BIODIVERSITY AND ECOSYSTEM FUNCTIONING IN CONTRASTING MARINE HABITATS: PATTERNS, DRIVERS, AND IMPLICATIONS FOR CONSERVATION PLANNING

by

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Abstract

The patterns and drivers of marine biodiversity and ecosystem functioning and how biological communities influence ecological processes remain poorly understood, especially for deep-sea and other remote environments. Such constraints impair effective protection of important organisms and ecosystem functions from human impacts and global change through conservation strategies such as Marine Protected Areas (MPAs). This thesis explores different aspects of biodiversity and ecosystem functioning in deep-sea sedimentary habitats, focusing on macrofaunal biodiversity and organic matter remineralization, which can be quantified through measurement of inorganic nutrient flux rates at the sediment-water interface. I examine the roles of biogenic (*e.g.*, sea pen fields) and geophysical (*e.g.*, submarine canyons) habitats along the Northwest Atlantic continental margin in regulating biodiversity and functioning. Through literature review and experimentation, I explore how biological traits of organisms influence the ecology and functioning of biological communities and can potentially inform MPA design and improve conservation outcomes. My findings demonstrate the important role of biogenic and geophysical habitats in shaping macrofaunal communities, mostly by altering food availability and creating habitat heterogeneity, and the central role of food availability in driving macrofaunal diversity at regional scales. The interacting effects of several abiotic and biotic factors that act over different spatial and temporal scales complicated efforts to discern patterns of organic matter remineralization. Some macrofaunal taxa and measures of biodiversity clearly influenced variation in benthic flux rates, reiterating the importance of biological communities in driving ecosystem processes. Biological trait expression analysis helped in understanding patterns and underlying drivers of community structure, despite poor correlations between traits and benthic flux rates, highlighting the need for further studies. The findings of this study highlight the importance of

protecting multiple ecologically important and sensitive marine habitats in order to maintain biodiversity and functions, but also punctuate the need for further studies to characterize biological traits of under-studied organisms and effectively apply trait-based approaches to improve conservation outcomes.

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List of common nomenclatures and abbreviations

AIC	Alkaike Information Criteria
AICc	Alkaike Information Criteria corrected
ANOVA	Analysis of Variance
AOI	Area of Interest
BEF	Biodiversity and Ecosystem Functioning
BIO	Bedford Institute of Oceanography
BTA	Biological Trait Analysis
CBD-UNEP	Convention on Biological Diversity-UN Environment Programme
Chl *a*	Chlorophyll *a*
CHN	Carbon, Hydrogen, Nitrogen
CHONe	Canadian Healthy Ocean Network
CCGS	Canadian Coast Guard Ship
CSSF	Canadian Scientific Submersible Facility
CTD	Conductivity/Temperature/Depth
dbRDA	Distance-based Redundancy Analysis
DFO	Department of Fisheries and Ocean Canada
DistLM	Distance-based Linear Models
DW	Dry Weight

EBM	Ecosystem-Based Management
FAO	Food and Agriculture Organization
INREST	Institut de Recherche en Environnement et en Santé au Travail
IUCN	International Union for Conservation of Nature and Natural Resources
MGS	Mean Grain Size
MPA(s)	Marine Protected Area(s)
MUN	Memorial University of Newfoundland
NAFO	Northwest Atlantic Fisheries Organization
nMDS	Non-metric Multidimensional Scaling
NOAA	National Oceanic and Atmospheric Administration
NSERC	Natural Sciences and Research Council of Canada
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
p	p-value
PA	Protected Area
PCA	Principal Component Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Homogeneity of Multivariate Dispersion
Phaeo	Phaeopigments

PSU	Practical Salinity Unit
R^2	R-squared
ROPOS	Remotely Operated Platform for Ocean Science
ROV	Remotely Operated Vehicle
SIMPER	Similarity Percentage Analysis
sp.	species
spp.	species pluralis
TL	Total Lipids
TN	Total Nitrogen
TOC	Total Organic Carbon
TOM	Total Organic Matter
Tot Pig	Total pigments
WW	Wet Weight

List of appendices

Co-authorship statement

The research detailed in this thesis was designed and conceptualized by Marta Miatta in collaboration with supervisor Dr. Paul Snelgrove and with assistance from committee members Drs. Anna Metaxas and Evan Edinger. All data were collected and analyzed by Marta Miatta, and all manuscripts resulting from this research were composed by Marta Miatta. Dr. Paul Snelgrove provided assistance with sampling and with delineating the content and structure of Chapters 1-6, and committee members provided editing assistance for Chapters 3-5 (published as Miatta and Snelgrove, 2021a, 2021b, 2022). Chapter 2 was written in collaboration with Drs. Amanda Bates and Paul Snelgrove and was published as Miatta et al. (2021).

CHAPTER 1 – INTRODUCTION AND OVERVIEW

The global ocean covers over 70% of the Earth's surface and provides almost 99% of the living space on the planet (Costanza, 1999). It encompasses an immense variety of marine ecosystems and habitats, including open oceans, seas, salt marshes, intertidal zones, estuaries, lagoons, mangroves, coral reefs, continental margins, deep seas, the sea floor, submarine canyons, hydrothermal vents, and many others (Kaiser and Roumasset, 2002). Marine systems sustain high biodiversity of organisms (Sala and Knowlton, 2006), defined as the variability of life at multiple levels of organization (*e.g*., genes, species, ecosystems); this biodiversity provides a wide range of goods and services essential for human life (Costanza, 1999; Worm et al., 2006). Beyond providing essential goods such as food, minerals, and oil (Costanza, 1999), the ocean provides vital services such as water reserve, global climate regulation, a major sink for the CO_2 produced by the burning of fossil fuels (Pachauri et al., 2014), almost half of the planet's primary production, and 70% of the oxygen we breathe (Lalli and Parsons, 1993; Muller-Karger et al., 2005).

With a world population of 7.9 billion people (World Population Clock, 2021), anthropogenic activities increasingly threaten marine ecosystems (Halpern et al., 2008; Swartz et al., 2010). Multiple anthropogenic stressors, such as resources exploitation, overfishing, aquaculture, pollution, climate change, ocean acidification, coastal erosion, habitat loss, and the introduction of invasive species all affect marine environments (Jackson et al., 2001; Duarte et al., 2007; Halpern et al., 2007; Ling et al., 2009). As a result, we now maximally exploit over 58% of the global fish stocks and overexploit 31% (FAO, 2016). More than 30% of the coral reefs are already severely damaged and close to 60% may be lost by 2030 (Wilkinson, 2002; Hughes et al., 2003). Despite its remoteness, climate change and human activities including deep-sea mining, pollution, and trawl fisheries now impact even the deep sea (Koslow et al., 2000; Ruhl et al., 2004;

Byrne et al., 2010; Keeling et al., 2010; Purkey et al., 2010; Stramma et al., 2010; Helm et al., 2011; Smith et al., 2013; van Cauwenberghe et al., 2013; Woodall et al., 2014; Danovaro et al., 2017). Both natural and human-driven changes can modify the physical, chemical, and biological properties of marine systems, altering their functioning. For example, reduction of marine biodiversity, changes in species composition, and homogenisation of habitats all affect ecosystem functioning and reduce the goods and services that human well-being relies on (Worm et al., 2006; Hewitt et al., 2008; Cardinale et al., 2012).

The depleted and degraded state of the ocean around the world and the consequent social, health, and economic impacts have prompted numerous international efforts to consider options for returning the ocean to a healthy state (Gelcich et al., 2014). Marine conservation strategies include, for example, the promotion of sustainable exploitation of marine resource (*e.g.*, through fisheries management), and marine spatial planning, such as the creation of Marine Protected Areas (MPAs; Palumbi, 2004). In the last few decades, governments have been calling for quantitative targets for ocean protection, such as increasing the extent of MPAs (CBD-UNEP, 2010), noting that less than 8% of the global ocean currently received protection (UNEP-WCMC and IUCN, 2021). Nevertheless, the need to advance ecological knowledge of marine systems as a key step toward effective conservation has become increasingly apparent. Whereas the design and implementation of MPAs have evolved from opportunistic to science-based and quantitative approaches that consider the potential benefits to fisheries and biodiversity (Leslie, 2005; Lundquist and Granek, 2005), traditional approaches still lack inclusion of important ecological information. For example, MPA designs solely based on measures of taxonomic biodiversity, endemism, or rarity and focused on protecting single species or habitats, either threatened, endangered, or economically important, often fail to achieve many conservation needs (Roberts et

al., 2003). Arguably, proactive fishery closures and the creation of no-take MPAs offer a beneficial tool to protect species, habitats, and functions from current and future threats, and might even contribute to protecting unknown organisms or processes that could have future social and economical value. Thought difficult to argue this point, we must acknowledge that such measures do not always suit socioeconomic conditions. A scenario in which humans seek to use as much ocean space and resources as possible requires wise and targeted management actions. For example, managers now recognize the essential need for knowledge of fish ecology and behaviour to manage stocks successfully (Wilen et al., 2002; Abbot and Haynie, 2012). Ecosystem-Based Management (EBM) – *the comprehensive integrated management of human activities based on the best available scientific and traditional knowledge about the ecosystem and its dynamics, in order to identify and take action on influences which are critical to the health of marine ecosystems, thereby achieving sustainable use of ecosystem goods and services and maintenance of ecosystem integrity* (OSPAR-HELCOM, 2003) – further elevates the importance of understanding ecological processes and threshold effects. The International Union for Conservation of Nature (IUCN) defines any Protected Area (PA; including MPAs) as "*a clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values*" (Dudley, 2008). By including the long-term conservation of ecosystem services provided by natural systems among PA requirements, this definition calls for better scientific knowledge of aspects of ecosystem functioning, which will enable effective management and protection of natural systems.

