb activity was higher in the core from the impact pond, which had a starting activity of 861.74 Bq kg^{-1}; starting activity was only 572.38 Bq kg^{-1} in the reference core (Appendix I - Figure AI2). Both cores displayed an exponential decay of ^{210}Pb activity through their depth. Based on the constant rate of supply model, the core from the impact pond represented ~138 years between the depths of 0 cm to 8.25 cm, while the reference core decayed rapidly between in the top layers of the core (from 572.38 Bq kg^{-1} at 0.25 cm to 11.65 Bq kg^{-1} at 4.25 cm), and only represented ~108 years between the depths of 0 cm to 1.75 cm (Appendix I - Figure AI2). The average sedimentation rate of the reference core (0.0191 cm $year^{-1}$) was considerably lower than that of the impact core (0.1063 cm $year^{-1}$). We decided to date each 0.5 cm of the reference core between 0 and 2 cm due to the slower sedimentation rate of the reference pond. At a depth of 2 cm, the reference core had reached a date of approximately 1914, while in the core from the impact pond that same depth reached the year 2005.

c) Multiproxy analysis

Overall, the geochemical values obtained from the chl *a*, δ^{15}N values, and metal(loid) analyses were higher at the top of the impact pond's core compared to the same values obtained from the reference core (Figure 1). Dates preceding the timeframe of the 1700s (corresponding to ~13 cm) were excluded from consideration for the impact core in this assessment of the results because of limitations in the reliability of extrapolating the dates to extend further into the past. For all the dates prior to the 1700's, core depth (cm) is used to describe the results instead.

The analysis of chl *a* concentration in the impact and reference cores revealed distinct patterns of change over time. In the impact core, chl *a* concentration remained relatively stable from the bottom of the core (~25.75 cm), where it was equal to 0.005 mg g^{-1} dry wt., up to ~6 cm (corresponding to the year 1931), where it was equal to 0.02 mg g^{-1} dry wt. In the reference core, chl *a* concentration between the similar depths was overall lower, with values at 20.25 cm equal to 0.002 mg g^{-1} dry wt., and at 6.25 cm equal to 0.005 mg g^{-1} dry wt. Chl *a* values in the core from the impact pond increased from 0.02 mg g^{-1} dry wt. in 1931 (~6 cm) to 0.1 mg g^{-1} dry wt. in 2021 (~0 cm). The core from the reference pond had values which decreased from 0.005 mg g^{-1} dry wt. at 6.25 cm, to 0.02 mg g^{-1} dry wt. at 0.25 cm. We also observed a short period of decrease in chl *a* in the top of the impact core from 2001 (~2.75 cm) to 2012 (~1.5 cm), and then a longer period of increase from 2012 to the highest concentrations in 2021 (~0 cm).

Next, we assessed the isotopic values of our core samples and then compared them to the values obtained from the two gannet fecal samples. Between ~25 cm to ~8.75 cm in the impact core (prior to 1895 according to the dating profile shown in Table S3, near the time period gannets were first seen nesting on Bird Rock [11]) δ^{15}N values fluctuated between 1.2‰ and 1.3‰, respectively, while similar depths in the reference core had δ^{15}N values that fluctuate from -3.4‰ at 20.25 cm, to 0.2‰ at 8.25 cm. Near 1913 (~7.75 cm), we observed a large increase in δ^{15}N values in the impact core up until

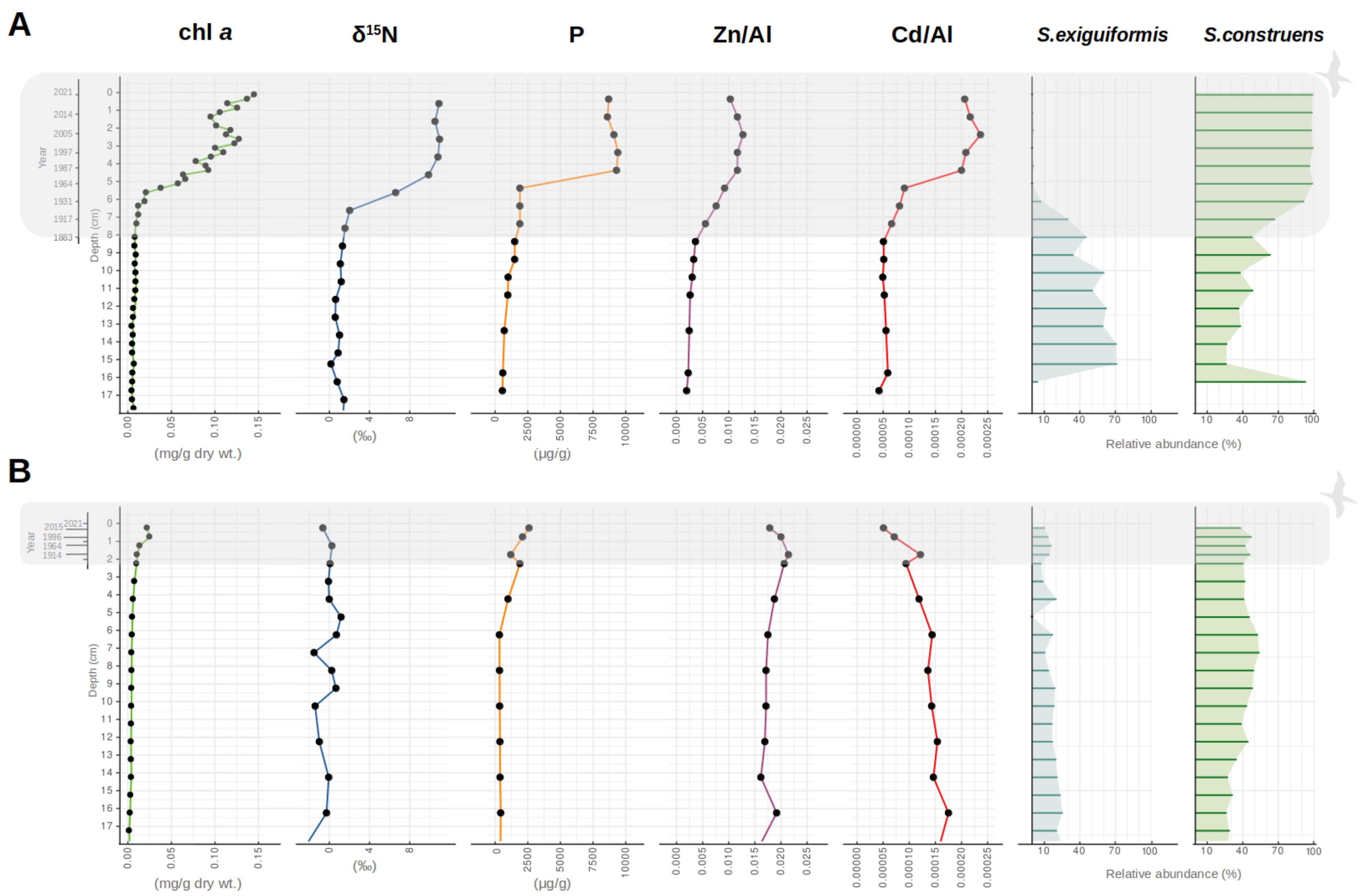

Figure 2.1 Multiproxy stratigraphy of the impact pond core [A] and reference pond core [B]. The secondary y-axis shows the ^{210}Pb dates that were assigned to selected layers of the core using the CRS model, and the dates are aligned by midpoint depth (cm). Grey shaded bars across the stratigraphy represent the time period gannets are known to be nesting in Cape St. Mary' s, based on the data found in Table 2.1.

1992 (~3.75 cm), from 1.6‰ to 10.8‰, respectively. Between similar depths in the reference core, we observed a slight increase in $\delta^{15}N$ from -1.5‰ at a depth of 7.25 cm, to -0.6‰ at a depth of 0.25 cm. From 1992 to 2021 (~0 cm), $\delta^{15}N$ values of the impact core plateau, with a range of 10.8‰ to 10.9‰ (µ =10.8‰), respectively. Values for $\delta^{13}C$ in the impact core ranged from -26.5‰ in 1863 (~9.75 cm) to -24.7‰ in 2017 (~0.75 cm). $\delta^{13}C$ values in the reference core ranged from -25.9‰ in 1992 (3.25 cm) to -26.6‰ in 2021 (0.25 cm); uncorrected values through the entire depth of the reference core ranged from -24.7‰ at a depth of 20.25 cm to -28.1‰ at a depth of 0.25 cm. Finally, the $\delta^{15}N$ values for the two gannet fecal samples were 11.3‰, and 16.8‰, and the uncorrected $\delta^{13}C$ values for these samples were -20.7‰ and -20.5‰, respectively.

Metal concentrations throughout the entire depth of the impact core were higher than the reference core. There was an increase in metal concentrations over the depth of the impact core that was not observed in the reference core to the same extent. From 16.75 cm to 6.5 cm (corresponding to the period prior to the 1930's) in the impact core, concentrations of P increased from 560 µg g^{-1} dry wt. to 1900 µg g^{-1} dry wt., Zn/Al from 0.002 and 0.008, and Cd/Al from $4.2x10^{-5}$ and $8.1x10^{-5}$, respectively. While in the bottom of the reference core, between the depths of 20.25 cm and 6.25 cm, P concentrations were 370 µg g^{-1} dry wt. and 330 µg $g^{=1}$ dry wt., Zn/Al were 0.01 and 0.018, and Cd/Al were $1.2x10^{-4}$ and $1.4x10^{-4}$, respectively. In the top layers of the impact core, we observed a large increase in P, Zn/Al and Cd/Al in from 1956 (~5.5 cm) to 2019 (~0.5 cm), where P increased from 1900 µg/g dry wt. to 8700 µg/g d.w, and the ratio of Zn/Al and Cd/Al increased from 0.009 to 0.01 and $9.1x10^{-5}$ to $2.1x10^{-4}$, respectively. The large increase in metal(loid)s observed in the impact core was not observed in the reference core, where P only increased from 990 µg/g dry wt. at 4.25 cm, to 2600 µg/g dry wt. at 0.25 cm, Zn/Al remained stable from 0.02 at 4.25 cm to 0.02 at 0.25 cm, and Cd/Al decreased from $1.2x10^{-4}$ at 4.25 cm to $5.1x10^{-5}$ at 0.25 cm. Even though the overall concentration of Cd was higher at some depths in the reference core compared to the impact core, it is important to note that concentrations of Cd/Al in the impact core increased over time, while in the reference core they decrease (Figure 1). Furthermore, the concentration of Cd/Al was lower at 4.25 cm in the reference core (5.1×10^{-5}) and then peaked at 1.75 cm (1.2×10^{-4}), before increasing again up to the 0.25 cm mark. Overall, concentrations of P in the top of the reference core increased, however much less than they did in the impact core. Although we observed higher values of Zn/Al in the top layers of the reference core compared to the impact pond, the Zn/Al values fluctuated less throughout the entire depth of the reference core, while the impact pond's core showed a more distinct shift between 1854 (~9 cm) and 1910 (~8 cm). Additionally, the breakpoint analysis conducted on all 30 metal(loid)s showed the greatest change between 1895 (8cm) and 1916 (7 cm) in the impact core (Appendix 1 - Figure AI3), but further analysis showed a rapid change in ornithogenic metalloids such as P between 1940 (5 cm) and 1986 (4 cm) (Figure 1).

The impact core showed a shift in the dominant diatom species that was not present in the reference core. At 1964 (~6 cm) in the impact core, the shift in dominant diatom species occurs matches the timing of the increase in chl *a* (Figure 1). At ~6 cm, the dominant species of *Stauroforma exiguiformis* disappeared and was replaced by two subspecies of *Staurosira construens* that remain the dominant species to 2021. Interestingly, the reference pond had the same dominant diatom species as the impact pond; however, their abundances stay stable throughout the depth of the reference core.

Overall, the impact core had a greater range in relative abundance of *S. exiguiformis* (range = 0% - 93.3%) and *S. construens* (range = 4.8% - 99.6%) than the reference core, which had more stable abundances of *S. exiguiformis* (range = 0 - 26.9%) and *S. construens* (range = 15.0% - 54.2%).
Next, we reviewed the years where derivatives of significant increase/decrease were identified for each ornithogenic-related proxy (Table 2.3) from the GAM (Appendix I - Figure AI5). The k-index and p-value of each proxy are used to assess the dimensionality of the basis functions used in the GAM. A low p-value ($p\text{-value} < 0.05$) suggests that the basis dimension (k) may be too low [40]. Overall, we found a

statistically significant increase across the proxies studied in the impact pond's core that are associated with ornithogenic nutrient transfer over the last ~140 years. In general, the significant periods of change identified for Zn/Al and $\delta^{15}N$ values occurred before the 1880's, when gannets first began nesting on Bird Rock; while significant periods of change for chl *a,* Cd/Al and P occurred after the 1880's. The GAM showed a significant period of increase in *S. construens* throughout the entire core (Table 2.3), however the stratigraphy in Figure 2.1 showed a more specific increase in the dominant diatom species, where abundance increased from 4.7% at the bottom of the core, to 67.5% at a depth of ~7.25 cm, and 99.6% at a depth of ~0.25 cm. The low k-indices and p-value for chl *a* and $\delta^{15}N$ shows that the basis dimensions (k) may have been too low, but the k-value was set to its maximum limit (equal to sample size) and resulted in a full convergence for chl *a* and $\delta^{15}N$ after 7 and 8 iterations, respectively, and a positive definite hessian, which suggest a well-performing model [40]. For the remaining proxies, the basis dimensions are not low, and the model performed appropriately, resulting in higher k-indices and p-values.

Table 2.3 Generalized additive model results for each proxy, including k-indices, p-values, and range of years where a derivative of significant increase was found. No periods of significant decrease were found for these data.

Proxy	k-index	p-value	Sig. increase
Chl *a*	0.54	$2x10^{-16}$	1914 -2021
$\delta^{15}N$	0.59	0.01	1869-2017
P	0.95	0.32	1895 - 2019
Zn/Al	0.77	0.14	1868 - 1989
Cd/Al	0.87	0.34	1895 - 2019
S. construens	0.97	0.35	1603 - 2021

Next, we reviewed the significant periods of change in the reference core from the bottom of the core to the top to compare trends seen in the impact core's temporal series. According to the GAM for the reference core, the relative abundance of *S. construens* (%) was shown to increase significantly from the bottom of the core (20.25 cm) to a depth of 8.99 cm, where we observed a slight, but significant, decrease between 5.37 and 4.97 cm. Moreover, the stratigraphy in Figure 2.1 shows a slight overall increase in abundance from the bottom of the reference core to the top, from 28.5 % at a depth of 0.25 cm, to 54.2 % at 7.25 cm and 15.03 % at 20.25 cm. We found smaller periods of significant increase in our proxy values from the bottom layers of the reference core (corresponding to the oldest sediments) to the top, for chl *a* between the depths of 0.25 cm and 4.17 cm, for $\delta^{15}N$ between 13.71 cm and 20.25 cm, for P between 0.025 cm and 6.78 cm, and Zn/Al between 0.25 cm and 20.25 cm (Table AI4).

In Figure 2.2, we combined selected ornithogenic-related proxies into one figure that includes an arithmetic mean (μ) fit with a polynomial regression to display the overall changes in the multiproxy data from the early 1800's to core collection in 2021. A cut-off was set at a depth of 11.25 cm, which corresponds to approximately 1808, to obtain a continuous set of data because some of the proxy data did not extend to the same depth. The dates used on the x-axis of Figure 2.2 are extrapolated from the ^{210}Pb dates across the depth of the cores using a polynomial regression (Figure AI2). In Figure 2.2, we observed a dip in chl *a* in the top layers of the core, which was also found in the $\delta^{15}N$ data, to a lesser extent, while the concentrations of P, Zn and Cd decrease but are not seen to increase again like they do in chl *a* and $\delta^{15}N$.

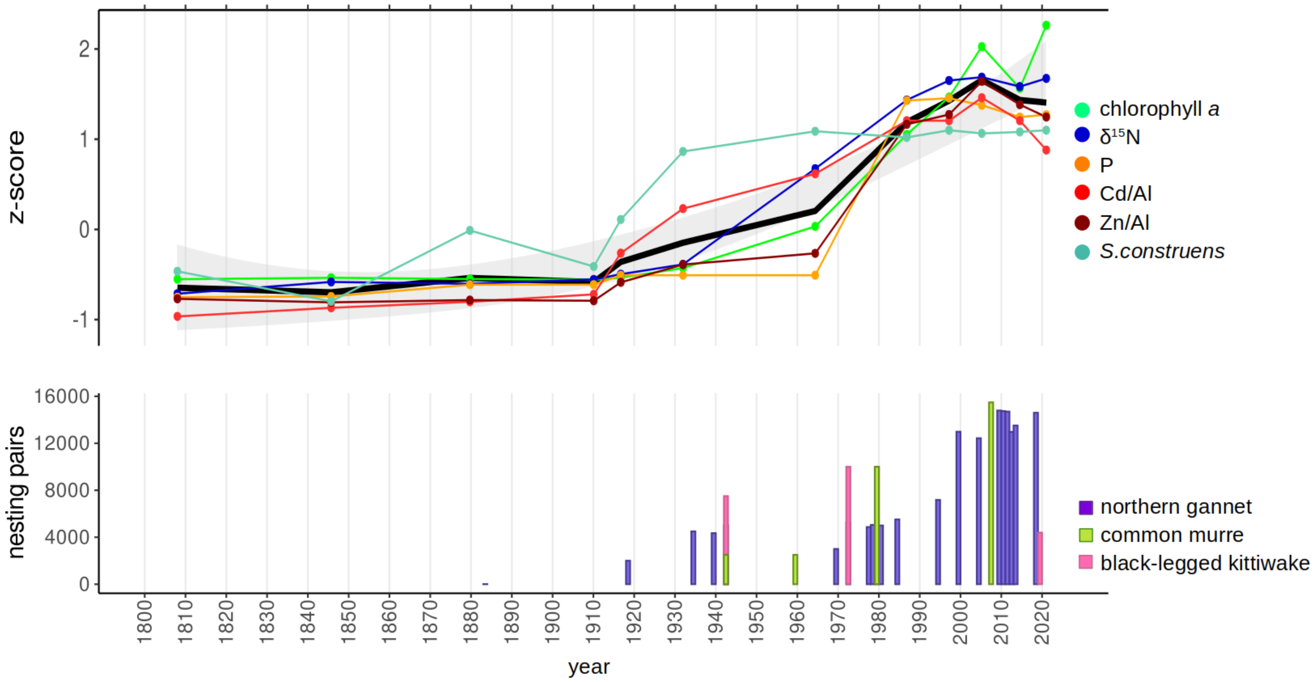

Figure 2.2 [A] A summary of ornithogenic-related proxies measured in the impact pond, where each point represents a z-score value for our selected proxy data. A final arithmetic mean (thick black line) was calculated across the points in the series and fit with a polynomial regression using ggplot2 in R. [B] The historical monitoring data for northern gannets (*Morus bassanus*), common murres (*Uria aalge*) and black-legged kittiwakes (*Rissa tridactyla*) nesting on Bird Rock and surrounding mainland areas aligned with the final proxy data by year (data referenced in Table 2.1).