Nevertheless, our scientific knowledge of marine systems remains limited, especially for the most remote and inaccessible ecosystems, such as the deep sea (Kennedy et al., 2019). A large

proportion of marine species remain unknown to humans (Sala and Knowlton, 2006; Costello et al., 2010; Appeltans et al., 2012) and, even for most known species, their biology, ecology, and distribution remain poorly understood (Tyler et al., 2011; Curley et al., 2013, Menegotto and Rangel, 2018). Similarly, many geophysical (*e.g.*, submarine canyons, abyssal plains), geochemical (*e.g.*, hydrothermal vents, cold seeps) or biogenic habitats (*e.g.*, deep-sea coral reefs) remain under-explored or even unknown. Hydrothermal vents, for instance, were first discovered in the late 1970s, illustrating how our planet still has surprises to offer the scientific world (Tunnicliffe, 1991). Even more notably, a few, geographically restricted studies form the basis of most knowledge of how human activities and ecosystem change may alter key ecosystem processes (*e.g.*, organic matter remineralization) and associated goods and services (*e.g.*, carbon sequestration and global climate regulation), limiting any generalization (Gamfeldt et al., 2014). The complexity and heterogeneity of these processes can further complicate their understanding and prediction (Middelburg, 2018; Snelgrove et al., 2018). Nonetheless, effective conservation of the ocean requires scientific knowledge of species, habitats, and ecosystem processes.

1.1 The concept of ecosystem functioning

Noting the absence of a precise and standard definition (Bremner, 2008), many researchers define ecosystem functions as the physical, chemical and biological processes that transform and translocate energy or materials in an ecosystem (Naeem, 1998; Paterson et al., 2012), and define ecosystem functioning as the combined effects of individual functions (Reiss et al., 2009), that contribute to the goods and services ecosystems provide to humans (Costanza et al., 1997) (**Figure 1.1, a**). Ecosystem functioning encompasses diverse phenomena and is, therefore, difficult to describe or quantify (Hooper et al., 2005); physical, chemical, and biological processes and characteristics influence each other and the overall functionality of ecosystems (**Figure 1.1, a**).

Consequently, the most appropriate way to characterize ecosystem functioning must consider multiple variables (Duffy and Stachowicz, 2006), keeping in mind that no individual parameter can describe the functioning of entire ecosystems (Giller et al., 2004; Bremner, 2008). Researchers widely recognize the major implications of biodiversity for ecosystem functioning (Solan et al., 2004; Hooper et al., 2005) and that organisms'characteristics or properties, known as biological traits, mediate their effect on functions (Díaz and Cabido, 2001; Mlambo, 2014; Pawar et al., 2015; Wong and Dowd, 2015) (**Figure 1.1, a**). Traits refer to any measurable morphological, physiological, or phenological features of an individual that affect its performance and are influenced by its environment (Violle et al., 2007), and the evaluation of trait occurrence over biological assemblages, therefore provides a way to describe multiple aspects of ecosystem functioning (Bremner, 2008).

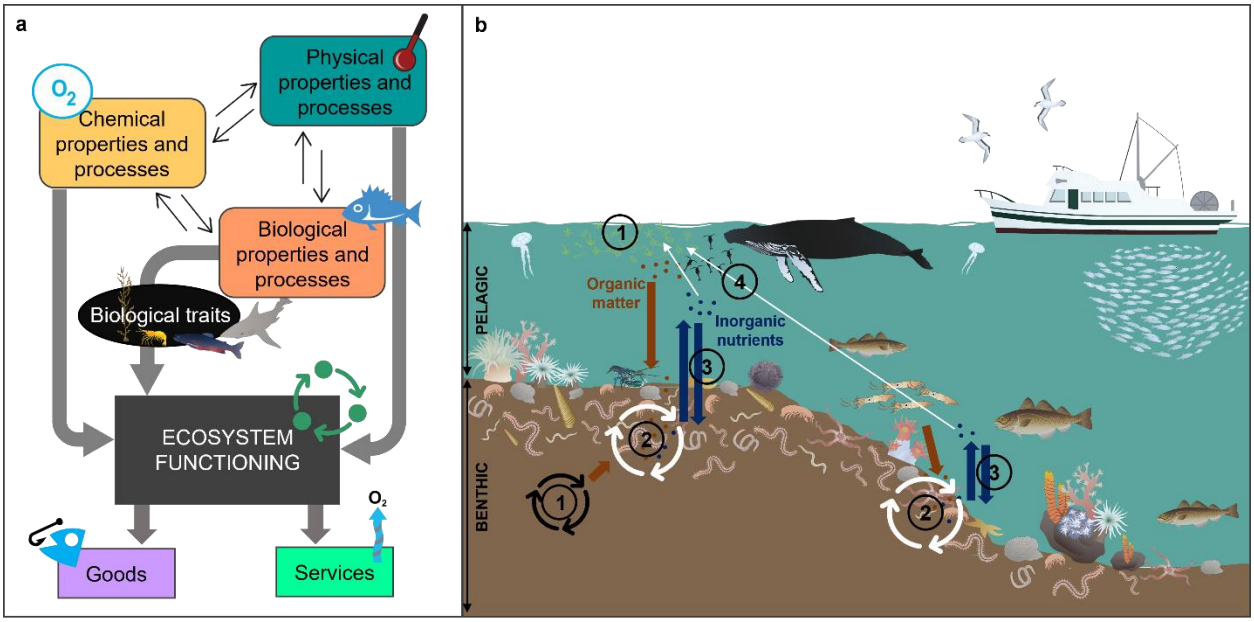

Figure 1.1: **a**) Schematic representation of the cascade of processes and properties that affect marine ecosystem functioning and contribute to the goods and services that sustain human life. **b**) Schematic representation of marine system encompassing benthic and pelagic habitats and illustrating some of the associated ecosystem processes: Primary production (1) in surface waters by photosynthesis (or in deep-sea sediments by chemosynthesis) generates organic materials that are deposited on the seafloor through vertical and lateral transport; remineralization of organic matter in marine sediments (2) results in fluxes of inorganic nutrients between the sediment and the overlying waters (3), whereupon currents eventually transport these nutrients to surface waters (4), where they fuel primary productivity (1), closing the cycle. Icons were from Integration and Application Network (ian.umces.edu/media-library) and organisms are not always scaled correctly.

1.2 Biodiversity and functioning in deep-sea sedimentary habitats

Over 63% of the Earth's surface is covered by deep-sea (beyond continental shelf depths, ~200 m) seafloor, most of which is covered by sediments (Snelgrove, 1999; Hüeneke and Mulder, 2010), making deep-sea sedimentary habitats the largest habitat on our planet. However, researchers can only access the deep sea with submersibles and remote sampling gear, making explorations logistically difficult and expensive, and leaving much of the diversity, ecology, and functioning of deep-sea environments unexplored (Snelgrove, 1999; Danovaro et al., 2014). In the past few decades, following important advances in the technologies available to access and sample

deep-sea environments (*e.g.*, remotely operated and autonomous underwater vehicles, fibre optic communications, new imagining tools, and molecular technologies), new discoveries have challenged many paradigms of deep-sea ecology and raised new scientific questions (Danovaro et al., 2014).