4. Discussion

Our multiproxy paleolimnological approach suggests that the pond 240 m away from Bird Rock has historically received and continues to receive seabird nutrients via wind transport, and possibly direct input from birds flying overhead and defecating in the pond, starting between 155 and 109 years ago (Table 2.3). Based on the derivatives of significant increase produced by the GAM, the chl *a* data suggests that the impact pond's primary production began to significantly change in 1914, while $\delta^{15}N$ values suggest that significant changes in nutrient transfer began in 1869, and Zn/Al in 1868. Both P and Cd/Al values indicated that the significant shift began in 1895.

In tree-less, windy coastal environments, especially in tundra barrens like those found at CSM, seabird nutrients can have large impacts on terrestrial ecosystems located above the cliffs on which the seabirds nest. For example, a large population (~400,000 breeding pairs) of thick-billed murres occupying the steep cliffs on Digges Island, Nunavut, was found to influence the water chemistry, sediment $\delta^{15}N$ values, metal(loid)s, and P concentrations in a pond situated above, but <100 m away from the nesting colony [43]. In our study, the timing of the notable nutrient input from seabirds to the impact pond varied with the proxy we utilized. Nevertheless, all proxies indicate a response to the nesting of gannets, with $\delta^{15}N$ values and Zn demonstrating a response about 17 years prior to the first documentation of 10 nesting pairs at Cape St. Mary's in 1883, and chl *a* data showing a response 29 years later. The rapid increase in sedimentary P may be a result of post-depositional processes and redox mobility, rather than just seabird nutrient input, corroborating findings from Ginn et al. [44], in Lake Simcoe (Ontario). However, the increase still aligns with the other proxies used in this analysis, and the period of significant increase in both Cd/Al and P most closely corresponded with the first monitoring report, commencing from 1895 onward (Table 2.1).

For the reference core, we did not observe large shifts in the proxy data. The sedimentary record from the reference core captured a very slow sedimentation rate relative to the impact pond and a stable aquatic ecosystem over hundreds of years (Figure 2.1). The distinct change in the dominant diatom species in the impact pond's core was likely related to a shift in water chemistry from a more acidic to circumneutral pH. Most importantly, we find that the striking changes in the ornithogenic-related proxies from the impact pond match the population data available for the northern gannets nesting on Bird Rock.

The concentration of chl *a* and its diagenetic products (hereafter referred to as chl *a*) in aquatic sediments has been used extensively in paleolimnology as an effective proxy to track shifts in primary production over long time periods. We found that concentrations of chl *a* at the bottom of the impact pond's core were similar to that of the reference core, but in the 1930s (~6 cm), the core from the impact pond showed a shift in primary production that was not seen in the reference core (Figure 2.1). Of all the proxies studied for the impact pond, we observed that the concentration of chl *a* began to significantly increase the latest, beginning in 1914, and extending to the present day.

Several multi-proxy studies have successfully linked shifts in stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and geochemistry within sediment cores to the population shifts of northern fulmar (*Fulmarus glacialis*) [45], common eiders (*Somateria mollissima*) [46], and Leach's storm-petrels (*Hydrobates leucorhous*) [47]. According to the GAM, $\delta^{15}N$ values increased significantly, beginning around 1869 and continuing to 2017, which was not recorded in the reference pond (Table 2.3). From 1992 to 2017, we noticed a deceleration in the rate of increase for $\delta^{15}N$ values over the course of the 20th century (Figure 2.2), which occured during a time-period when the gannet colony increased by ~7,419 nesting pairs from 1994 to 2018 (Table 2.1). Previously Duda et al. [33] showed that once a pond on Grand Colombier Island became saturated with storm-petrel $\delta^{15}N$, it could no longer track increases in the population. Since the impact pond has a maximum $\delta^{15}N$ value similar to that of the gannet guano (11.0‰), it is possible the impact pond experienced a plateau associated with a saturation of gannet $\delta^{15}N$ in the local environment.

Between 2007-2009, Tait et al. [48] measured the $\delta^{15}N$ values of feces collected from a total of 27 gannets sampled on the islands of Alisa Craig (μ = 15.9±3.37‰, range= 13.1‰ - 16.3‰) and Bass Rock (μ = 13.5±1.1‰ , range = 13.2‰ - 15.8‰), and a total of 213 black-legged kittiwakes sampled on the island of Rùm (μ = 9.1±0.53‰, range = 8.5‰ - 9.6‰), the Isle of May (μ = 10.9‰, range=8.3‰ - 12.8‰) and the Dunbar harbour (μ = 9.9‰, range = 7.1‰ - 12.9‰), in Scotland. The $\delta^{15}N$ values from our gannet fecal samples (11.3‰ and 16.8‰) were either slightly below or above the values found by Tait et al. [48]. Since the maximum $\delta^{15}N$ value in the Cape St Mary's impact core was 11‰ when it began to plateau, then it is unlikely that the kittiwakes are the primary drivers of nutrient transfer to the impact pond. However, it is difficult to draw any conclusions on this since we did not obtain $\delta^{15}N$ values specific to kittiwakes nesting in Newfoundland, where diets may vary considerably depending on the sample region and year.

While there was no significant difference in $\delta^{13}C$ values between the impact and reference site, the value for corrected $\delta^{13}C$ in the top layer of the CSM impact pond (-24.7 ‰) is lower than the two gannet fecal samples. Autochthonous aquatic primary producers tend to have negative $\delta^{13}C$ values relative to terrestrial vegetation and marine primary producers and/or organisms that feed within the marine food web [49, 50]. While gannet nutrients entering the impact pond seem to have increased autochthonous primary production, as inferred from the increase in chl *a* and elevated modern chl *a* concentrations relative to the reference pond, it is likely that sediment $\delta^{13}C$ values are a mixed result of negative $\delta^{13}C$ primary producers and the more positive $\delta^{13}C$ of gannet guano. This obscures the use of sediment $\delta^{13}C$ values in tracking gannets.

Overall, the concentrations of P, Zn/Al and Cd/Al were enriched by guano deposition in the CSM impact pond. While we found ratios of Zn/Al were overall higher in the reference core than the impact core, the ratios in the reference core remained stable over time (Figure 2.1). We quantified a significant period of increase in Zn/Al in the impact core between 1868 and 1989, and a similar increase in P from 1895 to 2019 (Table 2.3). However, referring to the data in Figure 2.2, we observed the concentrations of Zn/Al and P decreased from 1994 onward, and concentrations of Cd/Al decreased from 2003 onward.

Diatoms are another common ornithogenic indicator used in paleolimnology because they are sensitive to shifts in water chemistry and preserve well in sediments. Used in conjunction with $\delta^{15}N$ and other proxies, shifts in diatom assemblages have previously been used as a proxy for seabird-derived nutrient transfer [51]. We observed the dominant diatom taxa shift in the impact core at a similar depth as the shift in primary production occurs. The increase in the diatom taxa *S. construens* in the impact core occured at the same depth as the decrease of *S. exiguiformis* (formerly known as *Fragilaria virescens*), while our reference core showed no significant shift in dominant diatom species over time (Figure 2.1). Duda et al. [52] showed *S. exiguiformis* increased when the petrel colony on Baccalieu Island increased, although those ponds went from neutral to acidic while our pond goes from basic to circumneutral. The taxon *S. exiguiformis* was a very common diatom in the subarctic lakes sampled by Rühland et al. [53], occurring in 55 of 77 lakes with a maximum abundance of approximately 44%. In particular, this taxon was found in slightly acidic lakes with lower DIC (and a pH ~6.5) [54]. *S. construens v. venter* is often found in higher pH lakes, but like other benthic fragilarioid taxa is found in many environments, while *S. exiguiformis* is a benthic taxon that is most commonly associated with lower pH. In other subarctic lakes, *S. construens* had higher DIC and pH optima than *S. exiguiformis*.

For the water chemistry analyses, we observed an overall higher conductivity and concentration of TN and TP in the impact pond water compared to the reference pond water, which was an indication that the impact pond recieved an influx of seabird-derived nutrients. There were elevated concentrations of Ca, Mg, Na, and K in the surface water samples of the impact pond in July 2021 compared to samples from the previous year, but our September 2021 sample showed that these elevated concentrations were maintained throughout the 2021 breeding season (Table 2.2).

Our results, combined with the available monitoring data, suggest that the northern gannet nesting colony in CSM Ecological Reserve has reached a historical maximum. The available monitoring data for gannets on Bird Rock extends back to 1883, when 10 nesting pairs were reported on the stack [11]. Our sediment record for the impact pond, with dates reliably extrapolated back to the 1700's, indicated that there was no significant increase of nutrients into the pond before 1868.

Gannet populations in the North Atlantic have currently reached historic highs and most colonies have plateaued, which aligns with the proxy data collected here. According to the monitoring data available, the gannet population at Cape St. Mary's has grown from ~4,500 nesting pairs in 1934

[11] to ~14,600 nesting pairs in 2018 [19]. During the 1960's, the Cape St. Mary's colony survived a collapse due to human disturbances [12]. It is also worth noting that toxic chemical contamination from the insecticide, DDT, was responsible for low hatching success between 1966 and 1970 for the gannet population nesting on Bonaventure Island, which made up 50% of the species' total population in North America [55]. The nesting areas and adjacent lands and waters of Cape St. Mary's were officially protected under the provincial Wilderness and Ecological Reserves Act in 1983, as a Seabird Ecological Reserve [22]. Federally, the Canadian Wildlife Service is responsible for the seabirds in the Reserve, with regulations set under the Migratory Birds Convention Act enacted in 1918, last revised in 1994 [22]. Since then, the population of gannets on Bird Rock recovered, has begun expanding to the cliffs on the mainland, and steadily risen to >14,000 nesting pairs (Table 2.1).

One reason we observed a decrease or plateau in the impact pond's proxy data near present day may be due to the expansion of the gannet colony onto the mainland nesting areas, which began around 1980 [55]. For example, as the gannet colony shifted over time and more gannets nested on the mainland cliffs due to overcrowding on the main stack, the distance from the nesting colony to the pond increased, and gannets nesting on these mainland areas may have no longer been contributing to nutrient input into the pond. We noticed a chl a shift at the core's uppermost part: a brief decline followed by a substantial increase. This aligns with the dip in gannet nesting pairs from 2009 (14,789 pairs) to 2012 (12,970 pairs). Subsequently, nesting pairs rebounded to 13,515 in 2013 and reached 14,598 by 2018 (Figure 2.2).

The eutrophication of the impact pond may have also been influenced by other seabirds nesting within Cape St. Mary's Ecological Reserve, such as black-legged kittiwakes, which mostly breed on the mainland areas away from Bird Rock. Their population within Cape St. Mary's Ecological Reserve has declined since 1979, from 10 000 nesting pairs [20] to 4,391 pairs in 2009 [16]. The decline in the population at Cape St. Mary's is consistent with the decline observed across eastern Canada in the early

1990s due to low breeding success that was linked to gull predation and a cold-water event affecting capelin biomass [56]. The 1992 cold water event and fishing moratorium [57, 58] cut the kittiwake population in half across their breeding range in Newfoundland. Similarly, the kittiwake population on Great Island (Witless Bay, NL), once one of the largest known colonies in Canada, observed a decrease in nesting pairs by 60.9% between 1994 and 2003 [23]. Another large colony on Baccalieu Island also experienced a significant decline of >50% between 1983 and 2012 [23]. While the kittiwake population on Gull Island, Witless Bay, in Newfoundland, which is the same size as the Baccalieu population, also experienced a 50% decline, going from a historic high of 10,000 pairs in 1971, down to 5000 in 2001, and annual surveys show it as remaining at that level since (e.g., 2019 survey had 4300 pair) [23]. If kittiwakes were the main contributors of nutrients to the impact pond, given the current trends in the kittiwake population on Bird Rock, we believe we would have seen a significant decline in our proxy data beginning around the early 2000s.

The growth of the gannet colony is certainly contributing to the nutrient input into the impact pond, however the decline in the kittiwake population during the same time periods may likely be contributing to the plateau we see in our proxy data and perhaps less kittiwakes bathing in the pond could have contributed to a slowing increase. Furthermore, another factor to consider is the saturation effect which can occur in ponds experiencing ornithogenic nutrient inputs and would have likely led to a plateau in the proxy data, in this case with multiple species contributing to the saturation over long time periods. While a more conservative approach to analyzing shifts in nutrient content to this pond over time would be to consider the influx of nutrients from multiple species in one habitat, our study illustrates how the population data of one species in a colony, in this case the northern gannets in Cape St. Mary’s, may align more accurately with shifts in nutrient content over time than other, less dominant species of the same habitat.

5. Conclusion

Our results for water chemistry, metal(loid)s (Cd/Al, Zn/Al, and P), δ^{15}N values, and diatom assemblages show that ornithogenic inputs are being deposited into the pond 240 m away from Bird Rock. This approach emphasizes the importance of combining monitoring data with paleoecology for a complete understanding of seabird colony trends. By utilizing a multi-proxy analysis approach, we were able to effectively reconstruct the population trends of seabirds nesting on Bird Rock over the past 110 years. Our findings were corroborated by the historical monitoring data available for seabirds nesting in CSM and aligned particularly well with the northern gannet population data. Our reconstruction also showed that seabirds' inputs were not present before gannets were documented at this site, suggesting the colony at CSM is a relatively newly established colony. While gannets appear to have been the dominant driving force of nutrient input into the impact pond, the development of new biomarkers and/or application of environmental DNA would be advantageous to distinguish species-specific nutrient inputs.

The Eastern Hyper-Oceanic Barrens is a small (1603.4 km^2), widely fragmented ecosystem around the island of Newfoundland, which hosts a wide diversity of migratory seabirds every summer. We demonstrate the considerable impact seabird nutrients can have on such ecosystems, particularly drawing attention to the pivotal role gannets play as vectors of nutrient transport in Cape St. Mary's Ecological Reserve. Their guano and excrement provide a rich source of nutrients for the surrounding ecosystem, while their movement and feeding behavior help to distribute these nutrients and support the growth of plants and other organisms. The findings from this study contribute to a growing body of literature on the impact of ornithogenic nutrient transfer and highlight the importance of monitoring these effects in other coastal regions, particularly those with large seabird populations.

6. Data Accessibility

Datasets supporting this article have been uploaded as part of the electronic supplementary materials and can be accessed through the links provided in the appendices.

7. Acknowledgments

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CHAPTER 3

Investigating the bacterial diversity and community structure of sediments impacted by seabird nutrients with respect to seabird fecal microbiomes

Authors Note:

This chapter has been prepared and structured in accordance with the guidelines set forth by the intended publication venue, Proceedings of the Royal Society B.

Abstract

Seabirds play an integral role in the transfer of nutrients from sea to land, across ecosystem boundaries mainly in the form of guano (feces). While many paleolimnological studies have examined ecosystem shifts in ponds nearby seabird colonies experiencing nutrient transfer, the microbial ecology of such ponds has been largely overlooked in scientific literature. Previously, we reconstructed the colony size of the seabirds breeding site in Cape St. Mary’s Ecological Reserve, Newfoundland, using a multi-proxy paleolimnological approach and analysis of sedimentary records from a pond downwind of the colony. Since this pond is impacted by multiple seabird species, there is a need for a species-specific proxy that could identify which specific seabirds are contributing the most to the observed nutrient transfer. Thus, we investigate the potential use of fecal bacterial communities from different species of seabirds as an indicator of ornithogenic nutrient input into pond sedimentary records. We compare bacterial composition in sediment samples from two seabird-impacted ponds, and one control pond, to the fecal microbiomes of four pelagic seabird species using 16S rDNA amplicon sequencing. Our aim in this study was to (a) characterize the community structure and diversity of sediments impacted by seabird nutrients and (b) determine if there is a relationship between the fecal bacterial communities and the impact core samples. Sediments impacted by the input of seabird nutrients over time had significantly lower bacterial community diversity when compared to the control pond. Diversity among seabird fecal sampling groups did not reveal significant differences in α-diversity; however, northern gannets (*Morus bassanus*) had the most differential community structure among the four studied seabird species. Our results suggest that even in the absence of a species-specific link between seabirds and the sediments of ponds which they impact, seabird nutrient input does impact bacterial diversity in ponds near the breeding site. Furthermore, it may be possible to target specific bacterial genera present in seabird feces and sedimentary records to further develop DNA-related proxies for quantifying palaeoecological trends at important breeding sites.

1. Introduction

Characterization of microbial communities in aquatic sediments using next generation sequencing has emerged as a powerful new tool in paleolimnology [1]. In addition to identification of bacteria and archaea, sedimentary DNA (*sed*DNA) can be used in paleolimnology when morphological features of eukaryotic organisms are not preserved in sediments, allowing for the reconstruction of past biodiversity and ecosystem dynamics even in the absence of distinctive fossil remains. In combination with other proxies, *sed*DNA analyses have revealed the composition of past vegetation [2] and the presence of mammals, including regional appearances of animal husbandry [3, 4], with recent work pushing the captured time-frame back 2 million years in the Arctic [5]. Our ability to analyze species-specific connections within limnology has revealed promising prospects at the convergence of paleolimnology and conservation biology. For example, Duda et al. [6]. revealed how *sed*DNA, in combination with geochemistry and isotopes, could be used to assess the breeding site fidelity, avian behavioral responses to environmental shifts, and historical changes among Andean Condors (*Vultur gryphus)* over a long time period.

At large seabird colonies, ornithogenic nutrients may be transported into nearby aquatic systems when the colony is next to a pond or lake [7], or through indirect means such as wind transport [8]. As sedimentation occurs over time, a record of these seabird-derived nutrient inputs is preserved. Using classic paleolimnological techniques, shifts in seabird-derived nutrients are tracked and related to historical changes of a given seabird population [9]. Some common proxies that have been used to reconstruct seabird populations include stable nitrogen isotopes [8], metal(loid)s such as phosphorus (P), cadmium (Cd), and zinc (Zn) [7], fossil pollen assemblages [9], and invertebrates [10]. However, most paleolimnological proxies associated with ornithogenic inputs are not species-specific (i.e., they cannot always distinguish between different species of seabirds nesting in a lake catchment). Multiple seabird species often nest at one site, and they can release the same chemical biomarkers within their feces (e.g., enriched stable nitrogen isotopes and elevated P and Cd), which makes it difficult to confirm the exact origin of the nutrient input.

Seabird gut microbiomes can have compositions that are specific to diet, sex, environment, genetics, and social factors of the individual [11, 12, 13], and have the potential to be a useful indicator of ornithogenic nutrient input into ponds. The presence of the bacterial genus *Catellicoccus* tracks gull fecal contamination in coastal environments [14] and it was also found as a dominant genus in the first description of the thick-billed murre (*Uria lomvia*) fecal microbiome [11]. Therefore, this genus or other dominant taxa from seabird gut microbiomes could potentially be used to track avian trends if they are present in sediments.

We first assessed the gut microbiome variations among various seabird species in Atlantic Canada to understand species-specific bacterial composition. We then explored whether the DNA from bacteria found in the feces of different seabird species can be detected in the sediments of ponds near where these birds' nest. We used 16S rDNA amplicon sequencing to compare the bacterial composition of sediment samples taken from ponds impacted by seabird-derived nutrients to the taxa found in the fecal microbiome of four different species of pelagic seabirds that nest in this region. The seabirds included in our analysis are Atlantic puffin (*Fratercula arctica*), black-legged kittiwake (*Rissa tridactyla*), common murre (*Uria aalge*), and northern gannet. Sediment cores were collected from two seabird-impacted ponds, with one near a multi-species nesting site at Cape St. Mary's (CSM), Newfoundland, where northern gannets are the dominant nesters, but black-legged kittiwakes and common murres also nest, and the other from a pond in the Little Fogo Islands that is directly impacted by only one species of seabird, Atlantic puffin. We also collected a reference sediment core from a pond at CSM that does not experience seabird nutrient inputs. We sought to determine if there was a relationship between the fecal microbial communities and the associated impact core samples. We also examined how the bacterial community of the CSM impacted pond compared to those of the other sites that had only one species' input or none at all.

2. Methods

a) Ethics

Handling of live northern gannets was done using methods established by the Canadian Council on Animal Care (CACC) under an Environment and Climate Change Canada (ECCC) Scientific Permit to Capture and Band (Permit No. 10332K). Collection of samples from Cape St. Mary's Ecological Reserve was done under Wilderness and Ecological Reserve Permit #03294 from the Newfoundland and Labrador. Bird carcasses used in this study from nesting areas of Newfoundland are property of ECCC and held under the Scientific Permit #SS2803 of CWS.

b) Study site and sample collection

Two sediment cores were collected within CSM Ecological Reserve, the first on 25 June 2021 from a pond approximately 240 m away from the seabird nesting colony, referred to here as the 'CSM impacted site' (CSM-I, coordinates: 46° 49' 12" N, 54° 10' 48" W), and the second on 8 September 2021 from a shallow pond approximately 2.56 km away from the colony that does not experience any seabird nutrient inputs, which we refer to here as the 'CSM reference site' (CSM-R, co-ordinates: 46° 50' 24" N, 54° 10' 12" W), CSM Ecological reserve is a protected area of Newfoundland's highly-fragmented Eastern Hyper-Oceanic Barrens ecoregion, with peatbogs and headlands containing many shallow, and typically acidic, freshwater ponds [15]. A third core was collected on 4 August 2021, in the Little Fogo Islands, where only one species, Atlantic puffins, burrow directly adjacent to a pond which we refer to as the 'Little Fogo Island site' (LFI, coordinates: 49.8104N, -54.111E). Little Fogo Island is part of a tiny archipelago contained within the North Shore Forest ecoregion that borders the north-eastern coastlines of Newfoundland [16]. The island also contains a large population of Leach's storm petrels (38,000 pairs), but they are not found using our study pond [17].

A push-corer [18] with a polypropylene tube was used to collect the cores from the deepest part of the ponds. A vertical extruder [19] and plastic scraper tool were used to section the sediment cores. The CSM-I core was sectioned at 0.25 cm intervals, the CSM-R core and LFI core were sectioned at 0.5 cm. While sectioning, we took sub-samples to be used for DNA extractions from every centimeter of each core using a sterile plastic loop and gloves, making sure to sample from the middle of the core tube to avoid contamination from drag at the side of the tube [20]. Subsamples were put into plastic centrifuge tubes that were kept on ice until returning to the lab, where they were placed in a -80 °C freezer.

Fecal swabs were collected from live northern gannets whereas the samples from the other bird species were obtained from dead birds in the property of Environment and Climate Change Canada. The northern gannet samples were taken using polyester swabs and the tips were broken into microcentrifuge tubes. The frozen birds were thawed, and samples were taken using polyester swabs that were placed into microcentrifuge tubes containing SL1 buffer solution from the NucleoSpin® Soil

Kit (Macherey Nagel). All samples were stored at -80 °C until subsequent processing for DNA extraction.

c) DNA isolation and 16S rRNA gene sequencing

DNA isolations were performed in a dedicated PCR-free lab with sample manipulations performed in a biosafety cabinet. We targeted sediment samples from 5 intervals of each core, including the CSM-I core intervals 1-1.25 cm, 2-2.25 cm, 4-4.25 cm, and 8-8.25 cm, the CSM-R core intervals 1-1.5 cm, 2-2.5 cm, 4-4.5 cm, and 8-8.5 cm, and the LFI core intervals 0-0.5 cm, 2-2.5 cm, 4-4.5 cm, and 6-6.5 cm. Sediment samples (~250 mg) were weighed on an analytical balance for subsequent processing. DNA extractions were performed using the NucleoSpin® Soil kit. To increase DNA yield and decrease PCR inhibitor contaminants, we optimized extraction methods for both sediment and fecal samples as per kit instructions (Appendix II – Supplementary text, Supplementary figures AII.1 and AII.2). After adding the SL1 buffer and SX enhancer, we homogenized our samples in a bead beater at 5500 rpm for 45 seconds. After adding SL3 buffer and centrifuging samples, the supernatants were transferred to new centrifuge tubes, 2 μL of RNase A (10 mg mL^{-1}) was added and the samples were incubated at 37 °C for 30 minutes. The tubes were then inverted 25 times by hand and incubated on ice for 5 minutes before continuing with the manufacturer's protocol, with the SE elution buffer incubated at 70 °C for 3 minutes before use. In addition to the above-noted sediments, DNA samples that were deemed suitable for subsequent analysis were obtained from the guts of five northern gannets, five Atlantic puffins, five black-legged kittiwakes, and four common murres. DNA samples were stored at -80 °C.

Reaction set-ups for PCR were performed in a laminar flow hood in a dedicated pre-PCR, amplicon-free room. Fragments were PCR-amplified in duplicate using Q5 Hot-Start High-Fidelity 2X master mix and 0.5 μL of the universal 16S rDNA V4-V5 region primers (Forward = 515FB, 5'-GTGYCAGCMGCCGCGGTAA-3'; Reverse = 926R, 5'-CCGYCAATTYMTTTRAGTTT-3') [21, 22]. PCR protocols were optimized with changes to the annealing temperature and template volumes for both sediment and fecal samples until we observed sufficient amplification for every sample. Based on our visualization of PCR results, we found the highest yield in amplification when using 2 µL of DNA for northern gannet and kittiwake gut samples, 1 µL of DNA for common murre and puffin fecal samples, and 1 µL diluted in 10 µL of water for sediment samples. The final thermal cycling parameters we used included an initial denaturing temperature of 98 °C for 30 s, followed by 35 cycles of a denaturing temperature at 98 °C for 15 s, an annealing temperature of 52 °C for 15 s, and an 72 °C for 10 s, a single cycle of 72 °C for 30 s and finally 4 °C for 10 min. For a more detailed description on how we optimized our PCR methods see Appendix II – Supplementary text. PCR products were purified using the Qiagen MinElute Reaction Cleanup Kit as per manufacturer instructions and DNA concentration quantified using a Nanodrop spectrophotometer and on a Qubit fluorometer using the High-sensitivity dsDNA Kit (ThermoFisher Scientific). The purified DNA was sent to the Integrated Microbiome Resource (Dalhousie University, Halifax, CA) for sequencing on an Illumina MiSeq using 300+300 bp paired-end V3 chemistry [23].

d) Sequencing Data Analysis

The quality of the reads was assessed using FastQC [24] and MultiQC [25]. The FastQC reports were generated using the default settings, and the final MultiQC results were used to identify low quality samples or unusual characteristics. QIIME2 [26] was used to analyze and visualize the microbial community data. The paired-end FASTQ files were imported into QIIME2 using ‘qiime tools import’, and the 16S V4-V5 primer sequences were removed using ‘qiime cutadapt’. The paired-end reads were joined using ‘vsearch join-pairs’ and amplicon sequence variants (ASVs) constructed using ‘qiime deblur 16S’ [27], specifying a read trim-length of 240 bp based on the Phred scores produced from the MultiQC output.

Next, taxonomic IDs were assigned to each ASV using a Naive Bayes classifier trained with the Silva 138 database [28] via 'qiime feature-classifier classify-sklearn' [26]. In QIIME2, each feature (in this case, ASV) can only be assigned to one taxon, but one taxon may have numerous features assigned to it [26]. The integrity of the taxonomic assignments was assessed by comparing them to the top BLASTn hits of the ASVs using 'qiime feature-table tabulate', allowing the ASVs in the dataset to be randomly searched against the NCBI nucleotide database. We filtered out any ASVs with taxonomic labels indicating that they were mitochondrial or chloroplast sequences, as well as any ASVs that were not classified to the phylum level using 'qiime taxa filter-table'. Any features with a frequency less than 10 across the samples were also removed. We exported the table into a readable format along with the representative sequences and taxonomic classifications using the *R*/v4.2.2 package *qiime2R*/v0.99.6 [29].

Euler diagrams were plotted using *eulerr*/v.7.0.0 [30], with the feature and taxonomy data first prepared by calculating the sum of feature counts across each sample group and summarizing the taxonomy data to the genus level. Taxa found in the sediment core sites that were also present in the seabird fecal samples from different species were identified using the *R* package *dplyr*/v1.0.10 [31].

Shannon entropy values (H) were calculated using QIIME2's α-diversity plug-in. The QIIME2 artifact files were exported and the *qiime2R*/v0.99.6 and *phyloseq*/v1.42.0 [32] *R* packages were used to estimate Shannon diversity [33], the Simpson's index [34], and the number of unique features observed for each group [35]. Next, the *qiime2R*/v0.99.6 package in *R* was used to plot a taxonomic bar graph for the top 20 phyla present across the two sample types, and taxonomic heat maps for the top 20 genera present across all of the seabird fecal samples and sediment samples, independently. Finally, we assessed taxonomic composition of the sediment samples most impacted by seabird nutrient transfer by filtering the top 10 most abundant genera present in the top layers of each core from the three sites (1 cm in CSM sites, 0 cm in LFI site), and plotted the abundance of each genera using *ggplot2*/v3.4.2 in *R*.

After assessing taxonomic composition of the bacterial communities in our samples, β-diversity among the samples was estimated using an unweighted and weighted UniFrac principal component analysis (PCA) to compare the presence or absence of specific microbial taxa across samples and determine the differences among the microbial communities [36]. To assess β-diversity using UniFrac distance metrices, a phylogenetic tree was constructed using the 'q2-fragment-insertion' plug-in for QIIME2. SEPP generates a multiple sequence alignment, inserting sequences into a reference phylogeny based on the Silva (version 128) SEPP reference database, discarding sequences that are >75% identical to any sequence in the tree, it then removes errors, uninformative sites and repetitive regions, and builds a rooted tree [37]. The statistical significance of these differences was estimated via a pairwise permutational multivariate analysis of variance (PERMANOVA) with 999 permutations for the unweighted and weighted PCA distance matrices using 'qiime diversity β -group-significance' [38].

3. Results

a) Sequence and feature characteristics among sample types and groups

The 16S amplicon sequencing resulted in a total of 4 632 839 sequences from the 19 seabird fecal samples and 2 337 088 sequences from the 12 sediment samples. For seabird fecal samples, we obtained 132 576 to 400 690 (μ = 243 834) sequences from each sample and for sediment samples, we obtained 118 530 to 210 414 (μ = 155 806) sequences from each sample. Based on the MultiQC analysis, the average (μ) percentages of duplicate reads in seabird fecal and sediment samples were μ = 95.8% and μ = 89.1%, respectively. There was a total of 4797 individual ASVs (referred to as features on the QIIME2 platform) identified across the 31 samples before filtering contaminants (mitochondria and chloroplast), low feature counts, and unclassified features. After filtering there were 3877 features identified in sediment samples, and 776 features in seabird fecal samples (4555 total; a 5% loss). When considering the counts across all identified features, there was a total of 2 101 610 feature counts across the seabird fecal and sediment core samples, and after filtering there was a total of 2 063 500, representing a decrease of 1.8%. The lost features were either unclassified at the phylum level (200 features total) or assigned to mitochondrial or chloroplast 16S sequences (46 features total). All chloroplast sequences were from the sediment core samples, while mitochondrial assignments were only found in the common murre samples, which had ASVs assigned to the species white spruce (*Picea glauca*) and narrow-leaved glade fern (*Dizpazium pycnocarpon*) (Appendix II – Supplementary table AII.3).

Across the 4555 post-filtering features, the depth of taxonomic assignments varied, with 78 features assigned only to the phylum level, 162 to the class level, 126 to the order level, 450 to the family level, 2109 to the genus level, and 1630 to the species level (Appendix II – Supplementary file AII.3). Bacteria within the seabird fecal samples were classified into 1 domain, 21 phyla, 38 classes, 78 orders, 129 families, 177 genera, and 57 species. The ASVs for the sediment samples were classified into 2 domains, 57 phyla, 124 classes, 249 orders, 319 families, 393 genera, and 160 species.

Euler diagrams were plotted to compare the number of shared and unique taxonomic assignments among sample types (not taking into account ASV abundances), including the four seabird species and three sediment coring sites (Figure 3.1). The CSM-I samples had the highest number of total taxonomic assignments among the sediment groups, closely followed by CSM-R and LFI. The two CSM sites shared the greatest number of taxa, followed by CSM-I and LFI, and lastly, CSM-R and LFI. The CSM-R site had the most unique taxa, followed by LFI and CSM-I. Among seabird fecal samples, common murres had the highest number of total taxonomic assignments, followed by Atlantic puffins, black-legged kittiwakes and northern gannets. The highest number of shared taxa between two seabirds was found for common murre and Atlantic puffin samples, while the lowest was found for black-legged kittiwake and northern gannet samples. The common murre samples had the highest number of unique taxa, followed by northern gannets, Atlantic puffins, and black-legged kittiwakes. Overall, there were more taxa shared among sediment sample groups than among seabird sample groups (Figure 3.1).

The taxa we found in both sediment and fecal samples had relatively low abundance compared to other taxa in the groups. A total of nine taxa, comprising 1 family, 7 genera, and 1 species, were exclusive to one sediment coring site and one seabird fecal sampling group (Table 3.1), with no overlap in other sampling locations or groups. The CSM-I samples included the bacterial family Nocardioidaceae that was also found in a common murre sample, the genus *Mucispirllum* that was found also found in northern gannet samples, and *Epulopiscium* and *Candidatus woesebacteria* that were also found in Atlantic puffin samples. The CSM-R samples included the family Azospirillaceae and an uncultured genus of Rhodothermaceae that was also found in common murre samples. Finally, the LFI site samples included *Kaistia* that was also found in common murre samples, *Christensenellaceae_R-7_group* also found in northern gannet samples, and *Tumebacillus* that was also found in Atlantic puffin samples. While there were no taxa that were found only in black-legged kittiwake

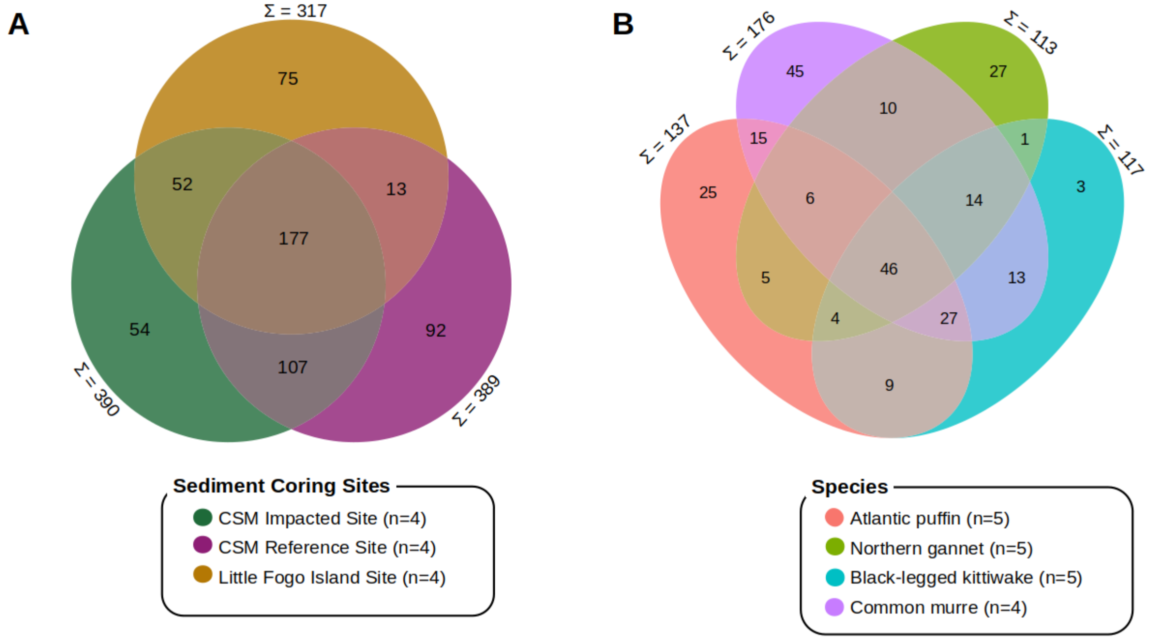

Figure 3.1 Shared and unique taxonomic assignments across the sediment sample groups [A] and the seabird fecal sample groups [B] after summarizing feature and taxonomy data to the genus level. Sample size (n) is included within each legend, and the sum of taxonomic assignments for each group (Σ) is included outside of each ellipse.

samples and one sediment site, an "uncultured genus" of Thermodesulfovibrionia and *PHOS-HE36* were found in black-legged kittiwake samples and at all of the sediment sites.