In the past, the lightless, cold, high-pressure, and typically food-limited characteristics of deep-sea environments led scientists to consider the deep sea a life-less desert. We now know this to be false. In reality, the deep sea hosts a high diversity (and sometimes biomass) of organisms, often exceeding those in shallow-water habitats (Sanders, 1979; Gage, 1996). Most deep-sea diversity resides in sediments (Rex, 1983), where macrofauna - here defined as animals sufficiently largeto retain on a 300-µm sieve (Snelgrove, 1998) - typically dominates the biota in terms of biomass. Polychaete worms typically dominate macrofaunal communities in marine sediments, which also include crustaceans, molluscs, and many other phyla, and some authors suggest that macrofauna might represent the most diverse metazoan assemblage on Earth (Snelgrove, 1998). Ecologically, macrofaunal communities are important secondary producers in marine food webs that contribute significantly to sedimentary processes through their ability to alter the physical and biogeochemical properties of marine sediments (Braeckman et al., 2010; Kristensen et al., 2012; Mermillod-Blondin, 2011). Several theories attempt to explain high deep-sea biodiversity, including the role of environmental stability and long-term competition that can facilitate speciation (Sanders, 1968, 1979), as well as predation pressure that can reduce the importance of dominant species (Dayton and Hessler, 1972), both resulting in increased local biodiversity. More recently, the patch mosaic theory proposed by Grassle and Sanders (1973), attributed high diversity in the deep sea to small-scale patches of organic matter (*e.g.*, sinking phytodetritus or carcasses) and disturbance (*e.g.*, organism activity that alters the environment, presence of biogenic

structures) that create a mosaic of micro-environments that allow the coexistence of different species and life stages (Jumars et al., 1990; Snelgrove, 1998; Snelgrove and Smith, 2002). On larger scales, the presence of various geologic, chemical, or biogenic features such as submarine canyons and seamounts, chemosynthetic environments, and deep-sea coral and sponge reefs, creates habitat heterogeneity and complexity that also contributes to high biodiversity (Levin and Dayton, 2009; McClain and Barry, 2010; Zeppilli et al., 2012; Danovaro et al., 2014). For example, numerous studies reported increased density and diversity of organisms and distinct communities in submarine canyons (De Leo et al., 2010; Levin and Sibuet, 2012; De Leo et al., 2014; Robertson et al., 2020), in relation to their role as conduits for organic matter that increase food availability in the food-deprived deep sea (Levin et al., 2010; Harris and Whiteway, 2011; Puig et al., 2014; Amaro et al., 2015). However, contrasting findings and high heterogeneity of processes (Bianchelli et al., 2008: Pusceddu et al., 2010) highlight the need for more studies to clarify biodiversity patterns in these habitats. Similarly, deep-sea coral reefs sustain a diversity of species that rivals tropical coral reefs, as suggested by some authors (Buhl-Mortensen and Mortensen, 2005; Baillon et al. 2012; Baillon et al. 2014). However, only a few studies have focused on these biogenic habitats, usually limited to specific geographic locations and assemblages, leaving many other biogenic habitats (*e.g.*, sea pen fields) largely unexplored.

Besides hosting high biological diversity, deep-sea sediments play important functional roles and provide invaluable goods and services. For example, researchers recognize oxygenated deep-sea sediments as regions of rapid diagenesis of organic materials, where regeneration of inorganic nutrients that fuel primary production occurs (Danovaro et al., 2014; Strong et al., 2015). Photosynthesis in the photic zone produces most of the organic materials that sustain heterotrophs in the deep sea and reach the seafloor through vertical or lateral fluxes (Danovaro et al., 2014).

However, researchers now recognize the important contribution of autochthonous carbon fixation by chemoautotrophs, a process not limited to hydrothermal vents or cold seeps, but occurring widely in deep-sea sediments (Danovaro et al., 2014). For instance, chemosynthetic primary production by benthic Archaea may account for up to 20% of total heterotrophic biomass production in the deep sea (Molari et al., 2013). The remineralization of various organic materials in deep-sea sediments plays a major role in marine ecosystem functioning (**Figure 1.1, b**), driving benthic-pelagic coupling (Snelgrove et al., 2014) and influencing global cycles of carbon, nitrogen, silica and other elements, as well as global climate and productivity (Meysman et al., 2006; Danovaro et al., 2014; Snelgrove et al., 2018). Microbes play a primary role in converting organic matter into inorganic forms (Jorgensen, 2006). However, larger organisms such as meio-, macro-, and mega-fauna also affect the rates and efficiency of organic matter remineralization, both directly and indirectly. Faunal activities such as burrowing, feeding, excretion, and ventilation (Aller, 2001; Welsh, 2003; Lohrer et al., 2004; Meysman et al., 2006) alter the properties of sediments and organic matter particles, affecting how effectively microbes remineralize organic matter (Laverock et al., 2011; Mermillod-Blondin, 2011). They also alter the fluxes of particles, including inorganic nutrients, between sediments, porewater, and the overlying water through bioturbation and bioirrigation activities (Aller, 1988; Kristensen and Andersen, 1987; Huettel and Gust, 1992; Kristensen and Holmer, 2000; Heilskov et al., 2006; Meysman et al., 2006). Organic matter remineralization also depends on other factors such as the input of organic materials, temperature, and oxic conditions, among others (Berelson et al., 1996; Jahnke, 1996; Link et al., 2013; Alonso-Pérez and Castro, 2014; Belley et al., 2016). Organic matter remineralization can be quantified effectively by measuring fluxes of oxygen and/or inorganic nutrients at the sediment-water interface (Giller et al., 2004) through *in situ* benthic chambers deposited on the seafloor

(Devol and Christensen, 1993; Berelson et al., 1996; Berelson et al. 2013), or *ex situ* incubations of sediment cores (Rowe and Phoel, 1992; Link et al., 2013; Belley et al., 2016). Some studies that estimated organic matter remineralization in deep-sea sediments highlighted the complexity of these processes, apparently controlled by a variety of abiotic and biotic factors, often interacting with each other at different spatial and temporal scales (Aller, 1994; Thompson et al., 2017). Better understanding and prediction of benthic processes to generalize patterns at large scales therefore requires larger-scale and more extensive studies and manipulative experiments (Snelgrove et al., 2014). A better understanding of these processes will also clarify how current and future changes may impact these cycles and how marine management can act to mitigate change and preserve the functionality of marine systems.

1.3 Format and content of this thesis

This study explores aspects of the biodiversity and functioning of contrasting marine habitats and discusses how better ecological knowledge of these systems can inform marine conservation strategies (*e.g.*, MPA design) and improve conservation outcomes. I arrange this thesis in 6 chapters, including this introduction and overview (Chapter 1). In Chapter 2, I review the literature to provide a synthesis of how biological traits of organisms can elucidate community dynamics and patterns, ecosystem functioning, and vulnerability of organisms and communities to anthropogenic impacts. Building from these ideas, I discuss how conservation strategies can incorporate trait-based approaches to improve their effectiveness in protecting marine systems, drawing on examples from different marine and terrestrial habitats. This chapter was published in *Annuals Review of Marine Science* as Miatta et al. (2021). Chapters 3-5 report original research data and were written as stand-alone manuscripts for publication in peer-reviewed scientific journals according to the journals' formatting requirements. For this reason, some repetition of

materials occurs among chapters, particularly in the Methods sections. The first two data chapters (Chapters 3 and 4) explore different aspects of the ecology deep-sea sedimentary habitats within the Laurentian Channel Marine Protected Area (MPA), a recently designated large MPA located on the edge of the continental shelf between Newfoundland and Nova Scotia, in Eastern Canada. In these two chapters, I investigate for the first time the link between Pennatulacean octocorals (sea pens), macrofaunal communities, and organic matter remineralization in sedimentary habitats. This study aims to fill knowledge gaps on the ecological role of these important and under-studied habitat-forming organisms. Both these studies, which were part of a bigger collaborative project sponsored by the NSERC Canadian Healthy Oceans Network (CHONe), also aim to evaluate and inform conservation strategies for the Laurentian Channel MPA and propose cost-effective monitoring protocols for this and other similar MPAs. Specifically, in Chapter 3, I compare macrofaunal density, taxonomic diversity, vertical distribution, community composition, and biological trait expression in sea pens fields versus bare sedimentary habitats, as well as in cores containing sea pen specimens versus other cores, in order to assess the effect of sea pens at large and small scales. I also explore the contribution of a wide range of environmental variables, including sedimentary organic matter quantity and quality, mega-epifauna densities and physico-chemical variables to identify the main drivers of variation of macrofaunal community composition and biological trait expression. Chapter 3 was published in *Deep-Sea Research Part I*: *Oceanographic Research Paper* as Miatta and Snelgrove (2022). In Chapter 4, I explore patterns and drivers of organic matter remineralization by measuring inorganic nutrient fluxes at the sediment-water interface. I explore the role of sea pens for benthic nutrient fluxes, at both large and small scales, and I explore environmental and biological drivers of ecosystem functioning (organic matter remineralization) variability within the Laurentian Channel MPA. Chapter 4 was

published in *Deep-Sea Research Part I*: *Oceanographic Research Papers* as Miatta and Snelgrove (2021a). Chapter 5 contrasts deep-sea sedimentary habitats along the Northwest Atlantic continental margin, including continental shelf, slope, submarine canyons and inter-canyon areas. Specifically, I characterize patterns of sedimentary organic matter quantity and quality, benthic nutrient fluxes and macrofaunal diversity, and explore how these variables change among habitats, relate to each other, and link to environmental factors. This study represents the first attempt to contrast organic matter remineralization, through the measurement of benthic nutrient fluxes, in canyon and inter-canyon habitats, as well as the first examination of macrofaunal communities in inter-canyon sediments. In contrast to the previous chapters, the habitats sampled in Chapter 5 offer a wider range of environmental conditions (*e.g.*, depth, sedimentary properties), therefore helping to understand how these factors affect diversity and functioning. Chapter 5 was published in *Frontiers in Marine Science* as Miatta and Snelgrove (2021b). Finally, in Chapter 6 I combine all the data collected for this thesis to consider regional-scale drivers of macrofauna and benthic nutrient fluxes, and I also provide overall conclusions, highlighting the key findings of my doctoral research and the overall significance of this work to marine ecology and conservation. Finally, I suggest some areas of future research.

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CHAPTER 2 – INCORPORATING BIOLOGICAL TRAITS INTO CONSERVATION STRATEGIES*

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2.1 Abstract

Implementation of marine conservation strategies, such as increasing the numbers, extent, and effectiveness of protected areas (PAs), can help achieve conservation and restoration of ocean health and associated goods and services. Despite increasing recognition of the importance of including aspects of ecological functioning in PA design, the physical characteristics of habitats and simple measures of species diversity inform most PA designations. Marine and terrestrial ecologists have recently been using biological traits to assess community dynamics, functioning, and vulnerability to anthropogenic impacts. Here, we explore potential trait-based marine applications to advance PA design. We recommend strategies to integrate biological traits into (*a*) conservation objectives (*e.g.*, by assessing and predicting impacts and vulnerability), (*b*) PA spatial planning (*e.g.*, mapping ecosystem functions and functional diversity hot spots), and (*c*) time series monitoring protocols (*e.g.*, using functional traits to detect recoveries). We conclude by emphasizing the need for pragmatic tools to improve the efficacy of spatial planning and monitoring efforts.

2.2 Introduction

Biological (or functional) traits represent morphological, biochemical, physiological, structural, phenological, behavioural, and ecological characteristics of organisms, whether individuals or species (Diaz and Cabido, 2001). Attributes such as motility, body size, life span, trophic mode, reproduction modalities, and habitat characterize the ecological roles that organisms

play (Diaz and Cabido, 2001; Violle et al., 2014) and interactions between individuals and species and the environment (Lefcheck et al., 2015). Traits are therefore useful to infer the responses of organisms to their environment and the effects of organisms on ecosystem processes (Violle et al., 2007; Nock et al., 2016). Functional diversity metrics are designed to capture the number, type, and distribution of biological functions across organisms (Diaz and Cabido, 2001), with the potential to advance a mechanistic understanding of how ecological communities assemble and function. Both individual and species traits can underpin metrics of functional diversity – indices that describe the total variation in one or more traits across all species within a community (Faith, 1996). Individual traits describe variation across populations – for example, by quantifying the average or range of trait values (such as individual body size) across all individuals, whereas species traits (such as maximum body size) typically integrate across populations.

Biological trait-based approaches can clarify the mechanisms underlying the dynamics of ecological communities (Dray et al., 2014) and link species geographical distributions to their environment (Usseglio-Polatera et al., 2000; Bremner et al., 2006b; Jeliazkov et al., 2020). Functional trait diversity metrics also enable deeper insights into the role of diversity in the provisioning of ecosystem functions and services (Norberg, 2004; Hooper et al., 2005; Vogt et al., 2010; Tavares et al., 2019) and the resilience of biological communities (Walker et al., 1999; Petchey and Gaston, 2002). Indeed, numerous studies indicate that trait-based functional diversity indices predict ecosystem functioning more effectively than taxonomic diversity (*e.g.*, Diaz and Cabido, 2001; Bremner et al., 2003; Hooper et al., 2005, Anton et al., 2010; Loreau, 2010; Schleuter et al., 2010; Mouillot et al., 2011; Mora et al., 2015; Törnroos et al., 2015; Villnäs et al., 2018). Biological traits of organisms ultimately link to ecosystem functions, including ocean nutrient cycling (Norkko et al., 2013, Belley and Snelgrove, 2016), primary production (Lohrer et

al., 2015), secondary production (Bolam and Eggleton, 2014), and sediment erodibility (Harris et al., 2015).

Biological trait-based approaches incorporate diversity, functions, and responses to environmental conditions. These approaches, therefore, characterize populations, communities, and processes simultaneously in a framework that can inform conservation strategies through numerous pathways. Increasingly, Protected Areas (PAs), including Marine Protected Areas (MPAs), marine reserves, and parks, are recognized as a key management tool to achieve sustainable use of resources and to preserve and restore ocean health (Gaines et al., 2010; Veitch et al., 2012). Global prioritization of protection punctuates the need to improve our capacity to prioritize which species, processes, and regions to protect, and to track progress following conservation actions. Many countries now strive to increase the number and extent of PAs to meet international targets. For example, the Aichi Targets developed by parties to the Convention on Biological Diversity aim to expand the protection of the ocean to 30% by 2030. Efforts must prioritize the creation of well-designed and well-managed PAs that effectively protect and conserve natural systems (De Santo, 2013; Roberts et al., 2018). PA success in achieving conservation objectives hinges on incorporating clearly defined ecological criteria and scientific information at the design stage, rather than primarily socioeconomic factors (Roberts et al., 2003). The criteria also need to advance beyond simple consideration of individual species or features of habitats and taxonomic diversity, such as species richness, endemism, and vulnerability (Brooks et al., 2006; Jenkins et al., 2013). Focusing on species or habitats may underrepresent biologically unique species and their ecological roles within systems (Brum et al., 2017) and does not address how the suite of ecosystem components responds to different threats (Bremner, 2008).

Incorporating approaches that consider the biological traits of organisms in PA design and evaluation increases the potential to achieve greater conservation gains (Bremner et al., 2006a,b; Bremner, 2008; Frid et al., 2008; Strecker et al., 2011; Brum et al., 2017). Specifically, biological traits can improve marine conservation efforts (Bremner, 2008; Vandewalle et al., 2010) by providing information on the diversity, structure, and dynamics of ecological communities and the associated ecological functions (Usseglio-Polatera et al., 2000). Trait-based approaches also complement biodiversity patterns based on taxonomic diversity, including identification of new hot spots of diversity (Stuart-Smith et al., 2013), and can help predict the responses of biological communities to current and future human impacts, including fishing and climate change (Bremner et al., 2004, 2006b; Darling et al., 2010; Beauchard et al., 2017).

Trait-based metrics in PA design build on the use of ecosystem-based management approaches that establish PAs to protect specific functions and associated services (Foley et al., 2010; Degen et al., 2018; Villnäs et al., 2018). In addition, metrics based on biological traits provide a common currency of diversity that transfers across species and habitats (Vandewalle et al., 2010), enabling comparisons of functional biodiversity across geographic locations (Statzner et al., 2001; Hodgson et al., 2005). Therefore, trait-based approaches offer a valuable tool to identify ecologically important areas, PA boundaries, and conservation objectives (Frid et al., 2008) and even to develop monitoring protocols. However, despite the potential applicability of biological traits in conservation, there are few examples of the use of trait-based approaches to develop conservation strategies and inform PA design. Challenges include a lack of rigorous trait data for many species and logical methods that include traits (Lefcheck et al., 2015); these deficiencies may limit uptake by scientists and managers.

Here, we explore the potential for trait-based applications to advance marine spatial planning efforts. We begin by providing a general overview of biological traits in the context of conservation (**Figure 2.1**). We review the uses of biological traits in PA design and monitoring protocols, highlighting how management can use biological traits to help improve the efficacy of PAs to meet conservation objectives. We recommend integrating traits into conservation strategies, including assessments of the sensitivity and vulnerability of communities to human impacts, predictions of species extinction risk, and the identification of resilience in natural systems. Traits can also inform PA spatial planning in mapping new hot spots of diversity and functions and in developing monitoring protocols that are responsive to population and community change. We further explore the advantages of trait-based approaches over traditional taxonomic approaches. We then address the challenges of transferring concepts and approaches developed on land to the ocean, recognizing intrinsic differences between the realms and logistical difficulties specific to marine conservation. We conclude by emphasizing the importance of including functional diversity metrics based on biological traits in PA planning and the need to identify pragmatic tools applicable to different contexts to support spatial conservation planning and monitoring efforts.

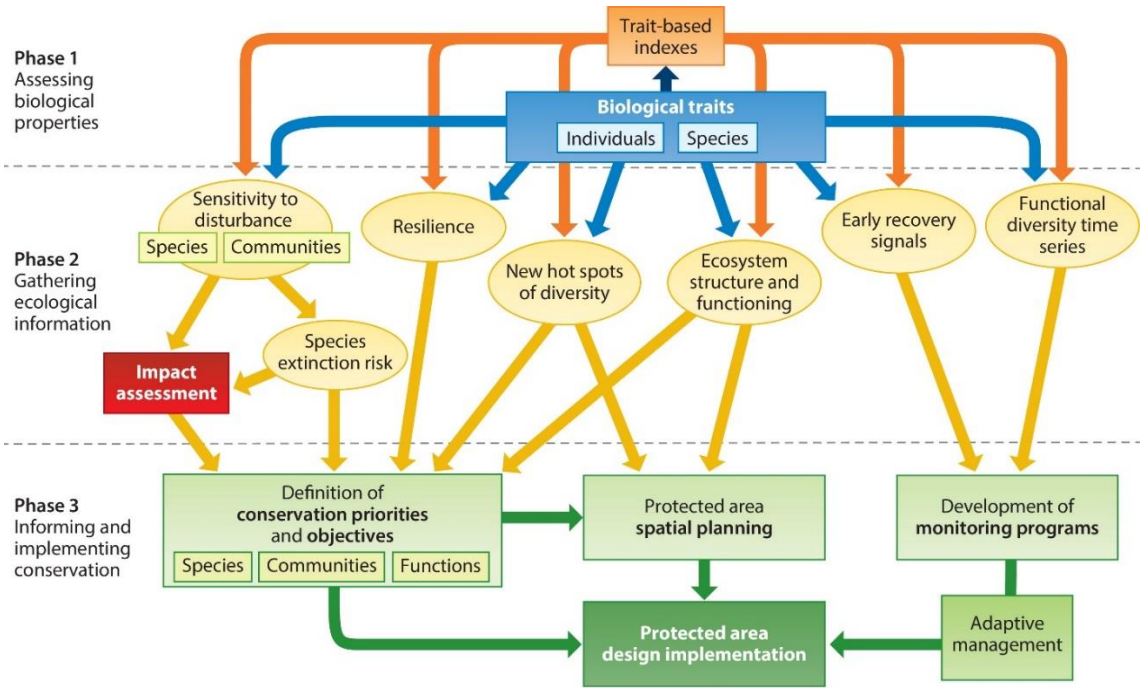

Figure 2.1: Phases for integrating biological traits into conservation strategies. These phases include the assessment of biological properties of populations and communities (**phase 1**); the gathering of ecological information on the vulnerability, structure, functioning, and responses of systems (**phase 2**); and the implementation of protected area design by informing prioritization, spatial planning, and monitoring protocols (**phase 3**).