When considering all the seabirds, there were five taxa that were also found in the two sites where seabird nutrient transfer occurs (CSM-I and LFI). These were the genera *Sporosarcina, Breznakia,* and *Ezakiella* and the family Peptostreptococcales-Tisierellales and the class Clostridia. Taxa found exclusively between the CSM-R site, where no seabird nutrient transfer occurs, and all the seabird species included the genera *Actinomyces*, *Fastidiopila,* and *Veillonella*, the family Lachnospiraceae, and the order Bacillales. Taxa that were found exclusively in CSM-I and all the seabird species were the genera *Peptoniphilus* and *Cetobacterium.* Taxa found exclusively in LFI and all of the seabird species were the genera *Varibaculum, Corynebacterium, Aequorivita, Lactobacillus, Gottschalkia, Psychrobacter,* and the families Flavobacteriaceae, Xanthomonodaceae.

Table 3.1 Feature counts (FC) of taxa present only in one sediment coring site and one seabird species

Core Site	Taxonomic assignment	Rank	Sediment samples		Seabird samples	
			Depth	FC	Sample ID	FC
CSM-I	Nocardioidaceae	Family	1 cm	5	Common murre (n=1)	21
			2 cm	3		
			8 cm	2		
	Mucispirrilum	Genus	8 cm	2	Northern gannet (n=5)	15 203
	Epulopiscium	Genus	2 cm	2	Atlantic puffin (n=2)	7950
	Candidatus woesebacteria	Species	1 cm	2	Atlantic puffin (n=1)	33
CSM-R	uncultured Rhodothermaceae	Genus	2 cm	21	Common murre (n=1)	74
	Azospirillaceae	Genus	8 cm	4	Common murre (n=1)	45
LFI	*Kaistia*	Genus	0 cm	9	Common murre (n=2)	21
			2 cm	5		
			4 cm	2		
			6 cm	3		
	Christensenellaceae_R-	Genus	0 cm	2	Northern gannet (n=4)	401

7_group		2 cm	4		
		4 cm	3		
		6 cm	4		
Tumebacillus	Genus	0 cm	2	Atlantic puffin (n=2)	12

b) α-diversity metrics among sample types and groups

Diversity metrics were calculated at a rarefaction depth equal to the minimum sampling depth of 42,252 for the seabird fecal samples and 20,283 for the sediment samples. Overall, seabird fecal samples had significantly lower average values for observed ASVs ($\mu = 107.38$) and the Shannon ($\mu = 1.65$) and Simpson's ($\mu = 0.63$) indices compared to sediment samples ($\mu = 1022.5$ and 5.17, and 0.97, respectively) (Kruskal-Wallis H test; $H = 21.38$, $p = 4 \times 10^{-6}$) (Figure 3.2A). There were no significant differences in bacterial community diversity among the four seabird species for Shannon and Simpson's indices (Kruskal-Wallis H statistic; $H=2.27$, $p = 0.52$ and $H=2.7$, $p = 0.44$, respectively), but the number of observed ASVs was significantly different ($H=9.13$, $p = 0.028$) (Figure 3.2B). Average Shannon and Simpson's index values were highest in common murres ($\mu = 1.99$ and 0.74, respectively), followed by Atlantic puffins ($\mu = 1.87$ and 0.70, respectively), northern gannets ($\mu = 1.47$ and 0.55, respectively), and black-legged kittiwakes ($\mu = 1.32$ and 0.55, respectively). The average number of distinct ASVs among the seabird species was highest in common murres ($\mu = 150$), followed by northern gannets ($\mu = 117$), Atlantic puffins ($\mu = 92.2$) and black-legged kittiwakes ($\mu = 78.8$). One common murre sample was an outlier and had a much higher Shannon diversity than the other samples in the same group. The bacterial community diversity metrics differed significantly among the sediment coring sites for observed ASVs and the Shannon and Simpson's indices (Kruskal-Wallis H statistic; $H=9.85$, $p = 0.007$) (Figure 3.2C). The average number of observed features and Shannon and Simpson's indices were all highest in the CSM-R site ($\mu = 1433$, 6.23, and 0.99, respectively), followed by the CSM-I site ($\mu = 915.25$, $\mu = 5.22$, and $\mu = 0.98$) and the LFI site ($\mu = 719.25$, $\mu = 4.05$, and $\mu = 0.93$, respectively).

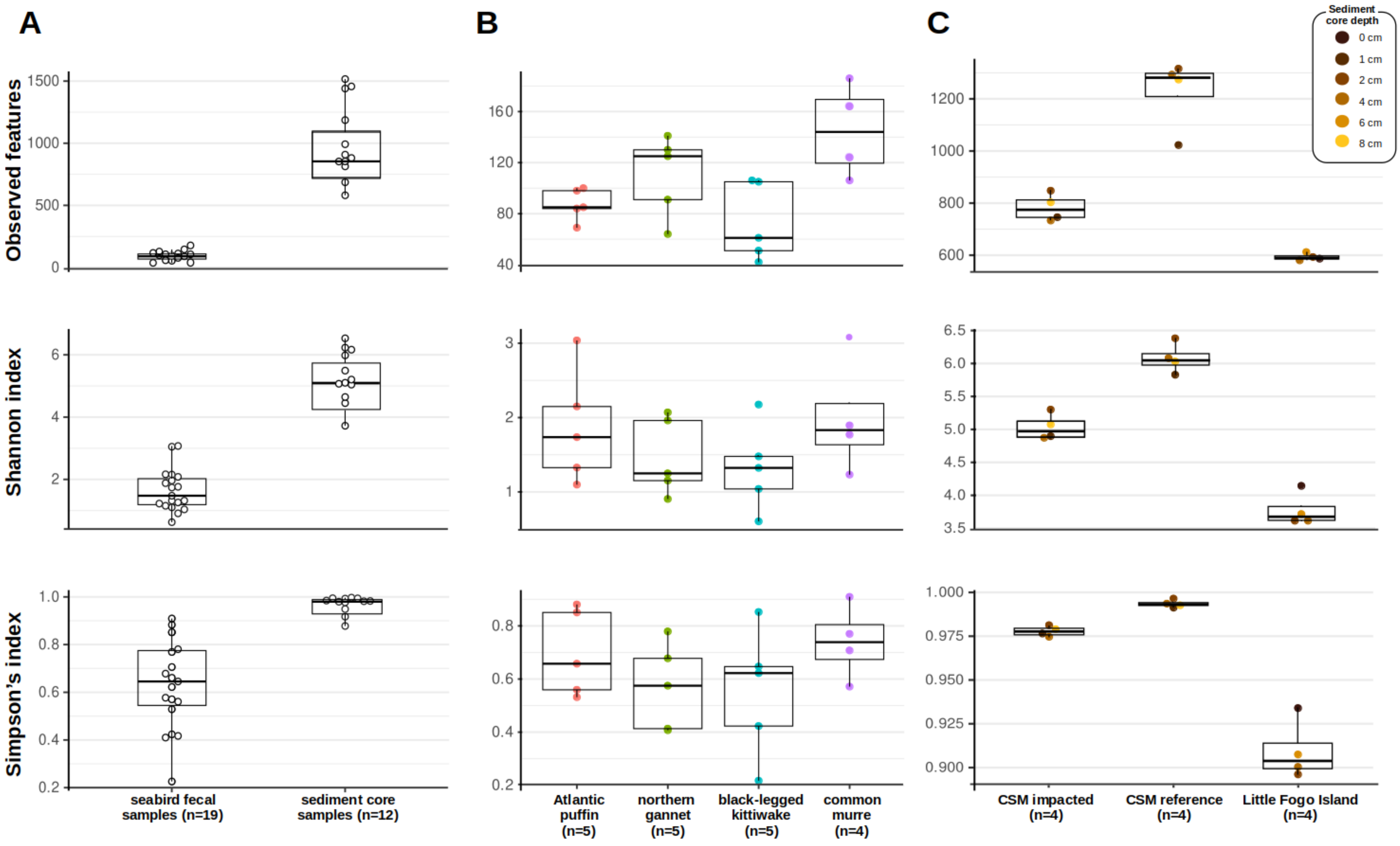

Figure 3.2 Diversity metrics in different sample types and groups. Boxplots are presented for the three metrics for the two sample broad types [A], the different seabird species' fecal samples [B], and the sediment sample sites (C). Each sediment sample is colored according to core depth in [C]. Sample size (n) is displayed on the bottom for each set of samples considered.

c) Community structure and abundance among sample types and groups

Next, we analyzed the twenty most abundant phyla across both sample types (Figure 3.3). Of the twenty phyla, four were abundant in both seabird and sediment samples, including Bacteroidota (feature count for sediments = 11 211, feature count for seabirds = 45 773), Proteobacteria (feature count for sediments = 113 928, feature count for seabirds = 470 130), Actinobacteriota (feature count for sediments = 18 137, feature count for seabirds = 53 167), and Firmicutes (feature count for sediments = 4 597, feature count for seabirds = 861 570). Five phyla were abundant in sediment samples that were completely absent in seabird fecal samples, including Zixibacteria, Methylomirabilota, MBNT15, Sva0485, and Latescibacterota. Fusobacteriota was abundant in northern gannet and common murre samples, but rare in sediment samples. Finally, ten of the twenty phyla shown in Figure 3.3 were abundant in sediment samples and rare in seabird fecal samples, including Verrucomicrobiota, Chloroflexi, Planctomycetota, Acidobacteriota, Desulfobacterota, Cyanobacteria, Campilobacterota, Gemmatimonadota, Spirochaetota, and Nitrospirota. It is important to note that some phyla were represented by a single species found in only one or few sampling groups, such as *Mucispirillum*, part of the phylum Deferribacterota, which was only found in gannets (feature count = 15 205) and also present in low amounts in the CSM-I site (feature count = 2) (Figure 3.3).

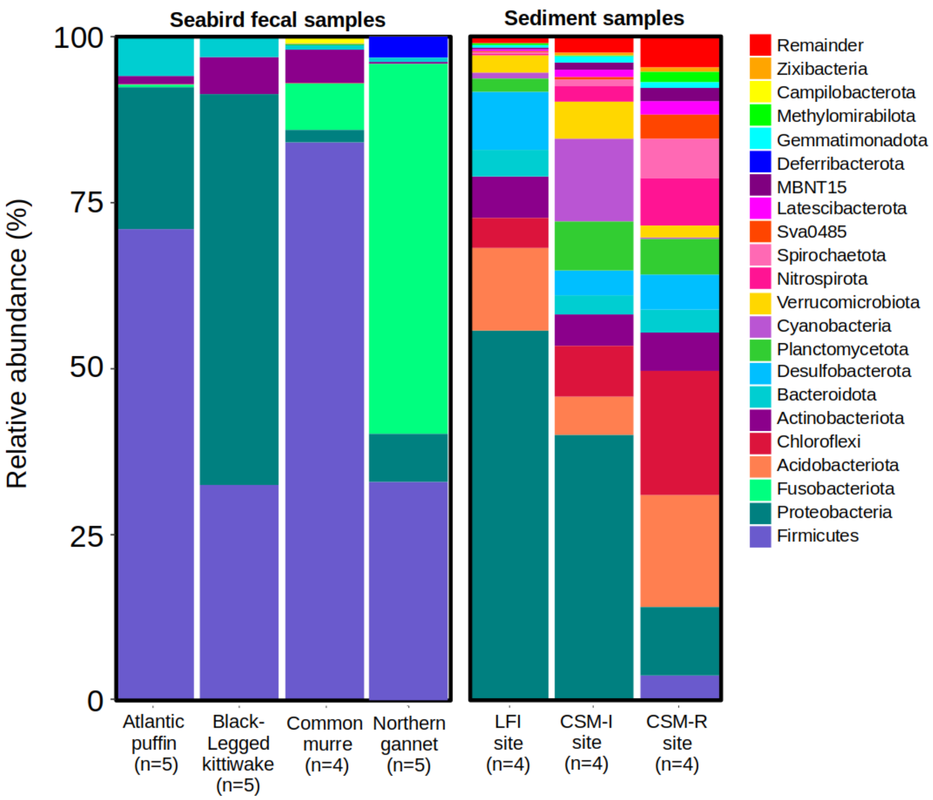

Figure 3.3 Relative abundance (%) of the 20 most abundant bacterial phyla were found across both seabird fecal and sediment samples. “Remainder” represents the fraction of taxa that were not among the 20 most abundant within the total dataset.

Next, we plotted a taxonomic heatmap for the relative abundance of the top 50 genera within the sediment samples (Figure 3.4) and seabird fecal samples (Figure 3.5). Genera assigned to “uncultured” taxa were categorized and assigned letter codes as outlined in File A1 of Supplementary Materials - Appendix II. For the sediment samples, the most abundant genera at LFI, that were less abundant at the other sites, included *Thiobacillus*, *Subgroup_23* (part of Thermoanaerobaculaceae) and *Sva0081_sediment_group* (part of Desulfosarcinaceae). The genera *LD1-PA32*, *Candidatus Udaeobacter, Aquisphaera*, *Cyanobium_PCC_6307*, *966-1*, and *SC-I-84* were most abundant genera in the CSM-I site but less abundant in the other sites. The genera *Sva0485*, *Spirochaeta*, an uncultured genus in Thermodesulfovibrionia, and *Aminicenantales* were most abundant in the CSM reference site, but less abundant in other sites (File A5, Appendix II - Supplementary materials).

For the top 50 most abundant genera found in the seabird fecal samples, genera that were abundant among all samples included *Staphylococcus*, *Corynebacterium*, *Lactobacillus*, *Fastidiosipila, Actinomyces*, and *Peptoniphilus* (Figure 3.5). Genera found only in northern gannet samples included *Sutterella*, *W5053*, and *Mucispsirillum.* The genera *Tyzzerella* and *Fusobacterium* were abundant in gannets (feature counts = 74 801 and 263 149, respectively), and less abundant in murres, kittiwakes, and puffins. Genera that were abundant in the seabirds other than gannets included *Breznakia, Mycobacterium, Acidobacterium, Streptococcus, Paludisphaera, Bosea, Terrisporobacter, Carnobacterium, Brevibacterium, Actinomycetaceae. Peptoclostridium, Tomitella,* and *Trichococcus.* Notably, *Catellicoccus* was abundant in the puffins (feature count = 206 054), kittiwakes (feature count = 97 430), and murres (feature count = 79 087), but nearly absent in gannets. *Breznakia* was also abundant in murres (feature count = 76 822) and kittiwakes (feature count = 62 618), less abundant in puffins, and completely absent in gannets. The genus *Paeniglutamicibacter* was abundant in puffins and absent in all other species. Genera that were abundant in common murre samples and less abundant in other seabird species included *Carnobacterium, Tissierella, Campylobacter, Dietzia* and an uncultured genus in Coriobacteriales (category J - File A1 of Supplementary Materials - Appendix II). *Escherichia-Shigella* was most abundant in puffins and kittiwakes, but nearly absent in murres and gannets (Figure 3.5).

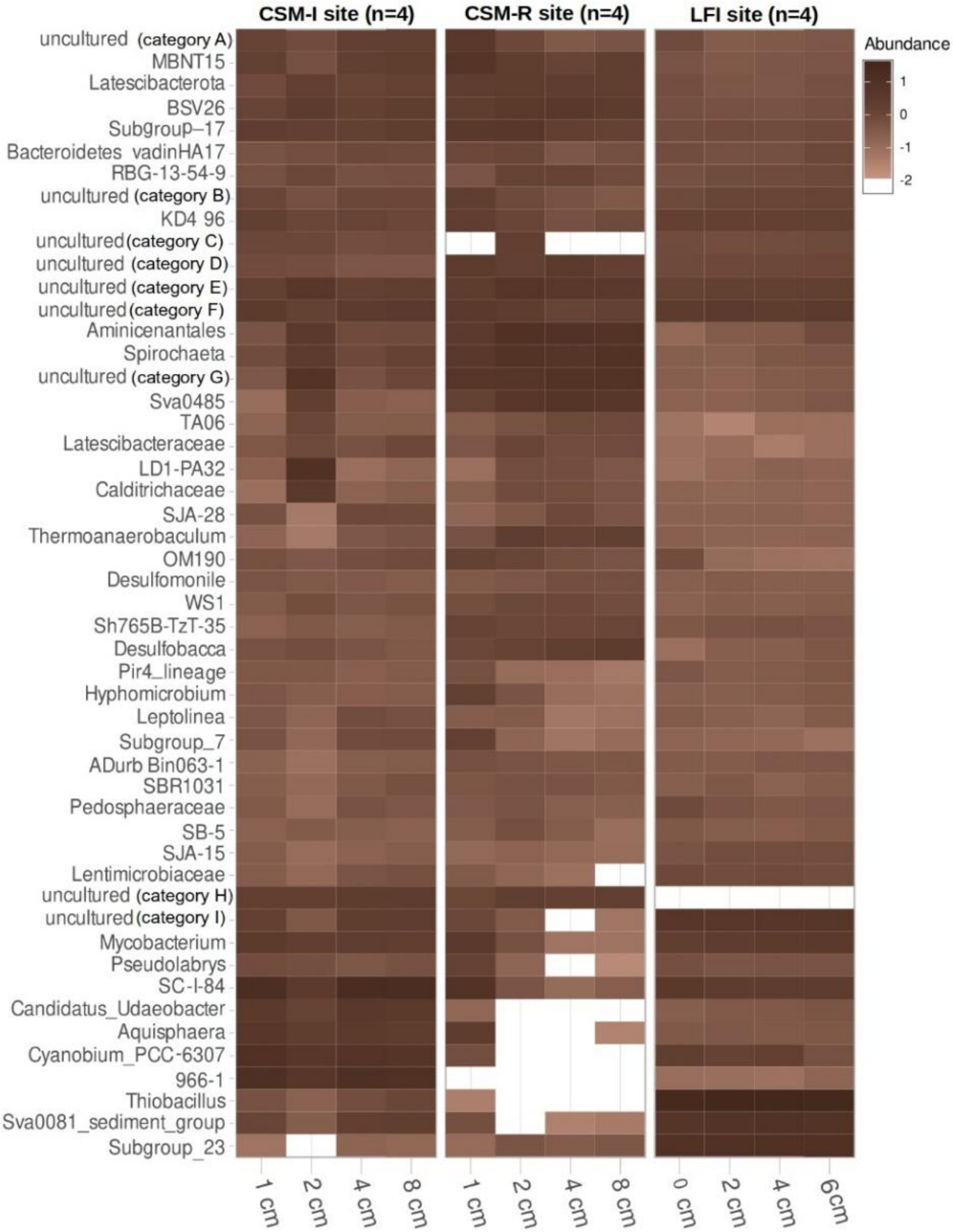

Figure 3.4 Heatmap of the 50 most abundant genera within the sediment samples. Relative abundance is represented on a logarithmic scale [$\log_{10}$(%)], and taxa with counts equal to zero are shown in white, while low abundance counts are light brown and high abundance counts are dark brown. Sediment subsamples for the two CSM sites were taken from the depths of 1 cm, 2 cm, 4 cm and 8cm of the cores, while sediment subsamples for the LFI site were taken from 0 cm, 2 cm, 4 cm, 6 cm of the core.