2.3 Common approaches for incorporating biological traits in ecological studies

In the last 50 years, scientists have used biological traits of organisms to characterize biological communities and gain insights into how communities function. In the early 1970s, plant ecologist J. Philip Grime (1974) incorporated traits of species to classify vegetation based on species strategies and to predict disturbance levels at particular sites. Subsequently, Southwood (1977) formulated the habitat templet theory, hypothesizing that the characteristics of the habitat select and favour specific sets of individual characteristics that determine which organisms persist. Southwood's work inspired other scientists to use traits in terrestrial and marine ecology. Traits have also been used to predict species distributions and community structure among habitats and along environmental gradients (Bournard et al., 1992; Townsend and Hildrew, 1994; Townsend et

al., 1997). For instance, Charvet et al. (1998a,b) used traits of macroinvertebrates to develop biomonitoring tools in freshwater streams by linking the trait patterns of a diverse set of species to environmental data.

Functional indices, such as functional diversity (the variation in one or more biological traits across all species within a community; Faith, 1996), offer one way to incorporate biological traits into ecological studies. Other traditional functional indices include metrics based on the sum (Walker et al., 1999) or average (Botta-Dukát, 2005) of functional distances between species pairs in multivariate functional trait space, the distances between species along hierarchical classification (*e.g.*, Petchey and Gaston, 2002), or the distribution of abundance along functional trait axes (*e.g.*, Mason et al., 2003).

The scientific community lacks a consensus on which index to use to quantify functional diversity because functional diversity encompasses at least four components. Some researchers argue that the first three – richness, evenness, and divergence – require separate quantification (Mason et al., 2005; Villéger et al., 2008). Functional redundancy adds a fourth dimension of diversity to trait analysis: species with overlapping ecological roles in communities and ecosystems (Lawton and Brown, 1993). Indeed, the presence of many functionally similar species in a given habitat, known as the portfolio effect (Doak et al., 1998), may protect against functional loss where changes in the community impact one or more species.

In some cases, functional group richness is used as a surrogate of functional diversity (even though richness is acknowledged as representing only one dimension of function diversity). Species are assigned to functional groups based on a few general and well-known key attributes, and specialists estimate the frequency of these categories through indices such as the Shannon or Simpson index (Stevens et al., 2003; Villéger et al., 2008). However, this approach ignores species

abundances and functional differences among species (Fonseca and Ganade, 2001; Villéger et al., 2008). Alternatively, some studies define subgroups of traits that describe specific functions or ecosystem properties and use them to estimate the cumulative expression of ecosystem properties at individual sites and, at larger scales, ecosystem multifunctionality, which refers to multiple, interacting functions in an environment (Villnäs et al., 2018). Other approaches use multiple functional traits (*e.g.*, body size, mobility, feeding strategy, habitat, and reproductive mode) measured for each individual or species and statistical techniques such as principal component analysis or co-inertia analysis to distinguish different communities and their associated functions (Bremner et al., 2003; Petchey and Gaston, 2006). Such approaches may add significant challenges in obtaining specific trait values for every species in a community, especially rare species, but offer a more complete understanding (Schleuter et al., 2010). In fact, analyses that include only some of the species that are present in a system and only a few traits (*e.g.*, feeding type or physiological tolerance) by definition exclude potentially important ecological information (Charvet et al., 1998b).

Biological trait analysis (BTA; Bremner et al., 2003; Bremner et al., 2006b) offers a comprehensive approach to describe multiple aspects of functioning based on features of the biological communities, links traits to environmental conditions, and predicts associated changes in ecosystem processes performed by communities (*e.g.*, nutrient regeneration and processing of pollutants in marine sediments). The analysis uses multivariate ordination to describe patterns of trait composition across all species within the entire assemblages, incorporating both multiple traits and multiple trait categories. While species are typically scored as falling within one trait category (*e.g.*, feeding type has categories of deposit feeder, filter/suspension feeder, opportunist/scavenger, and predator), fuzzy coding allows species (or the taxonomic unit of interest) to fit into more than

one trait category. Fuzzy coding, therefore, quantifies the affinity of a species across different categories of a trait. The analysis can then include the abundance or biomass of species as a weight and empirically describe differences in ecological strategies or functioning among communities. The traits that contribute most to these differences can subsequently be identified and linked with other variables, such as environmental parameters and anthropogenic stressors.

Biological traits also vary among individuals, sometimes even more than among species. Intraspecific trait variability results from either phenotypic plasticity or genetic diversity, where selection filters lead to population adaptations to local environments (Jung et al., 2010) and substantial shifts in ecological dynamics (Whitlock et al., 2007; Bolnick et al., 2011; de Bello et al., 2011; Lefcheck et al., 2015). Indeed, some species exhibit high intraspecific trait variability. For example, variation in several traits of European freshwater fish species, such as growth rate, mortality rate, and length of the breeding season, is greater among populations of the same species across latitudes than among species (Blanck and Lamouroux, 2006). Some species exhibit much higher intraspecific variability in traits than others and, more predictably, variability among ontogenetic stages of a species. For example, individual body size predicts the trophic level of fishes better than species identity (Jennings et al., 2001).

Depending on the specific conservation context, it may be important to consider population-level trait variability. For instance, obtaining functional trait estimates from existing data that represent average values across the full range of conditions under which a species occurs may miss important intraspecific variation, and thus under- or overestimate sensitivity. In fact, species with high population-level diversity may respond and adapt more successfully to environmental changes and therefore be more resilient to impacts. The importance of considering trait variability at the population level is particularly true if intraspecific variability is high or

subpopulations are adapted to local conditions (*e.g.*, Atlantic cod; Hu and Wroblewski, 2008). Conversely, average species traits derived from small-scale (single-environment) studies may not account for altered phenotypes driven by local adaptations and therefore may inaccurately describe populations from different geographic areas (Bolnick et al., 2011; Tucker and Cadotte, 2013).

The expression of biological traits can also vary within species over time, in response to natural or human-driven changes in environmental conditions (*e.g.*, in response to fishing pressure; Jennings and Kaiser, 1998). Incorporating intraspecific trait variability into trait-based community ecology may advance the theory on the ecological filtering processes that occur during community assembly by identifying which traits are successful (Jung et al. 2010). Intraspecific trait variability may also offer insights on stability and functionality (Bolnick et al., 2011). Indeed, authors now consider the functional variability of individuals within populations when interpreting ecological dynamics such as community composition, fitness, and competition (*e.g.*, Bürger, 2005; Kopp and Hermisson, 2006; Vellend, 2010; Bolnick et al., 2011).

Ultimately, integrating traits of individuals across species to describe communities offers the most accurate way to describe functional diversity and explain functional processes (Cianciaruso et al., 2009). This objective requires measuring traits from all individuals within a community during each sampling event (Cianciaruso et al., 2009), usually requiring prohibitive labour (Baraloto et al., 2009). A less intensive solution (de Bello et al., 2011) involves sampling a subset of the existing population of each species to capture representative trait distributions. Fuzzy coding approaches (described above) allow variation in trait categories within taxa (Chevenet et al., 1994) and partially incorporate such variation into the analysis when such variability is known. If the goal is to describe a community functionally, then individual trait expression can even be assessed without first identifying species taxonomically, such as in cases where similar species are

difficult to distinguish. The nonnecessity for taxonomic expertise may, therefore, offer an advantage in terms of time and cost. In addition, the lack of available data on traits for many species - or, more often, inaccuracy for certain populations - may require direct measurements of individual traits in populations of interest. By contrast, the difficulty or even impossibility of measuring some individual traits on preserved specimens may require drawing opportunistically from published data to attribute scores to traits representing, for instance, reproduction behaviour, life span, and feeding habits. However, taxonomic biases in basic biological information limit mining the literature to well-studied species, such as most fishes and some macroinvertebrates. Therefore, a mix of new observations, expert opinions, and published data could yield robust information (Chapman et al., 2019).