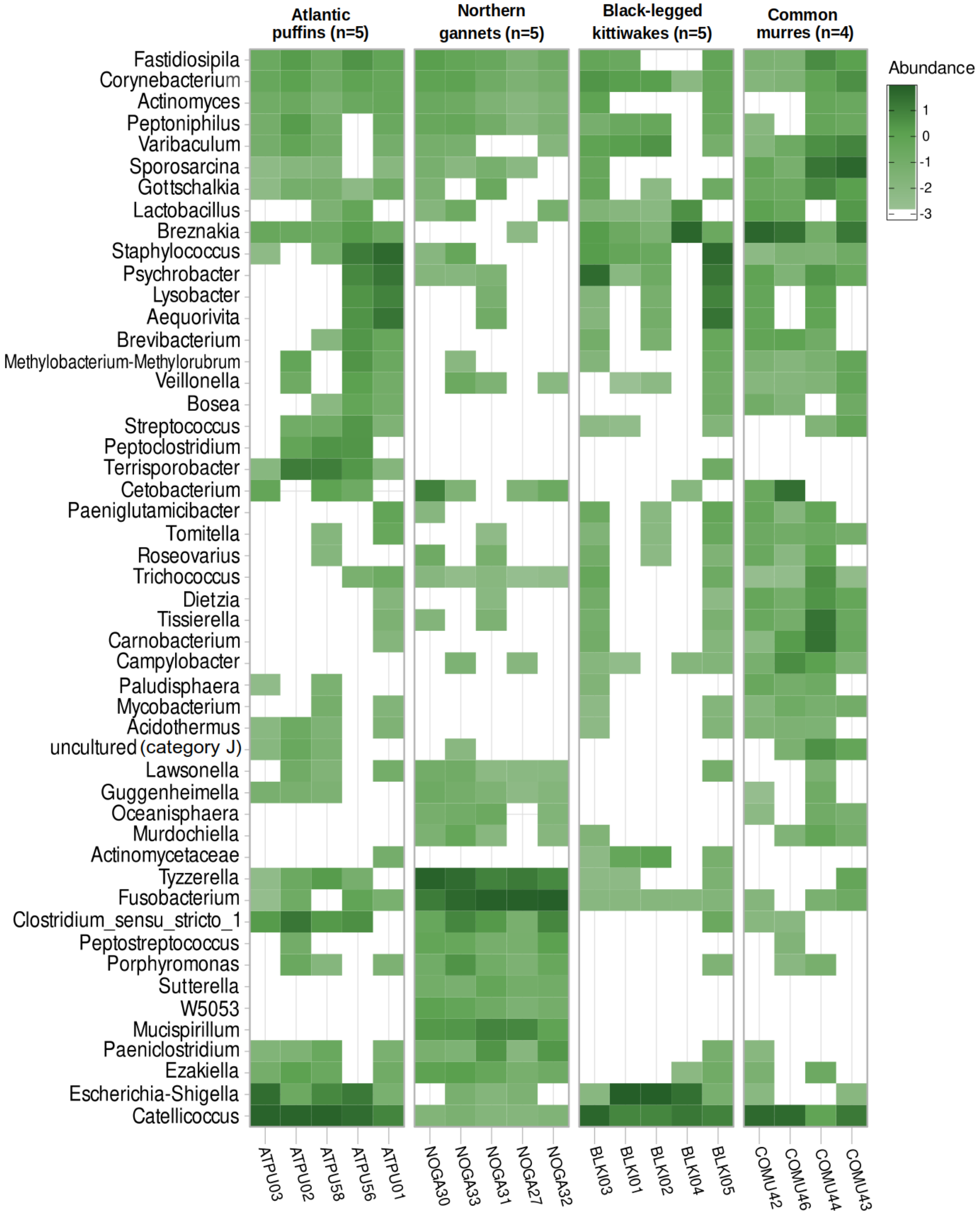

Figure 3.5 Heatmap of the 50 most abundant genera assigned to seabird fecal samples. Relative abundance is represented on a logarithmic scale [$\log_{10}$(%)], and taxa with counts equal to zero are shown in white, while low abundance counts are light green and high abundance counts are dark green.

d) Differential abundance of bacterial taxa in the top sedimentary layers

Next, we plotted the top 10 most abundant taxa found in each sediment coring site, from the top 1 cm interval of each core (Figure 3.6). The most abundant taxa found across the top layer of all sediment coring sites included *Mycobacterium, SC-I-84*, and an uncultured genus (category F, Appendix II – Supplementary file AII.1). *Subgroup_17* was most abundant only in the CSM sites, while *Cyanobium_PCC6307* was the most abundant genus shared among the CSM-I and LFI sites.

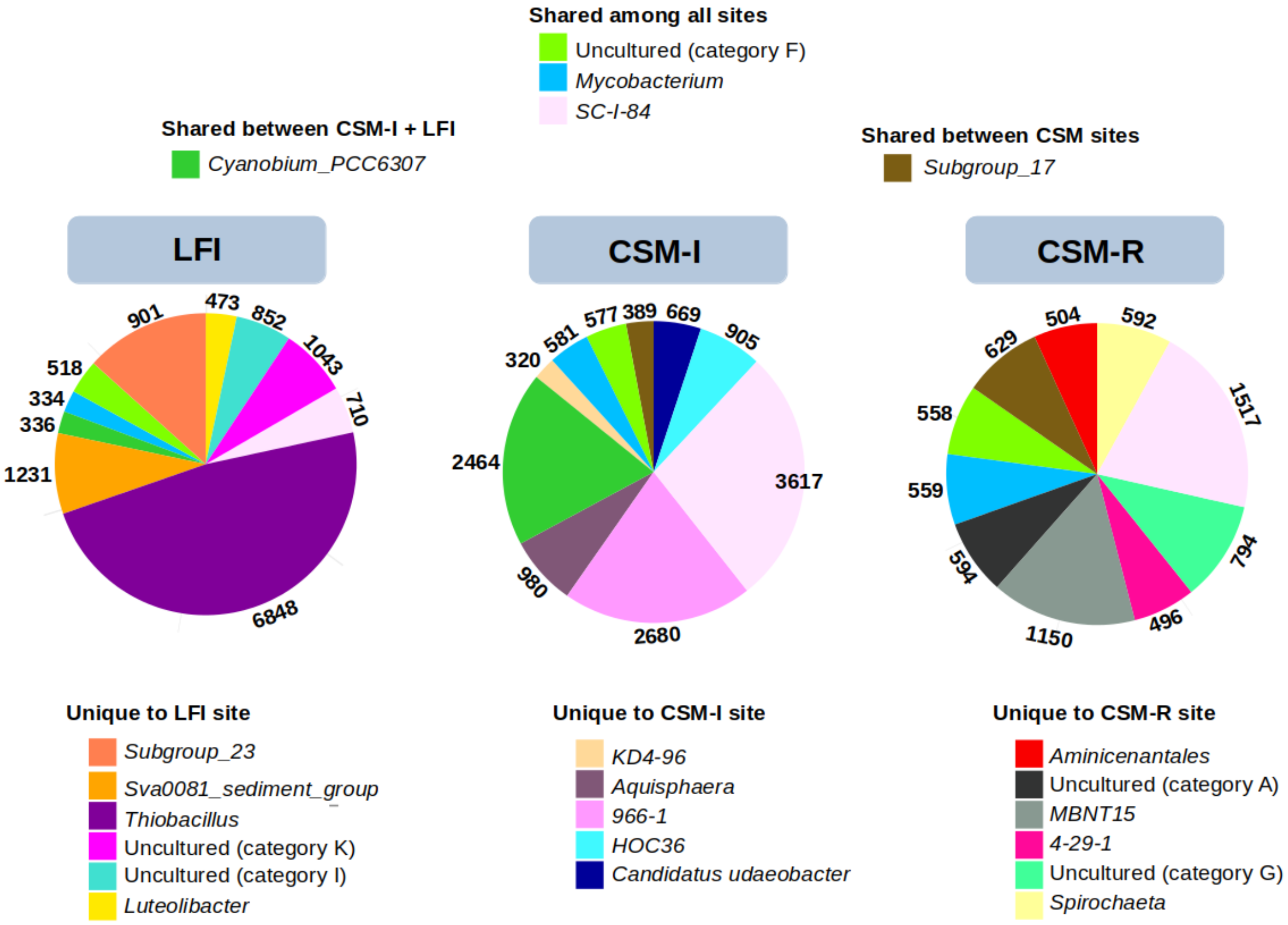

Figure 3.6 Comparison of taxa in the top sediment core layers from each site (1 cm for CSM-I and CSM-R, 0 cm for LFI). Each pie chart represents the top 10 most abundant taxa identified in each sediment core site. Uncultured taxa are listed in Appendix II - Supplementary file AII.1, and total ASV counts for each taxon are labelled on the pie charts.

The most abundant genera found in the LFI site were *Subgroup_23, Sva0081_sediment_group, Thiobacillus, Luteolibacter,* and two uncultured genera (category K and I, Appendix II - Supplementary file AII.1). The most abundant genera in the CSM-I site were *KD4-98, Aquisphaera, 966-1, HOC36*, and *Candidatus udeobacter*. Finally, genera which were most abundant in the CSM-R site were *Aminicenantales, MBNT16, 4-29-1, Spirochaeta,* and two uncultured genera (category A and G, Appendix II - Supplementary file AII.1).

e) β-diversity comparisons among sample groups

The abundance of taxa in the community (weighted UniFrac analysis) and the presence/absence of taxa without consideration of abundance (unweighted UniFrac analysis) were used to evaluate β-diversity (Figure 3.7). When considering the weighted UniFrac plot (Figure 3.7B), the northern gannet samples separated into a distinct group, whereas the other seabird species were more evenly distributed, and their 95% confidence intervals overlapped. Overlap between seabird groups was more prominent in the unweighted PCA (Figure 3.7A). β-diversity in sediment samples exhibited stronger site-specific patterns in both unweighted (Figure 3.7A) and weighted (Figure 3.7B) distances; the LFI site formed a distinct cluster, while the confidence intervals for the two CSM sites overlapped. In the CSM-I site, the 2 cm depth sample was less closely related to the clustered 1 cm, 4 cm and 8 cm samples. In the CSM-R site, the 1 cm depth sample was less related to the clustered 2 cm, 4 cm and 8 cm samples.

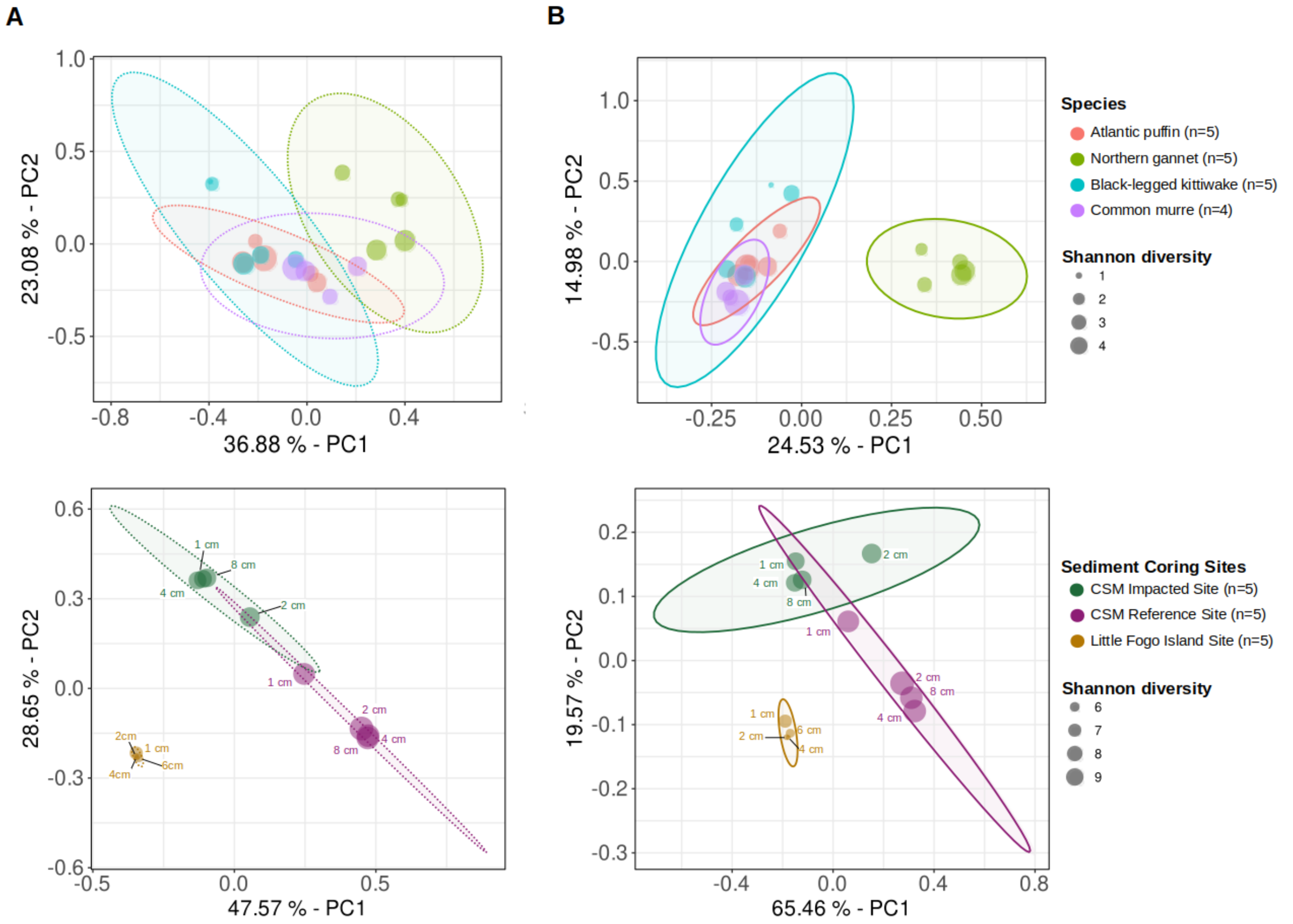

Figure 3.7 Unweighted [A] and weighted [B] UniFrac principal component analysis (PCA) plots for seabird fecal (top) and sediment (bottom) samples, showing the percentage of variance explained by axes 1 (PC1) and 2 (PC2). Each point represents an individual sample coloured according to sample group, and the ellipses represent the 95% confidence intervals for each group. Each sediment samples is labelled according to sediment core depth (cm). Dot size is proportional to the nearest integer value of Shannon diversity. The percentage of variance each axis explains is displayed in the axis labels.

β-diversity relationships among the seabird groups was further examined by running a PERMANOVA test, which assessed the β-group significance of samples for both the weighted and unweighted distance matrices (Table 3.2) [39]. The gannets consistently had a pseudo-F statistic that was higher than the test statistic across all its pairwise permutations, and q-values that represent a significant difference in β-diversity. There were no significant differences found for the weighted distance differences between puffins and kittiwakes or puffins and murres, despite most of q-values being below the threshold (0.05), because the pseudo-F statistic values between these groups were all below the F-test statistic. The q-values obtained from the pairwise analysis of unweighted and weighted distances between kittiwake and murre samples were both above the threshold, and the pseudo-F

Table 3.2 Statistical comparisons of differences in microbial community compositions among sediment and seabird sample groups based on the weighted and unweighted UniFrac distance matrices, including the pseudo-F statistic, p-values (p) and q-values (q) obtained for each test.

Group 1[1]	Group 2[1]	N[2]	Unweighted UniFrac[3] seabird test statistic = 3.33 sediment test statistic = 12.10			Weighted UniFrac[3] seabird test statistic = 4.54 sediment test statistic = 11.34		
			pseudo-F	p	q	pseudo-F	p	q
ATPU	NOGA	10	4.836	0.013	0.0195	7.894	0.007	0.0210
	BLKI	10	1.544	0.121	0.1210	1.645	0.229	0.2290
	COMU	9	2.080	0.013	0.0195	2.329	0.064	0.0768
NOGA	BLKI	10	4.639	0.011	0.0195	6.791	0.006	0.0210
	COMU	9	5.766	0.011	0.0195	6.210	0.022	0.0440
BLKI	COMU	9	2.091	0.045	0.0540	3.181	0.035	0.0525
CSM-I	CSM-R	8	8.37	0.021	0.034	7.88	0.070	0.070
	LFI	8	13.43	0.034	0.034	9.69	0.032	0.0525
CSM-R	LFI	8	16.38	0.027	0.034	19.15	0.035	0.0525

[1] Sample identification: Atlantic puffin (ATPU), northern gannet (NOGA), black-legged kittiwake (BLKI), common murre (COMU), CSM impacted site (CSM-I), CSM reference site (CSM-R), Little Fogo Island site (LFI)

[2] N = number of samples in the comparison

[3] F-test statistic values for seabird fecal sample and sediment core sample PAIRWISE permutations

statistic for each was lower than that of the test statistic, therefore the difference in diversity between these two samples was also insignificant.

We then compared the β-group significance between the sediment groups using the same PERMANOVA test (Table 3.2). When comparing the CSM-I and CSM-R sites, the pseudo-F statistic was lower than the test statistic for both unweighted and weighted distances. However, the q-value was significant for unweighted distances between these sites, while it was not significant for weighted distances. This can also be seen in Figure 3.7 where the confidence intervals overlapped more for the weighted compared to the unweighted clusters. When considering unweighted distances between the CSM-I and LFI sites, the pseudo-F statistic was higher than the test statistic and the q-values were below the threshold; diversity was therefore significantly different. However, when considering weighted distances, the pseudo-F statistic was below the test statistic value, which indicated that taking abundance of ASVs into account resulted in the differences being not significant. Finally, comparing the CSM-R and LFI sites using both weighted and unweighted distances, diversity was significantly different only in the presence/absence of ASVs, not their abundance. This was indicated by a higher pseudo-F statistic than the test statistic, even though the q-value for the weighted distance exceeded the threshold value.