2.4 The rationale for the use of biological traits in marine conservation

2.4.1 Traits to identify conservation priorities and inform conservation objectives

PA planning usually begins with the creation of databases and maps of ecological, physical, and socioeconomic characteristics of the region of interest. This information then underpins setting conservation objectives (**Figure 2.1, phase 3**), such as quantitative assessments of the minimum amount of a feature that needs to be conserved (Magris et al., 2017), including empirical targets for ecological outcomes (Game et al., 2013; Pressey et al., 2015). Conservation objectives define one or more feature targets for a PA, which can include sustaining species, biodiversity, ecosystem types, and functioning (Magris et al., 2017).

Traditional approaches used to inform conservation objectives fail to capture important aspects of functional diversity and ecosystem functioning. When developing conservation objectives in an area of interest, managers typically use taxonomic indicators, such as species richness and the presence of endemic and/or threatened species. This information is then combined

with socioeconomic data on the level of human use and impact. For example, one conservation objective might focus on identifying the species that are most vulnerable or resilient to specific threats. Neglecting biological traits in this assessment, by definition, omits important ecological context (Moretti and Legg 2009; de Bello et al., 2010).

While no single parameter effectively describes the functioning of an entire ecosystem (Giller et al., 2004; Rees et al., 2012), biological traits offer advantages over traditional taxonomic approaches. First, traits represent information about the sensitivity of species and communities to disturbance (**Figure 2.1, phase 2**). Traits can predict species and community responses to changes because biological traits link directly to the fitness of organisms (Vandewalle et al., 2010). Therefore, species with traits that increase vulnerability to a given disturbance and/or lower trait redundancy typically dominate sensitive communities (Walker, 1992; Williams et al., 2010; McLean et al., 2019a). Biological traits can also help researchers distinguish changes related to niche effects caused by anthropogenic or environmental stressors from random effects associated with natural community variability. This possibility exists because a disturbance tends to exclude or reduce the abundance of species with particular traits (Haddad et al., 2008). For example, McLean et al. (2019a) documented the high sensitivity of small pelagic and corallivorous fishes to ocean warming, where the trait structure and redundancy of temperate and tropical fish communities determined their respective sensitivities to a disturbance. Their study was among the first to use temporally and spatially expansive data, verifying the validity of considering biological traits to predict future disturbance impacts on biodiversity and ecosystem functioning across ecosystems and taxa (McLean et al., 2019a).

Second, based on the same principles that determine sensitivity, individual and species traits can help predict the extinction risk of populations and species (**Figure 2.1, phase 2**). These

predictions can inform priorities for conservation, including species or communities in locations not yet threatened, perhaps because current levels of anthropogenic pressure have not reached a critical level (Cardillo et al., 2006; Van Kleunen and Richardson, 2007; Cooke et al., 2019). These approaches offer the important advantage of distinguishing vulnerable species and detecting disturbance-related changes before local extinction occurs (Mouillot et al., 2013). For example, Cooke et al. (2019) used biological traits of more than 15,000 land mammals and birds to quantify current and predict future ecological strategies. Based on species' extinction probabilities, they predicted a change in strategies over the next 100 years, where mammal and bird species will shift toward becoming small, fast-lived, highly fecund, insect-eating generalists.

Third, traits can provide information on the capacity of natural systems to resist and recover after disturbances (*e.g.*, resilience), which also merits consideration when defining conservation objectives (**Figure 2.1, phase 2**). Management decisions should prioritize safeguarding ecological resilience in order to create PAs that maintain desirable ecosystem states, even under changing environmental conditions (West and Salm, 2003; Mori et al., 2013). High trait diversity or redundancy in communities usually indicates higher resilience to disturbances and invasions, whereas species diversity alone is not always a good indicator of resilience (Dukes, 2001; Bellwood et al., 2004; Bates et al., 2019). In a study carried out in the Amazon raiforest, Bregman et al. (2016) analyzed the functional trait structure of bird assemblages to explore the impacts of land-cover change on two ecosystem processes that maintain the structure and resilience of human-modified tropical forests, namely seed dispersal and insect predation. Their findings suggest that the loss of tropical forests reshapes the types of species that are present in the landscape, with dramatic consequences for ecosystem processes and resilience. They concluded that standard approaches used to understand environmental change based solely on species richness and

composition may overlook important implications for ecosystem processes that functional diversity indices effectively capture (Bregman et al., 2016).

Fourth, biological traits can help to identify functionally important locations for protection (**Figure 2.1, phase 2**) in order to conserve biodiversity hot spots with species that play different ecological roles, and therefore functions and services (Frid et al., 2008; Stuart-Smith et al., 2013). For instance, comparisons of fish biodiversity distribution based on traditional taxonomic metrics with those based on novel biological trait metrics identified new hot spots of functional diversity for reef fish in locations rarely prioritized for conservation, such as temperate rocky reefs (Stuart-Smith et al., 2013). Moreover, forecasts on how environmental change and anthropogenic activities impact ecosystem functions and associated goods and benefits provide a framework for informing PA designs that explicitly incorporate measures of ecosystem functioning where the intent is to protect or restore functional diversity and ecosystem services (Bremner, 2008; Frid et al., 2008). Yet explicit consideration of functioning in conservation planning has historically lacked the fundamental science to support such an approach (Frid et al., 2008; Beauchard et al., 2017). In some circumstances, PAs originally designated to protect specific features of habitats and biodiversity also fortuitously protect ecosystem functions and services. However, this is not always the case. For example, locations designated to protect rare or threatened species may rank low in overall functional importance in comparison to other habitats or locations (Potts et al., 2014). In other cases, management measures may not adequately conserve species that critically underpin functionally important PAs. For example, higher taxonomic diversity may not always coincide spatially with higher functional diversity and ecosystem functioning (Stuart-Smith et al., 2013).

Fifth, it is possible to generalize indicators based on biological traits (Vandewalle et al., 2010), enabling biodiversity comparisons among regions differing in biogeography, communities, and species composition (Statzner et al., 2001). As a result of environmental filtering, communities tend to be composed of species with similar traits (Poff, 1997; Statzner et al., 2001; Bremner et al., 2006b; Hewitt et al., 2008; Tolonen et al., 2016), which therefore leads to similar responses to the same anthropogenic stresses in communities from different regions (Statzner et al., 2001). Indeed, McLean et al. (2019b) showed temporal trait convergence in North Sea fish communities in response to ocean warming, despite divergence in species composition. This convergence in species with similar traits enables the development of general models of population and community dynamics and distributions of organisms within their environment that have widespread applicability (Blanck and Lamouroux, 2006; McGill et al., 2006; McLean et al., 2019b). Therefore, indicators based on traits can be particularly useful for environmental policies implemented across large geographic areas, where differences in species composition could otherwise complicate traditional, species-based assessments (Bremner et al., 2003; Villnäs et al., 2018). Examples include designing large PAs that encompass multiple biogeographic realms, comparing biodiversity among regions in order to define conservation priorities, and tracking how these communities develop following protection and with exposure to disturbance events.

Following the identification of priorities and objectives for conservation, spatial planning of PAs (**Figure 2.1, phase 3**) requires defining details such as PA size, boundaries, and allowed activities. Planning stages should aim to include conservation priorities and meet specific objectives. Ideally, PAs should be as large as possible (Edgar et al., 2014). Networks of PAs are critical to protecting habitats important for different life history stages and population connectivity (Cowen and Sponaugle, 2009; Burgess et al., 2014). Traditionally, PA spatial planning has focused

on the spatial distribution of key species or habitats, despite the dynamic and ecologically connected nature of marine systems, in which ecological processes extend across physical habitat boundaries (Frid et al., 2008). In marine systems, biological assemblages may depend on processes occurring elsewhere (Balbar and Metaxas, 2019). For example, distant locations may play a central role in supplying food, nutrients, and reproductive propagules, and migratory residents that leave the PA may be at risk (Morris et al., 2014). Therefore, the simple protection of physical habitats can be insufficient to preserve processes, and biological traits can help in identifying ecologically relevant locations for specific conservation objectives (Frid et al., 2008).

PA zoning should maximize protection for ecologically critical species, communities, and processes (Agardy, 2000). A case study from Bremner et al. (2006a) illustrates a protocol and practical application of biological traits in proposing PA boundaries (**Figure 2.2**). They used biological trait analysis based on species to explicitly incorporate the ecological structure and functioning of benthic communities in delimiting PA boundaries. They described multiple aspects of functioning using features of the biological ecosystem components and selected species traits as indicators of functions. Specifically, they examined frequencies of species traits across assemblages to determine the ecological structure and functioning of assemblages and how these relate to environmental parameters. To demonstrate a practical application of the protocol they developed, they applied it to two of the proposed Offshore Special Areas of Conservation in the United Kingdom (the Outer Thames sandbanks and the Eddystone Reef). The protocol enabled the generation of maps of ecological structure and functioning, which were used to delineate the boundaries of potential PAs that aimed to protect biological communities and the functions they support.

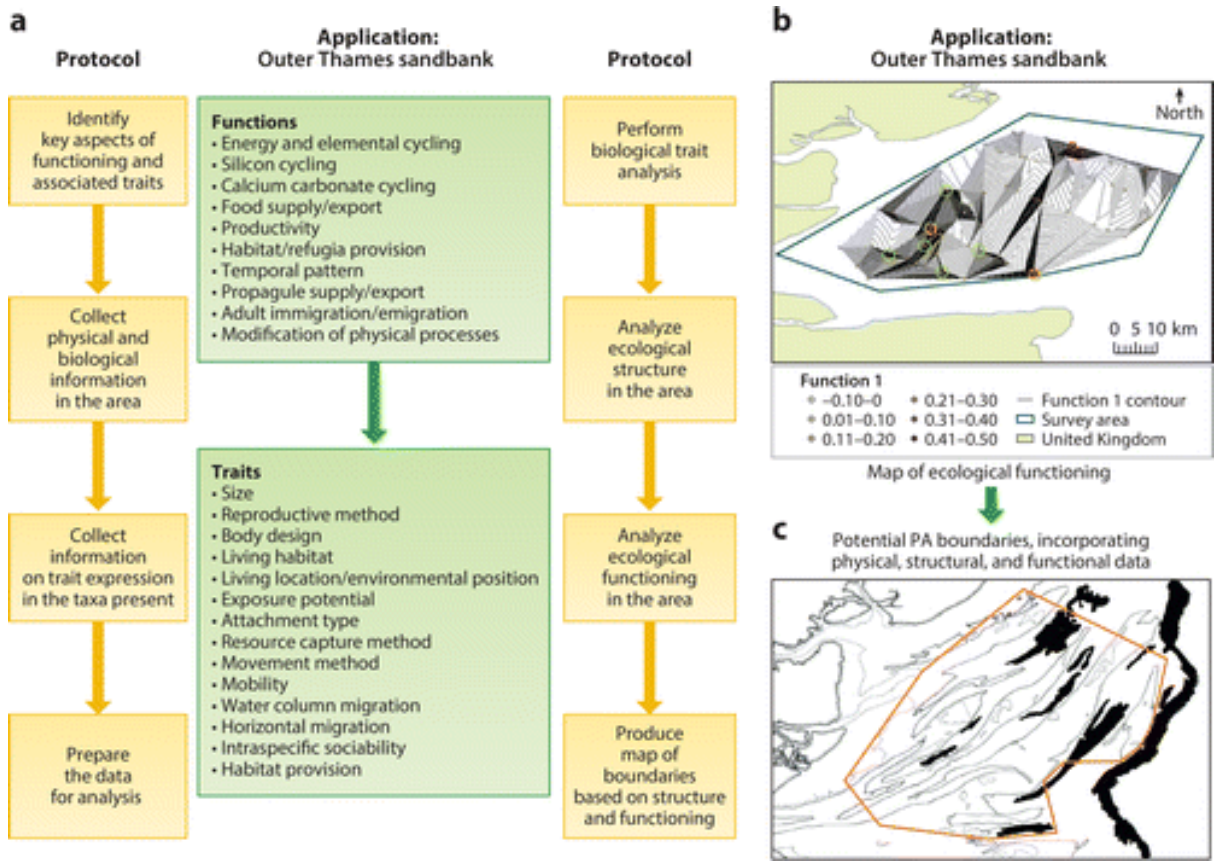

Figure 2.2: **a**) Key steps in the protocol used by Bremner et al. (2006b) to explicitly include ecological structure and functioning in delimiting the boundaries of special areas of conservation through biological trait analysis, along with examples of the relevant functions and traits in an application of such a protocol to the Outer Thames sandbank in the United Kingdom. **b**) Map of ecological functioning in the Outer Thames sandbank based on the co-inertia scores of the biological communities. Lines close together indicate the areas of greatest change, and lines farther apart indicate areas of similarity. Data points circled in red indicate outliers, whereas those in green indicate sampling of the same or adjacent locations using different techniques. **c**) Potential PA boundaries based on physical, structural, and functional data, including the results shown in panel b. Abbreviation: PA, protected area. Panels b and c adapted with permission from Bremner et al. (2006b).

Similarly, Rees et al. (2012) developed a framework that used biological trait analysis to incorporate indirect ecosystem services into PA planning and management and highlight the potential of biodiversity to provide indirect ecosystem services. The authors used their framework to define the spatial area over which benthic species deliver specific indirect services (in this case, nutrient cycling, gas and climate regulation, and bioremediation of waste) in Lyme Bay in

southwest England. This approach enables managers to link the provision of services with current conservation policies (Rees et al., 2012). These case studies demonstrate applications of biological trait analysis and indicate that simply considering habitat type or biodiversity measures when selecting PA boundaries ineffectively characterizes ecosystem function and services. They concluded that because ecological processes typically transcend physical habitat boundaries, an effective PA design should consider biological communities and associated functions (Bremner et al., 2006a; Frid et al., 2008). Also, conservation policy that focuses on biodiversity alone may result in the exclusion of functionally important but low-diversity locations during the planning process (Frid et al., 2008; Rees et al., 2012).

Biological trait analysis is one tool to quantify ecological functioning (Frid et al., 2008), with potential application to marine conservation in the design and management of PAs aimed at protecting functions provided by biological communities (Bremner et al., 2006b) and associated ecosystem services (Rees et al., 2012). Biological trait analysis can highlight changes in species composition arising from anthropogenic factors that impact ecosystem functioning (Rees et al., 2012). Whereas the incorporation of trait-based approaches in conservation strategies could potentially advance conservation outcomes, managers have not generally embraced the use of biological traits to advance marine conservation outcomes. Challenges include a lack of rigorous trait data for many species and logical methods that include traits (Lefcheck et al., 2015). In addition, the time- and data-consuming nature of biological trait analysis may discourage managers from applying such a methodology when planning PA design and management. Another downfall of this approach is that, by itself, trait analysis does not quantify the associated functioning and associated ecosystem services delivered. In fact, no methodology can quantify how much "function" biological assemblages deliver or is required to ensure human well-being. Therefore,

integrating ecosystem services into conservation planning and management remains a significant challenge (Rees et al., 2012). Acknowledging the need to keep working toward pragmatic tools to include ecosystem functions and services in marine conservation plans, managers could consider biological trait analysis as a current tool to advance marine conservation, which will progress as open trait databases are produced.

2.4.2 Monitoring outcomes in Protected Areas: using biological traits to track effects of protection

Enhancing PA effectiveness in achieving conservation goals hinges on the development and use of adequate and comprehensive indicators that measure the socioeconomic, biophysical, and institutional (governance) outputs and outcomes of PA management. Evaluation, or monitoring (**Figure 2.1, phase 3**), consists of reviewing the results of management actions and assessing whether these actions produced the desired outcomes (Pomeroy et al., 2005). This important step allows management to respond and adjust actions based on actual outcomes. Obtaining information about the conditions and changes that occur in PAs and assessing the effectiveness of management actions require a periodic and comprehensive assessment of the natural and social processes that occur within and outside PA boundaries.

Indicators based on traits can detect early responses, including important recovery signals (Mouillot et al., 2013; Coleman et al., 2015) (**Figure 2.1, phase 2**), to help assess the outcomes of protection. Where needed, trait indicators also allow rapid, adaptive management measures. Detection of responses of traditional metrics based on taxonomic diversity such as species richness or abundance sometimes takes up to 20 years (Edgar et al., 2009; Babcock et al., 2010) because subtle community-level changes, changes in long-lived species, or system variability may mask earlier detection (Coleman et al., 2015). In fact, trophic or functional changes often drive recovery following protection (Babcock et al., 2010). For instance, large and predatory species often benefit

directly from protection, leading to indirect community shifts as trophic levels are restored (Babcock et al., 2010; Coleman et al., 2015). Therefore, indicators that include biological traits often prove more sensitive in detecting early and subtle responses following protection, in addition to providing important information on key ecosystem processes (Mouillot et al., 2013).

PA monitoring that includes measuring the biological traits of species through time (**Figure 2.1, phase 2**) can, therefore, track the effects of management interventions and, when compared with a control site, allows attribution of protection from other variables that also shift through time, such as warming (*e.g.*, Bates et al., 2014b). Trait-based approaches can provide information on changes in the functionality of assemblages over time and confirm whether protective measures resulted in positive effects on functioning (de Bello et al., 2010; Vandewalle et al., 2010). Traits also offer an alternative for determining reference (or baseline) conditions, including functional aspects (Bremner, 2008; Bates et al., 2014a). Consideration of functional traits can help discern changes related to natural community assembly processes, associated with environmental shifts, and caused by anthropogenic stressors (*e.g.*, Usseglio-Polatera et al., 2000; Bremner et al., 2006b). For instance, de Bello et al. (2012) used biological traits of plants to discern niche differentiation effects (resulting in trait divergence) from competition effects (resulting in trait convergence) on species coexistence and community assembly. Understanding the mechanisms driving changes in biological assemblages over time is particularly important when evaluating conservation outcomes for attribution and context.