4. Discussion

We previously used a classic paleolimnological approach to identify shifts in isotopes, chlorophyll *a*, metal(loid)s and diatoms in the pond adjacent to the CSM colony that aligned well with the current population data available for northern gannets nesting there [40]. However, this pond is also frequented by black-legged kittiwakes and therefore they might also affect the sediments at this site. Here, we wanted to characterize the fecal microbial communities of relevant seabird species and look for evidence of those microbes within seabird-impacted pond sediments. Even in the absence of a direct link in terms of specific organisms, we expected there could be some effect of the seabird fecal/nutrient input on the microbial communities in general. Exploring these associations could allow microbes to be used as proxies to quantify seabird population trends and impacts at ecologically important sites.

Among sediments, the CSM-R site, which is not impacted by seabird inputs, had a more diverse microbial community compared to the two other sites that are impacted by seabird nutrients (Figure 3.2C). This fits with observations from other environments where increased nutrient inputs can result in decreased community diversity [41, 42. 43]. The diversities at the CSM-R and LFI sites were the least similar, likely attributed to the combination of the differences in environments and lack of seabird nutrients entering the CSM-R site. The CSM-R site had the highest α-diversity across all metrics, closely followed by the pond impacted by the multi-species nesting site, CSM-I, and lastly, the pond impacted by the single species, LFI. Despite the lack of seabird nutrients being introduced into the reference pond, β-diversity did not differ significantly between the two CSM sites (Figure 3.7), likely attributed to the similar overall environmental conditions at the reserve. The CSM Ecological Reserve is characterized primarily by barren peatlands with acidic water bodies and the main difference between these two collection sites is the input of the seabird-derived nutrients to the pond 240 m away from the colony.

In contrast, the diversity analyses among fecal sampling groups did not reveal significant differences in α-diversity among seabird species. The discrepancies in the H value and p values obtained from the Kruskal-Wallis tests on fecal sample groups are likely due to differences in the sensitivity of each metric to changes in the microbial community compositions. The number of observed ASVs (or features) is a metric that counts all presences, regardless of their abundance. On the other hand, the Shannon and Simpson's diversity indices consider both the number of unique ASVs and their relative abundances and are thus more sensitive to changes in the evenness of the microbial communities. The four seabird species did not differ significantly when considering Shannon and

Simpson's diversity indices. However, there were significantly different numbers of unique ASVs across seabird species, suggesting that there were certain microbes present exclusively in one species or another. The highest average Shannon and Simpson's index values were found in murres, followed by puffins, gannets, and kittiwakes. β-diversity analysis using the unweighted and weighted UniFrac distance matrices showed that gannet samples formed a distinct group, while the other seabird species were more evenly distributed (Figure 3.7), indicating that they have a unique bacterial community compared to other seabird species. This is supported by the presence of unique bacterial genera in gannet samples that are not found in other seabird species, such as the genera *Mucispirillum*, *Tyzzerella*, and *Fusobacterium* (Figure 3.6). The low number of overall taxonomic assignments found among the northern gannet samples could explain why there is no significant difference in α-diversity metrics (Figure 3.1B). It is also important to note that UniFrac analysis considers the phylogeny of bacterial communities in each sample, and may contribute to the observed differences in diversity among seabird species; which provides an explanation for why we may see no significant differences in diversity among seabird species when considering α-diversity metrics but do see significant differences when considering β-diversity.

Catellicoccus was abundant in murre, puffin and kittiwake samples but nearly absent from gannet samples. *Catellicoccus* has previously been found as a core genus of the gut microbiome in thick-billed murres (*Uria lomvia*) [11] and gulls [44], thought to play an important role in maintaining healthy weight during limiting feeding events [11]. Similar to our results, Góngora et al. [11] also found that ASVs assigned to the genus *Breznakia* was abundant in the murre gut microbiome. Despite their prevalence in these seabird species, *Catellicoccus* and *Brenakia* were not observed in any of our seabird influenced ponds.

Several studies have examined the relationship among the environment and diet of seabirds and their gut bacterial communities. Species-specific microbial assemblages have previously been discovered in the gut microbiome of the long-lived procellariform seabirds (mainly dominated by the phyla Firmicutes, Proteobacteria and Bacteroides) [45]. Certain studies have found distinct differences between the microbiome of several species in the same family, such as Dewar et al. [46] who found variations in the gastrointestinal microbiota of four penguin species. The seabirds we consider in this study have different diets and nesting behaviors. Northern gannets prefer to nest in close proximity to the coastline, particularly on sea stacks isolated from the mainland, where built up guano, seaweed and even plastic debris form mounds they use as nests [47]. Black-legged kittiwakes and common murres compete with gannets for nest space on Bird Rock, however they tend to nest on steeper cliff sides on the mainland and lower plateaus of the stack, whereas gannets dominate the top of the stack. In Newfoundland, gannets feed mainly on mackerel, herring and squid near the surface of the water [47], while common murres are deep-diving seabirds that are known to consume juvenile cod, squid, and historically, capelin [48]. Black-legged kittiwakes are historically known to consume capelin in Newfoundland [49]. On Little Fogo Island, Atlantic puffins nest in close proximity to the freshwater pond that we sampled. The diet of Atlantic puffins is diverse, and they are known to feed mostly on sand lance and capelin, as well as squid, polychaetes, and juvenile cod [50]. The dominant impacting factor on the gut microbiome of most animals is diet [51], and the differences in the diet of northern gannets presumably contribute to the more distinct community they contain. However, they are also more evolutionary divergent and in a different taxonomic order (the Suliformes) compared to the other species examined, which are all within the order Charadriiformes.

While we found no significant evidence that bacteria in the fecal microbiome of seabirds was present in the sediments taken from our study sites, certain species present in fecal samples were also present in very small amounts in the sediment cores. Notably, the taxa *Epulopiscium* that was found exclusively in puffin samples and found in the CSM impacted site at a depth of 2 cm. This taxon is a genus of gram-positive bacteria that is commonly found in the gut of raptors, and known to have a symbiotic relationship with hosts, specifically inhabiting the gut of fish [52].

Cyanobium_PCC6307 was abundant in the upper layers of seabird influenced sites, but almost absent in our reference site. This genus is a freshwater picocyanobacterium, commonly found in freshwater ponds, that has been found to inhibit the growth of co-occuring bacteria in pelagic environments [53]. Picocyanobacteria play a role in primary production within pond ecosystems, and could therefore be elevated in the ponds experiencing eutrophication caused by seabird nutrient inputs. In the CSM impact site, we found particularly elevated levels of chlorophyll *a* that aligned well with the other proxies in our previous analysis [40]. *Thiobacillus* was abundant in the top layers of the LFI core, and nearly absent in the CSM-I and CSM-R cores, potentially due to ecological differences between the two geographical locations, as it was not present in the Atlantic puffin samples. Overall, more work is still required in order to better understand how seabird nutrient input to freshwater ponds nearby colonies might impact bacterial diversity in these ecosystems.

5. Conclusion

Our results provide insights into the microbial diversity of seabird fecal samples and pond sediments, and suggest there may be microbial targets that could be exploited to evaluate seabird impacts on such environmental samples. It is important to note that our knowledge concerning the seabird gut microbiome is still limited. Current scientific literature lacks insights into the microbial ecology of ponds affected by nutrient transfer specifically from seabirds. Therefore, more investigation into both the structure of seabird gut microbial communities and their influence on sediment microbial composition is required to establish links between seabirds and their surrounding ecosystems. Interestingly, we illustrate that seabird-impacted sites have significantly lower bacterial diversity than unimpacted pond sediments, potentially implicating the high amounts of nutrient influx to the lower bacterial diversity. The ongoing development of species-specific geochemical and biological proxies is crucial for providing accurate paleolimnological reconstructions. Developing such proxies would have implications for monitoring and managing these areas, particularly at multi-species breeding sites like Cape St. Mary's Ecological Reserve.

6. Data Accessibility

Files containing data pertaining to this chapter can be found in Appendix II. A guide for the commands and code used in this tutorial is available on Github. The microbiome analysis accompanies the data used in the chapter and incorporates documentation from QIIME2, Compute Canada's User Guide, and the Microbiome Helper into a step-by-step workflow that can be used as a research tool for users who want to use QIIME2 to run a basic microbiome analysis on any laptop or desktop computer. We provide an in-depth review of the bioinformatics pipeline and commands used to perform an analysis on sediment samples and seabird fecal samples with QIIME2. The 'QIIME2_for_Graham' repository, licensed under The Creative Commons CC0 Public Domain Dedication.

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CHAPTER 4

1. General discussion

The Cape St. Mary's region located on the southwestern tip of the Avalon Peninsula (Newfoundland) is an important breeding site for colonial seabirds. Over 30,000 breeding pairs of seabirds, including northern gannets (*Morus bassanus*), common murres (*Uria aalge*), and black-legged kittiwakes (*Rissa tridactyla*), nest along the 4 km of mainland cliff and the isolated stack (Bird Rock) [1]. The daily foraging trips of seabirds to and from Bird Rock deliver marine-derived nutrients that have triggered significant ecological shifts in the terrestrial habitat surrounding the breeding area, including a nearby body of water. Understanding how ecosystems are influenced by seabirds transferring nutrients in ecologically important areas, as seen in various colonies globally [2, 3, 4, 5, 6, 7], helps us grasp the role of seabirds in transporting nutrients across ecosystem boundaries.

In the introductory chapter, I highlighted the recommendations of Capo et al. [8] for sedimentary DNA (*sed*DNA) analysis in paleo-studies reconstructing population shifts of biota in an ecosystem. They emphasized the importance of choosing a study site based on the ecological processes at question and using multiple proxies to validate results. Cape St. Mary's Ecological Reserve was an ideal study site for integrating a broader application of *sed*DNA in our palaeoecological approach because of the multiple species that nest on Bird Rock and the location of the pond relative to the main breeding site. Furthermore, Morrissey et al. [9] found that the predominant wind direction in the region of CSM in 2021 was from the SW for 18% of the year (Figure 4.1), which aligned with the location of the pond relative to the main nesting site, Bird Rock.

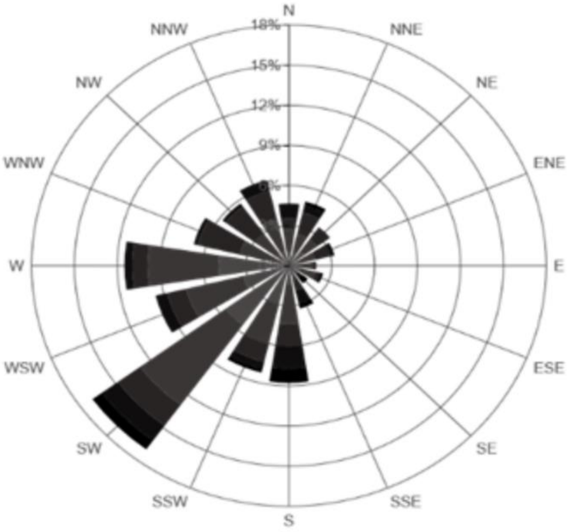

Figure 4.1. Wind rose for CSM region. The percentage values shown in the figure indicate the proportion of time the wind comes from each direction throughout the year. [Source: Morrissey et al. [9]]

In this final chapter, I summarize my findings and highlight the key role seabirds play in transferring nutrients across ecosystem boundaries on a global and local scale. Lastly, I provide further recommendations to examine the impact this colony has on the terrestrial landscape of Cape St. Mary's for future research.

a) Research summary and interpretation of results

In the first research chapter, I used a multiproxy paleolimnological approach to validate the monitoring data for the northern gannet nesting colony in Cape St. Mary's Ecological Reserve that suggested the colony was first established in the early 1880s [10]. The sediment cores obtained from CSM were approximately 26-30 cm, and lead-dating the sedimentary record allowed for a reconstruction of about 140 years. Extrapolating these dates using a polynomial regression extended the scope of this reconstruction to the 1800s. I found a significant increase in nutrients, metalloids, and chlorophyll *a* (chl *a*) starting around the time the first gannets were reported on Bird Rock, indicating that over the length of my sediment records, the gannet colony has indeed reached a historical maximum in present day.

The second manuscript explored the potential use of fecal bacterial communities from different species of seabirds as an indicator of ornithogenic nutrient input into pond sedimentary records. I improved the DNA yield from sediment and fecal samples by adapting the DNA isolation and recovery protocols, which allowed for successful characterization of bacterial diversity and community structure in the top 6-8 cm of sediment records taken from ponds near seabird colonies. Interestingly, my results illustrated how ponds influenced by seabird nutrients have overall lower bacterial diversity, which is a common occurrence in aquatic systems that experience eutrophication. I discussed the specific bacterial genera present in sedimentary records, and the seabird fecal microbiome, which should be considered in future studies that would like to develop DNA-related proxies used for quantifying palaeoecological trends at important breeding sites. Given the small section of the cores used to assess bacterial composition (0-8 cm), I observed no significant trend in bacterial diversity or community structure across core depth.

For the fecal microbiome analysis, I focused on the seabirds of conservation interest in the region of Cape St. Mary's (CSM) Ecological Reserve, which included the northern gannet, common murre, and black legged kittiwake [11]. Access to population data for these species was critical for our multiproxy approach and enabled us to draw insightful conclusions in the fecal microbiome analysis segment of the project. The population monitoring data for kittiwakes and common murres on Bird Rock are sparse compared to the data for northern gannets, which have been frequently surveyed since 1934 [10]. Atlantic puffins, the other species included in this research, are pelagic seabirds that construct deep burrows in organic soil to provide shelter and raise their young, and in the process they reduce the stability of top soils and alter plant communities [12]. The decision to include Atlantic puffins was driven by the fact that the core sample from Little Fogo Island originated from a pond mainly inhabited by puffins, with no signs of other species using this habitat. The puffins burrow along the perimeter of the pond, which also made them a suitable species of interest for our study since the colony in Cape St Mary's was more than 100 m away from the pond.

When the sediment cores were retrieved, the vegetation surrounding the reference pond (located further inland) was characteristic of flat, peatland habitats, made up of a thick layer of sphagnum mosses, and various species of grass, reindeer lichen (*Cladonia rangiferina*), and pitcher plants (*Sarracenia purpurea*). Comparatively, the perimeter of the impact pond in Cape St. Mary's was very rocky, and had a different flora compared to the reference pond, including Lemnoideae and various flowering plants. The reference pond was more acidic than the impact pond, the surface water had a significantly lower total nitrogen (TN) and total phosphorus (TP) concentrations, and our multiproxy analysis showed nutrient content remained at values that were stable or decreasing over time relative to the pond nearby the colony. Despite differences in the physicochemical characteristics of the reference pond compared to the impact pond, the bacterial community structure at the genus level was more similar in the two Cape St. Mary's sites than the impacted site and the Little Fogo Island site influenced by puffins.

My study's reference pond had a high concentration of TN (0.451 mg L^{-1}) and TP (50 µg L^{-1}), relatively low conductivity (120 µS cm-1) and a slightly acidic pH (6.7), compared to the water chemistry results taken from other studies' reference sites [3, 13]. Duda et al. [13] conducted water chemistry on samples collected from a mainland control pond near Baccalieu Island, belonging to the same ecoregion of Cape St. Mary's (Eastern Hyper-Oceanic Barrens ecoregion); their findings showed lower pH, conductivity, TN, and TP values compared to my reference pond. My reference pond exhibited similar pH levels, but lower conductivity, TN, and TP values compared to two control ponds in the Arctic habitat of Digges Island (Nunavut) that were studied by Hargan et al. [3]. Furthermore, Morrisey et al [9] also collected water chemistry data from twelve ponds in Cape St. Mary's, with increasing distance from Bird Rock, and found that values for TN, K^+ and Mg^{2+}, Na^+ decreased significantly with increasing distance from the colony. Seven of pondsthat were between 1.32 km and 5.46 km away from the colony (including the same reference pond I used in this research) had high values of TN (range = 0.451 – 0.738 mg L^{-1}) and TP (range = 28 – 283 µg L^{-1}) compared to the sites sampled by Duda et al. [13] and Hargan et al. [3]. The ponds also had an acidic pH (range = 4.2 – 6.7), and high conductivity (range = 80 – 180 µS cm^{-1}). Therefore, the higher values of TN, TP, and conductivity in the surface waters of my reference pond in Cape St. Mary's are likely a characteristic of the acidic, peatland environment that makes up this portion of the Eastern Hyper-Oceanic Barren ecoregion.

b) Implications of the findings

Cape St. Mary's is designated as an Ecological Reserve and Important Bird Area in the northeast coast of Newfoundland [11]. In Canada, northern gannets breed in six different colonies including Baccalieu Island, Cape St. Mary's and Funk Island in Newfoundland, and Anticosti Island, Bird Rocks and Bonaventure Island in Quebec. Since the species' diet is dependent on the availability of prey, it is reasonable to assume that changes in the marine environment impact the population dynamics of the gannets across all their breeding colonies. The rich diversity of prey found in the waters surrounding Cape St. Mary's likely offered a sustainable food source that attracted the gannets to Bird Rock in the early 1880s. Additionally, the stack being isolated from the mainland provides protection from land-based predators. As such, our reconstruction of the main nesting site in Cape St. Mary's provides valuable insights into the overall history of northern gannet populations in Canada, including those breeding in Newfoundland. This information could be used to further validate the historical monitoring reports available and help inform future management strategies for the species in the region.

The species composition of the fecal microbiome has also become an important part of studying animals. Most of what we currently know about the fecal microbiome comes from studying humans and domesticated animals. However, this approach tends to neglect the microbiomes of wild species. This is particularly true for migratory animals like seabirds who are hard to monitor over long time periods, as they spend their life travelling through a multitude of habitats. As a consequence of being exposed to a larger diversity of microbes, the fecal microbiome of migratory seabirds is of particular interest in conservation biology [14]. Diet plays a key role in shaping the fecal microbiome of most vertebrates [15, 16, 17]. Since pelagic seabirds rely on the varying availability of prey in the nearby foraging regions of a colony [18], and are also influenced by changes in population caused by fishing activities [19], it is important to remember that the diets of the seabird species in my research will change over time. Puffins and gannets in the North Atlantic generally have similar diets, feeding on herring, mackerel, and capelin [18], and a majority of the black-legged kittiwake diet is made up of sandeel [20]. Gannets will also eat squid and cod, and puffins will eat sandeel and zooplankton [18]. My research has provided microbiome data that can be considered in future studies which assess the influence diet and/or behavior has on bacterial communities that make up the fecal microbiome of seabird species. From a paleoecological perspective, identifying specific bacterial genera associated with seabirds will also help inform future studies that seek to develop more targeted DNA-related proxies for quantifying ecological trends at important breeding sites.

These findings also highlight the importance of seabird nutrient inputs for the microbial ecology of aquatic ecosystems. Previous studies have shown that seabirds cycling nutrients into terrestrial ecosystem can alter the soil microbial communities near their colonies [21, 22]. For instance, Ramírez-Fernández et al. [23] found that soils impacted by southern elephant seals (*Mirounga leonina*) and penguins (*Pygoscelis*) showed differences in nutrient composition compared to control soils, with the soils nearby the penguin colonies possessing the lowest bacterial diversity. Recently, Justel-Díez et al. [24] investigated the effects of yellow-legged gull (*Larus michahellis*) guano on microbial communities in sea water samples taken from the Cies Islands (Spain), and found that the addition of guano stimulated the growth of certain microbial groups in the surrounding coastal waters. The addition of guano led to an increase in chl *a* concentration and bacterial abundance, regardless of the initial nutrient levels in the water. The findings of Justel-Díez et al. [24] strongly indicate that certain compounds present in the guano are essential for the growth of phytoplankton. While my results may not show that guano promoted the growth of specific bacterial species in sediments influenced by seabird colonies, they do demonstrate the impact of seabird nutrients on the bacterial composition of pond sediments, showing that seabird-influenced ponds had significantly lower bacterial diversity than those not affected by seabirds. However, there could be other ecological factors impacting the diversity at my three different sites, including the presence of pollutants and the long-term history of ecological dynamics in the study area. Overall, these results add to the growing body of evidence that seabirds can have a significant impact on microbial communities in both terrestrial and aquatic environments and can inform conservation efforts aimed at protecting these important ecological relationships.

c) Limitations of the study and future research

In many ways, this research can serve as a preliminary investigation for future paleolimnological research within Cape St. Mary's Ecological Reserve. It is possible to extend the time period captured in our cores to determine whether any seabirds nested at Cape St. Mary's prior to the 1700's, for example, during purposed warmer periods (e.g., the Medieval Climate Anomaly) in the Holocene. While my results contribute to our understanding of the impacts seabirds have on the geochemical and biological characteristics of their habitats, they also emphasize the need for further research to fully understand the ecological implications of these changes.

The incorporation of a negative control, like the reference pond utilized in this study, was a valuable approach for cross-validating the multiple proxies with the sedDNA analysis, as outlined by Capo et al. [8]. Having a reference site aided in providing a reliable baseline for comparison and helped us gain a better understanding of the natural state of the Eastern Hyper-Oceanic Barren ecoregion making up Cape St Mary's. By continuing to collect paleolimnological data from both seabird-influenced sites and reference sites across multiple ecoregions in Newfoundland, we can further improve the validity and reliability of paleolimnological results and develop a comprehensive understanding of the diverse ecosystem dynamics that exist within the province. Future research on the influence seabird guano has on microbial composition and structure of pond ecosystems over long time periods should also consider other factors which could be influencing the bacterial diversity of their chosen water body.

Today, the analysis of sedimentary deposits using high-throughput genetic sequencing has shown how genetic records of past ecological communities are preserved over time. However, the extent to which genes are preserved in sediments is still relatively unknown [25]. A more in-depth analysis of the changes in bacterial community structure throughout the entire depth of the sedimentary record from the pond nearby Bird Rock may provide a more robust reconstruction of bacterial diversity over time and may even reveal that the pond once had a more diverse bacterial composition pre-colonization. To obtain a longer sediment core from this pond, a few options could be considered. One option could be to use a peat coring device, which would be an appropriate choice for the barren, peatbog terrain of CSM. Paleoenvironmental reconstructions often require high-resolution sampling and large sample sizes to compensate for the long-time period that is needed to be captured and the various analytical methods that are implemented [26], and peat cores offer reconstruction up to the millennial scale and offer the opportunity to gather a more robust reconstruction of seabird colonies, given the long sections they acquire.

After careful DNA isolation and PCR protocol optimization, I was able to successfully use the same extraction kit for both sediment and guano samples. Throughout the process, I identified and implemented crucial optimization steps that allowed for a more reliable extraction of DNA from both sample types. These optimization steps were documented and can serve as a valuable resource for future research that aims to extract DNA from sediment and guano samples using the same kit. Continuing to develop methods that improve the quality of *sed*DNA data and integrating it with data from more traditional methods, such as stable isotope and lipid analyses, will provide a more robust understanding of the effect seabirds have on their habitats. Additionally, most of the sequences stored in the Silva reference database [27], which we used to conduct our microbiome analysis through QIIME2, are derived from environmental sequencing. The database currently contains many unknown traits listed as “uncultured” taxa, which is one reasons why I obtained a lower number of taxonomic classifications for the seabird fecal samples compared to sediment samples. Future studies should consider the use of multiple reference databases while assigning taxonomic classifications to sequences.

2. Final conclusion

On a broad scale, predicting shifts in biota caused by abiotic and anthropogenic factors is difficult when there is a lack of in-situ measurements (monitoring reports, breeding success, habitat quality, disease prevalence, etc.) collected across shifting environmental conditions. My research aimed to tackle some of the key challenges in paleolimnology, specifically related to reconstructing biotic changes in a multispecies seabird colony, by producing a time series of ecological shifts in Cape St. Marys Ecological Reserve related to seabird presence and including a comparative microbiome analysis of sediments impacted by seabird nutrients. Our main findings illustrate the value of using a multi-proxy approach combined with paleogenetics to understand past changes in an ecosystem and assess the known period of colonization in an ecologically important seabird colony. Notably, we found that seabird impacted pond sediments have a tendency for lower bacterial diversity than non-impacted ponds; however, a more extensive reconstruction of past bacterial diversity and community structure of ponds impacted by seabird presence is needed to develop a species-specific proxy.

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APPENDIX I

a. Supplementary tables

Table AI1. Geographic and morphometric information of the sampling sites in CSM Ecological Reserve.

Pond Type	Impact	Reference
Core ID	CSM-IMP-2	CSM-REF-3
Sampling date (MM/DD/YY)	2021 June 25	2021 Sept 08
Latitude	46.82	46.84
Longitude	-54.18	-54.17
Distance from colony (km)	0.24	2.56
Coring depth (m)	1.12	0.79
Core length (cm)	26	30

Table AI2. List of metal(loid)s analyzed in CSM-IMP-2 and CSM-REF-3 sediment cores, including limits of detection outlined in SGS Canada Inc., 2020

Metal(loid)s (limit of detection – μg g^{-1} dry wt.)					
Mercury	(0.005)	Copper	(1)	Antimony	(< 6)
Silver	(< 1)	Iron	(0.01)	Selenium	(< 0.07)
Arsenic	(0.001)	Potassium	(0.01)	Tin	(< 0.05)
Aluminum	(0.2)	Lithium	(< 2)	Strontium	(0.0005)
Barium	(0.05)	Magnesium	(0.01)	Titanium	(0.02)
Beryllium	(0.05)	Manganese	(2)	Thallium	(0.002)
Bismuth	(5)	Molybdenum	(1)	Uranium	(0.002)
Calcium	(0.01)	Sodium	(0.01)	Vanadium	(0.01)
Cadmium	(1)	Nickel	(1)	Yttrium	(0.0005)
Cobalt	(1)	Phosphorus	(0.01)	Zinc	(0.002)
Chromium	(1)	Lead	(2)		

Table AI3. Dating profile for the impact core (CSM-IMP-2) with dates extrapolated using a polynomial equation

Midpoint (cm)	Year *
0.125	2021.04
0.375	2019.42
0.625	2017.80
0.875	2016.17
1.125	2014.55
1.375	2012.22
1.625	2009.89
1.875	2007.56
2.125	2005.23
2.375	2003.23
2.625	2001.22
2.875	1999.22
3.125	1997.21
3.375	1994.62
3.625	1992.03
3.875	1989.43
4.125	1986.84
4.375	1981.23
4.625	1975.62
4.875	1970.00
5.125	1964.39
5.375	1956.29
5.625	1948.19
5.875	1940.08
6.125	1931.98
6.375	1928.17
6.625	1924.36
6.875	1920.54
7.125	1916.73
7.375	1915.07
7.625	1913.41
7.875	1917.11
8.125	1910.09
8.375	1902.85
8.625	1895.37
8.875	1887.66
9.125	1879.73
9.375	1871.57
9.625	1863.18
9.875	1854.56
10.125	1845.71
10.375	1836.63
10.625	1827.32
10.875	1817.78
11.125	1808.02
11.375	1798.03
11.625	1787.80
11.875	1777.35
12.125	1766.67
12.375	1755.76
12.625	1744.62
12.875	1733.25
13.125	1721.66
13.375	1709.83
13.625	1697.78
13.875	1685.49
14.125	1672.98
14.375	1660.24
14.625	1647.27
14.875	1634.07
15.25	1613.84

* see Figure S3 for polynomial regression used to extrapolate year

Table AI3. Generalized additive model results for each proxy from the reference core (CSM-REF-3), including k-indices, p-values, and range of depths (cm) where a derivative of significant increase/decrease was found. Depths are shown instead of years due to the low activity of the sediment core, therefore periods of change are considered from the youngest interval (near top of the core) to the oldest (near the bottom of the core).

Proxy	k-index	p-value	Sig. decrease (cm)	Sig. increase (cm)
chl *a*	1.2	0.81	NA	0.25 - 4.17
δ^{15}N	1.12	0.58	NA	13.71 - 20.25
P	1.34	0.78	NA	0.25 - 6.78
Zn/Al	0.86	0.15	NA	0.25 - 20.25
Cd/Al	1.16	0.56	0.25 - 5.480	NA
S.construens	1.33	0.95	8.99 - 20.25	4.97 - 5.37

b. Supplementary figures

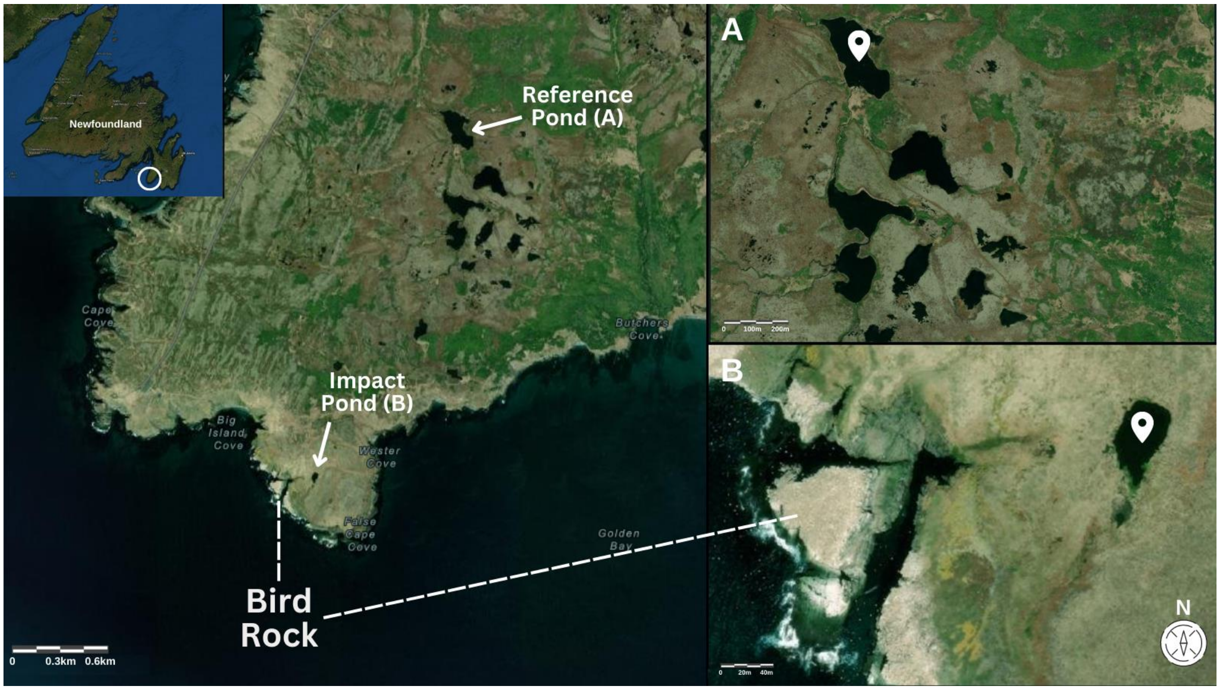

Figure AI.1. Map showing the CSM sediment coring sites relative to Bird Rock

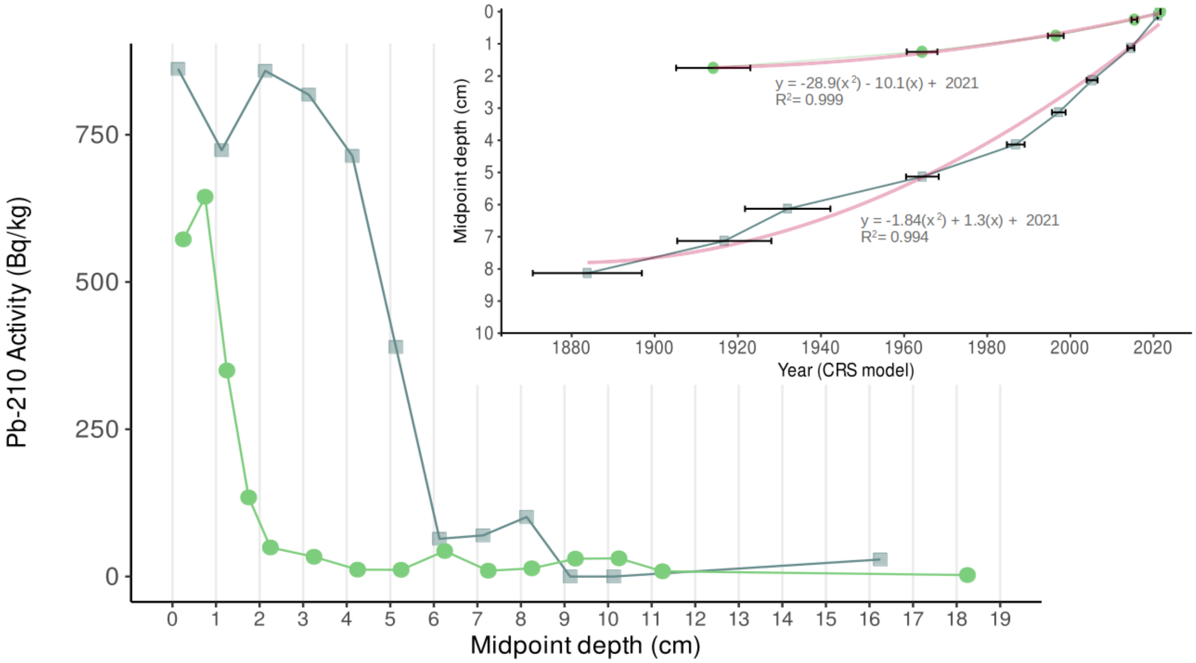

Figure AI2. ^{210}Pb activity (Bq kg-1) over the depth of the impact core (CSM-IMP-2, blue), and the reference core (CSM-REF-3, green). The ^{210}Pb dates assigned to the midpoint depth of the cores using a constant rate of supply (CRS) dating-model are shown in the smaller graph.

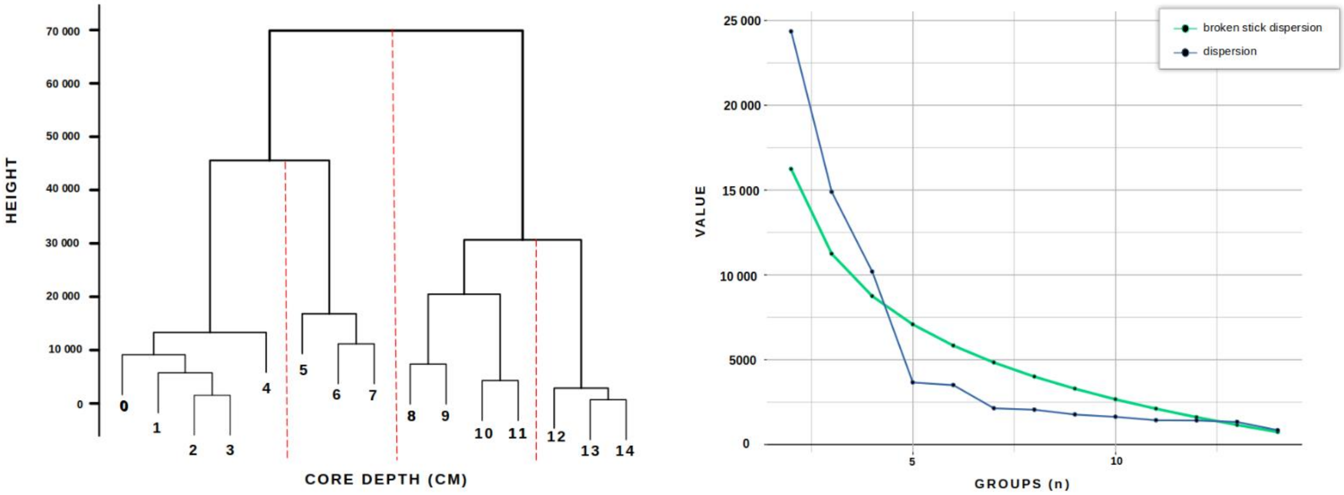

Figure AI3. Dendrogram (LEFT) showing the distinct breakpoints in the metal(loid)s data across the core depth (cm) of CSM-IMP-2, and a line plot (RIGHT) showing the broken stick dispersion plot for the breakpoint analysis, where value is a measure of the magnitude of the variable being analyzed. Created using the tidyverse and tidypaleo packages available in R (v.4.2.1) software.

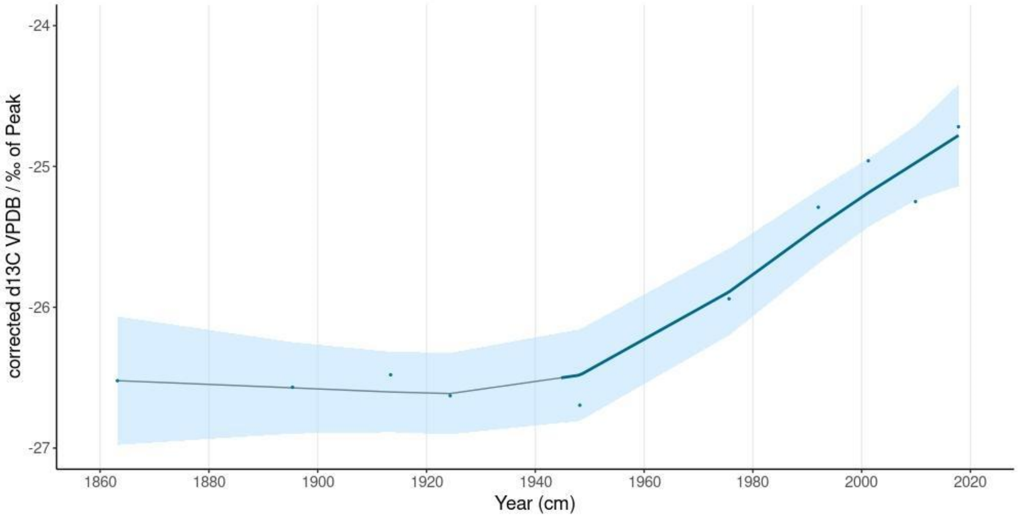

Figure AI4. Generalized additive model for the Suess-corrected values of δ13C plotted against the 210Pb dates. Each dot on the graph represents a value of $\delta^{13}C$ (‰), and the trend line includes significant periods of change in bold. The 95% confidence intervals are shaded.

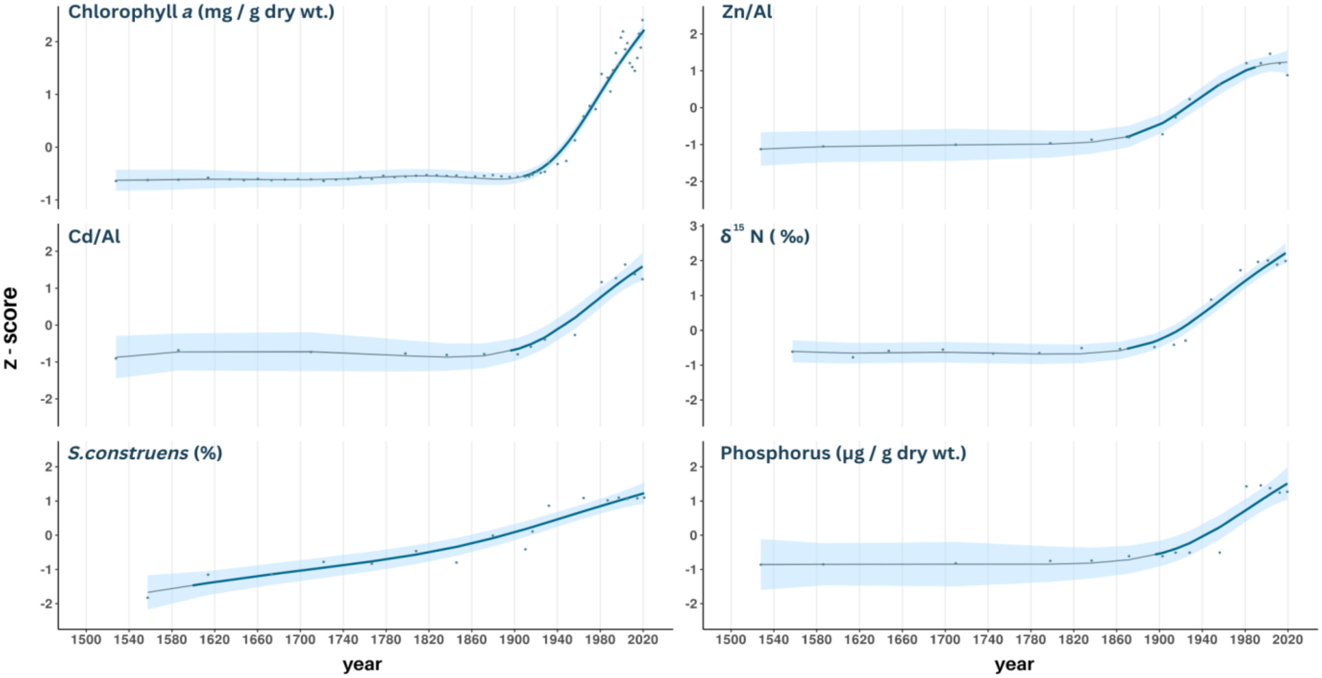

Figure A15. Generalized additive models (GAMs) for all proxies measured in CSM-IMP-2, aligned with extrapolated lead-dates. Bolded blue lines represent significant periods of change.

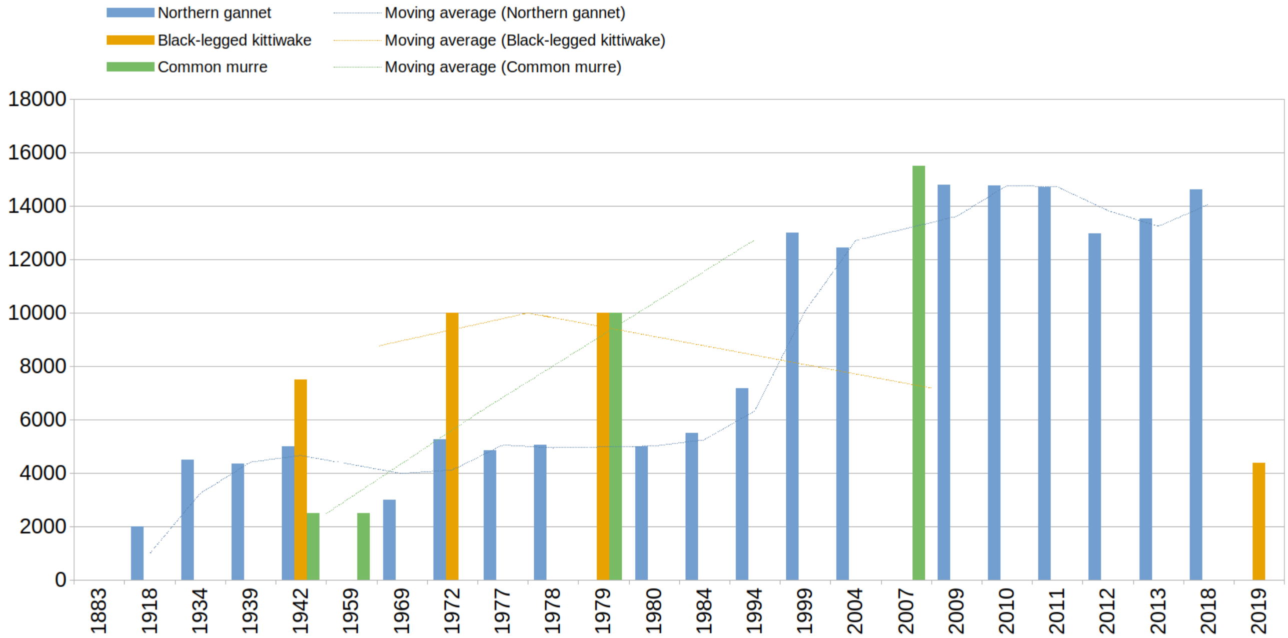

Figure AI6. Population counts for seabirds nesting in CSM (Data in Table 1)

1. Media release

New research on northern gannets in Cape St. Mary's Ecological Reserve (Newfoundland, Canada) reveals how sediment cores collected from an upwind pond, 240m away from the colony, contained traces of preserved seabird nutrients that revealed the colony's historical population trends. By analyzing nutrient levels in sediment cores, researchers confirmed that the establishment of the seabird colony only occurred in the late 19th century, illustrating the ability to use a multi-proxy approach to track colony trends, even when a nesting site may not be directly adjacent to a pond.

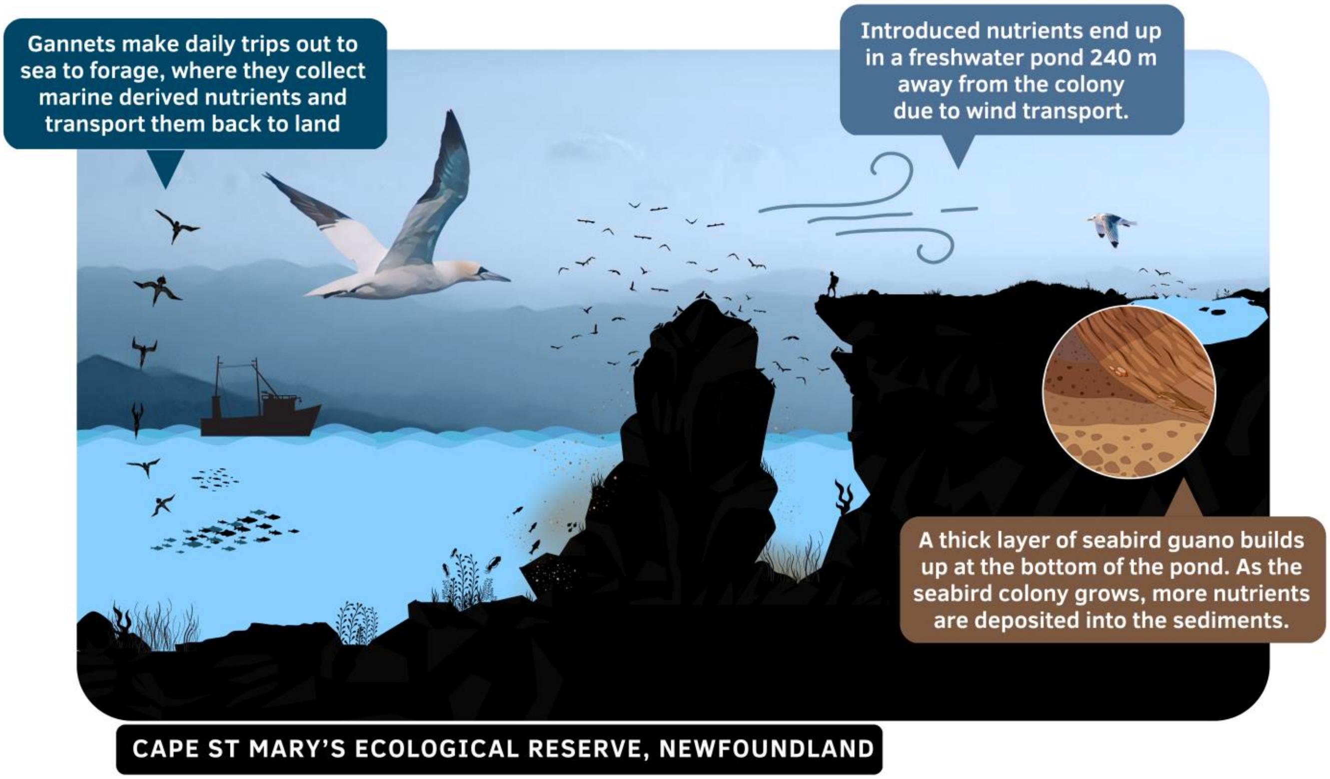

Figure AI7: Seabird nutrient transfer from Bird Rock, in Cape St. Mary's Ecological Reserve (Newfoundland) to a pond 240 m away from the seabird colony. Seen in the illustration are northern gannets (*Morus bassannus*) and black-legged kittiwakes (*Rissa tridactyla*), which both nest within the Reserve on an annual basis.

APPENDIX II

1. Supplementary files

→ File AII.1: feature ID's of uncultured genera found within heatmaps Figure 6 and Figure 7

→ File AII.2: multiQC reports from unoptimized Jan 2022 data and optimized Nov 2022 data

→ File AII.3: full review of taxonomic assignments at each taxonomic level

→ File AII.4: review of alpha-diversity metrics across sample types and groups

→ File AII.5: taxa feature counts for top 50 genera across seabird and sediment samples

→ File AII.6: collapsed feature count and taxonomy table for sediment samples (1 cm)

2. Supplementary text

We sequenced two different sets of the same fecal and sediment samples using amplicon sequencing to target the V4V5 region of the 16S rRNA gene. One set was based on PCR products that were not optimized in-house (referred to here as the 'unoptimized' set) and one set with PCR products we optimized in-house (referred to as the 'optimized' set). We used the same primers for each set and maintained the same protocols for sequencing both sets. However, for the set of samples optimized in house, we used a Q5 Hot-Start High-Fidelity 2X master mix and 0.5 µL of the universal (V4-V5^b - Forward = 515FB, Reverse = 926R) primer targets for the PCRs. We also ran several different reactions using 2 µL of DNA, 1 µL of DNA and 0.1 µL of DNA to see which would result in the best amplification for each sample. For the samples which were 'unoptimized' in-house, IMR used the high-fidelity Phusion+ polymerase and only one round of PCR was done. Six samples failed at the sequencing stage when unoptimized PCR products were used whereas none of the samples failed sequencing when our optimized PCR products were used. Overall, we observed a higher total read count when sequencing with optimized PCR products than unoptimized products (Table A1); however, we also obtained a higher percentage of duplicate reads for sequencing with optimized PCR products.

We constructed the amplicon sequence variants (ASVs) using the same protocols outlined in the methods section above. After assigning ASVs, we found that sequencing with optimized PCR products resulted in a higher number of taxonomic associations than the set of samples from the unoptimized PCR products.

3. Supplementary tables

Table AII.1 Review of sequence statistics for optimized and unoptimized sets of samples.

PCR Products	**Unoptimized**	**Optimized**
Total read count	4,883,684	7,503,593
Total unique reads	358,424	454,582
Total duplicate reads	4,525,260	7,049,011
% of duplicate reads	92%	94%
Average reads across all samples	167,481	210,299

Table AII.2 Feature counts before and after filtering unclassified ASVs and contaminants.

Sample	Feature counts (ASVs)	Feature counts after filtering contaminants and unclassified ASVs	Features lost after filtering
ATPU01	52551	52491	60
ATPU02	47621	47222	399
ATPU03	173060	172413	647
ATPU56	42252	42252	0
ATPU58	75175	75124	51
BLKI01	199403	199289	114
BLKI02	116300	116263	37
BLKI03	110304	110300	4
BLKI04	114527	114519	8
BLKI05	49288	49225	63
COMU-42	100061	99828	233
COMU43	69869	59405	10464
COMU44	43989	42989	1000
COMU46	78947	78305	642
NOGA27	116332	116161	171
NOGA30	71269	71163	106
NOGA31	100396	100360	36
NOGA32	106157	106127	30
NOGA33	87653	87282	371
REF1	23023	22523	500
REF2	25830	24778	1052
REF4	35057	33503	1554
REF8	29511	28259	1252
FOGO-0	27900	24376	3524
FOGO2	32441	28434	4007
FOGO4	33695	29720	3975
FOGO6	36734	31399	5335
IMP1	28400	27722	678
IMP2	20939	20283	656
IMP4	24389	23928	461
IMP-8	28537	27857	680
Total features before filtering			2101610
Total features after filtering			2063500
Features lost after filtering			38110
Percentage of features lost			1.81%

Table AII.3 Taxonomic assignments from the contaminants (belonging to mitochondrial or chloroplast 16S sequences) that were filtered out from the final feature table.

Feature ID	Sequence origin	Species	Sample group	Feature Count
fdf453f5b21bc752e7def06f99f3e152	Chloroplast	Chlorophyta symbiont	LFI	672
81b56ba658f5b12d98d44ec7b5028261	Chloroplast	Pseudochloris wilhelmii	CSM-I, LFI	1149
b3395b6328d5de8a7d65ce1c426fc5b3	Chloroplast	Chlorophyta symbiont	CSM-I, LFI	366
0dfde15766f9446e2f445b845b73cbe9	Chloroplast	Oscillatoriales cyanobacterium	CSM-I, LFI	76
fa36b1d619a01d4cdd25bfeda073ee9d	Chloroplast	Chlamydomonas sp.	LFI	26
0fbd7ff1852b8e2ed2653be8fcbca460	Chloroplast	Nannochloropsis gaditana	CSM-I, CSM-R, LFI	86
f0cdbabd84c160d3ddb9816461fcf070	Chloroplast	Picochlorum sp.	CSM-I, LFI	13
5c0337604432e0f04026a8be937f987f	Chloroplast	Pseudochloris wilhelmii	LFI	12
2f2385415b1e5763b5458b0f98b18ae1	Chloroplast	Mychonastes jurisii	CSM-I, CSM-R, LFI	13
1921ea120d8d437b13b8cee4b8ab1aa4	Chloroplast	Chlorophyta symbiont	CSM-I	55
ce771b89683bfd9562220d90f7d4349d	Chloroplast	Oscillatoriales cyanobacterium	CSM-I, CSM-R	323
25df4398a86af090f2126d84fa27b750	Chloroplast	Oscillatoriales cyanobacterium	CSM-I, CSM-R	98
f1547684d61a1cfb5a640af5ec9dc891	Chloroplast	Chlorotetraedron incus	CSM-I	36
8f370c18f1fe1f409a615dbd8f06ae8e	Chloroplast	Vischeria sp.	CSM-I, CSM-R	42
e14591087d2ba9437a1c162c21a2aea7	Chloroplast	Vischeria sp.	CSM-I, CSM-R	15
c56634ccf3d072c5fe173b6eb975085e	Chloroplast	Chlorotetraedron incus	CSM-I	16
1bb696bf5add2810112c65e1bee4775d	Chloroplast	Oscillatoriales cyanobacterium	CSM-I, CSM-R	21
9f50884c49f805fc94220a21726b8480	Chloroplast	Oscillatoriales cyanobacterium	CSM-I	14
025a61bb1941af93fe2e73d195fde345	Chloroplast	Koliella longiseta	CSM-I, CSM-R	10
2265ab9567c3a94a0033dde22d8f1717	Chloroplast	Ettlia pseudoalveolaris	CSM-R	18
be51d770bfe149a3b9868f74476b8772	Chloroplast	Trachydiscus minutus	CSM-R	10
fd0074bedd2ad1bee1efd35e49dda2a1	Chloroplast	Chlorophyta symbiont	CSM-R	10
aa2445d3a75f9b0db06100e8e2df914b	Mitochondria	Diplazium pycnocarpon	COMU	454
830ad3a804771337425f26c7be35bacf	Mitochondria	Picea glauca	COMU	686
70dbf659f76586b06613ab0ad97e2198	Mitochondria	Picea glauca	COMU	15

Table AII.4 Feature counts for phyla listed in Figure 4 taxa bar plot

Phyla	ATPU	BLKI	COMU	NOGA	LFI	CSM-I	CSM-R	total	total seabirds	total sediments
Firmicutes	276393	191231	235719	158227	257	259	4081	866167	861570	4597
Proteobacteria	83188	346956	5233	34753	63147	39557	11224	584058	470130	113928
Fusobacteriota	1496	32	19638	268104	8	8	10	289296	289270	26
Acidobacteriota	10	9	11	0	14207	5778	18408	38423	30	38393
Chloroflexi	280	10	226	0	5143	7618	20384	33661	516	33145
Actinobacteriota	4861	32853	14031	1422	7114	4735	6288	71304	53167	18137
Bacteroidota	22405	18422	1866	3080	4569	2877	3765	56984	45773	11211
Desulfobacterota	2	13	0	115	9963	3733	5771	19597	130	19467