2.4.3 Conservation in a changing world: how biological trait-based approaches can help mitigate against ecosystem change

PA design rarely incorporates climate change-related disturbances (Levy and Ban, 2013; Bates et al., 2019). The legislation around management tools such as MPAs also typically lacks

the flexibility to support adaptive management needed to cope with change. Conservation in a changing world should prioritize designing PAs that enhance ecosystem resilience to climate change (McLeod et al., 2009). The role of climate as an increasingly significant stressor in both terrestrial and marine ecosystems leads to biodiversity loss, shifts in communities, and species extinctions (Pereira et al., 2010; Doney et al., 2012; Bijma et al., 2013; Antão et al., 2020). Although the creation of PAs alone cannot fully mitigate the effects of climate change, protection from other threats can minimize the synergistic impact of multiple stressors, ultimately increasing ecosystem resilience to climate change impacts (Trakhtenbrot et al., 2005; Bates et al., 2019). Nonetheless, climate change can threaten the effectiveness of conservation by altering species distributions, community structure, and ecosystem properties of the established or planned PAs (Pressey et al., 2007).

Traits of species link directly to their sensitivity or adaptability to climate change (Jiguet et al., 2007; Dawson et al., 2011; Foden et al., 2013; Bates et al., 2014a; McLean et al., 2019a,b). This linkage can help to predict ongoing and future effects of climate change on biological communities and their functioning when designing PAs (Degen et al., 2018). For instance, Foden et al. (2013) identified the main traits associated with increased sensitivity and low adaptability to climate change in birds, amphibians, and corals. These traits include habitat specialization, environmental tolerances, dependence on environmental triggers or interspecific interactions that climate change could disrupt, rarity, low dispersal ability, and limited capacity to evolve and adapt. Combining information on species sensitivity with predictions of climate change extent in different regions can help define population vulnerability and identify priority conservation areas. Traits can also predict climate change effects on ecosystem processes and functioning. For instance,

Suding et al. (2008) proposed a framework that uses different functional traits to predict the effects of environmental changes on ecosystem functions.

In the same way that species traits can indicate sensitivity to climate change, traits can identify species with broad environmental tolerances and habitat requirements, strong competitors, and potential geographic spread (Kotiaho et al., 2005; Van Kleunen and Richardson, 2007; McKnight et al., 2016; Cardeccia et al., 2018). Therefore, traits related to physiological tolerance, life history strategies (Sol et al., 2012), and biotic interactions (Dick et al., 2002; Twardochleb et al., 2013) can predict invasion potential (Blossey and Notzold, 1995). For instance, Cardeccia et al. (2018) reported that high dispersal ability, high reproductive rate, and ecological generalization all characterized widespread nonindigenous species in European seas. Traits, therefore, provide the basis for tools that can simultaneously identify which species are most likely to become invasive in the future and which native species are most susceptible to the negative effects of invasions. Thus, trait-based approaches can help focus conservation efforts on strategies that prevent the spread of invasive species and protect the most vulnerable native species.

2.5 Challenges in transferability from terrestrial to marine systems

Marine conservation lags behind its terrestrial counterpart, in both the extent and effectiveness of conservation measures (Spalding et al., 2008). As of May 2020, PAs covered only 7.4% of the ocean, compared with 15.2% of land (UNEP-WCMC and IUCN, 2020). Furthermore, only 2.5% of the ocean received full protection (Marine Conservation Institute, 2020), with uneven representation across ecosystem types (Spalding et al., 2008). One issue is that the translation of methodologies developed for terrestrial conservation to marine areas rarely considers differences between the two realms (Allison et al., 1998; Carr et al., 2003; Hooker and Gerber, 2004; Agardy et al., 2011). Marine systems fundamentally differ from terrestrial ones because the ocean covers

most of the planet, with fewer physical boundaries to restrict dispersal (Hooker and Gerber, 2004). These differences result in ecological processes in the ocean that span larger spatial scales, often with greater connectivity (Carr et al., 2003). Moreover, many marine species have open populations, where offspring disperse over long distances and parental populations rely on the immigration of propagules arriving from other sources. By contrast, most terrestrial species are direct developers, with offspring that do not disperse far from parental populations (Carr et al., 2003). Thus, marine systems require larger, self-sustaining PAs or well-connected networks (Carr et al., 2003; Edgar et al. 2014).

Moreover, the hidden and relatively inaccessible marine environment (Edgar et al., 2016) creates greater challenges for sampling, observation, and manipulation of natural assemblages. Despite many technological and scientific advances in recent decades, such as seafloor observatories and genetic tools, research on and applications of biological traits in the marine environment are in their infancy for many taxa compared with those in the freshwater and terrestrial realms (Bremner, 2008; Madin et al., 2016b; Beauchard et al., 2017). Understanding the general relationships among traits, species, and environments requires advances in marine research, including funding allocation, in order to generate ideas about how the approach can guide marine conservation and management (Edgar et al., 2016). For example, recent efforts are increasing the availability and reliability of information on species traits through curated and open-source databases of trait information, such as the Coral Trait Database for corals (Madin et al., 2016a) and sFDvent for hydrothermal vent species (Chapman et al., 2019).

In the ocean, even more than on land, logistical and financial constraints limit the scope of many studies in space and time and therefore the current understanding of ecological patterns, biodiversity, ecosystem functioning relationships, and even the occurrence of threats (Whittaker

et al., 2001; Snelgrove et al., 2014; Edgar et al., 2016). In addition, marine ecosystems often incorporate diverse habitats and span environmental and anthropogenic gradients, complicating the extrapolation of local properties onto larger scales (Snelgrove et al., 2014). Thus, some of the approaches developed and tested in terrestrial systems that generalize from one region to another may be less accurate in dynamic marine systems. This problem also applies to biological traits because data collected in limited biogeographic areas form the basis of most trait databases. Such data may, therefore, represent populations from other areas and underestimate values for the species. Identifying different populations within a geographic region, though often complicated, may prove important when applying traits (as discussed above).

Even so, the potential to generalize results of trait-based analyses across similar habitats in different regions means that biological traits can help to address challenges in comparing vulnerability assessments, which often span different spatial scales (Vandewalle et al., 2010). Moreover, trait-based analyses are also applicable to a wide range of marine organisms and habitats (**Figure 2.3**), from coral reefs to deep-sea benthic sediments and the pelagic habitats in between.

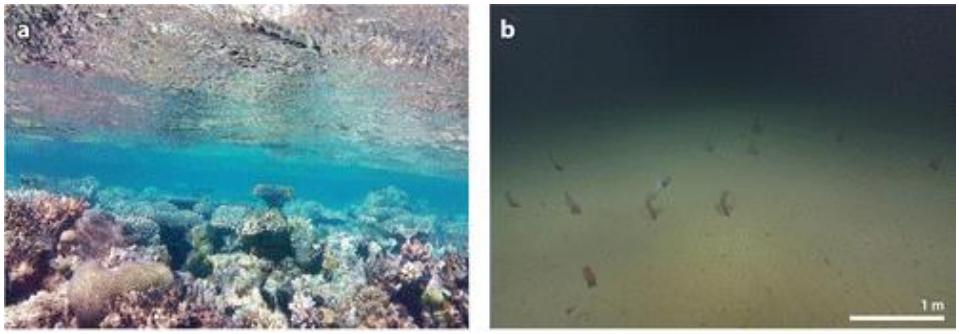

Figure 2.3: Example of marine habitats where biological traits can help advance marine conservation, illustrating the wide range of habitats for which managers could utilize trait-based approaches. **a**) The Great Barrier Reef, Australia. Extensive trait databases exist for fishes and corals to aid such an application. Photo by Paul Snelgrove. **b**) Deep-sea sedimentary habitats (the image shows approximately 15 m2 of mud with the scattered presence of epifaunal sea pens) in the Laurentian Channel Marine Protected Area, Canada, where conservation efforts consider functions. Scattered sources exist on traits for different groups of sedimentary fauna, though most extensively for polychaete worms. Photo taken from the Remotely Operated Platform for Ocean Sciences (ROPOS), courtesy of the Canadian Scientific Submersible Facility (CSSF).

2.6 From theory to practice: recommendations on how to practically incorporate biological traits into conservation science

Several major steps must occur in the creation of a new PA, and scientists and managers can incorporate biological traits into some of these steps (**Figure 2.4**). During the initial screening of a candidate area for conservation, biological traits can clarify the biological structure and ecology of populations, communities, and ecosystem functions of the area and how these link to environmental and anthropogenic factors. After the appropriate selection of traits and the collection of information on trait expressions in the taxa or individuals sampled to create a list of trait expressions in the area, application of the methodology of choice follows (*e.g.*, biological trait analysis or a similar approach, and/or the use of trait-based functional diversity indices). The results can be used to relate trait expression in populations or communities to physical features and threats and to understand differences among contrasting locations in the area and the traits that drive them. Because traits link to functions, these approaches enable the linking of communities

to ecosystem processes and the building of maps of ecosystem functioning. Based on the information gathered during the screening process, managers can then identify priorities and objectives for conservation, plan area boundaries, and define regulations. Biological traits can facilitate this phase by providing information on species or community vulnerability, important functions expressed (*e.g.*, which functions to protect in the area and which processes are more vulnerable to threats), and the traits related to these questions (*e.g.*, which traits provide those functions and which traits express vulnerability to threats). Boundaries and protection levels can then be set by using traits to identify areas in the region where important or vulnerable traits occur most often or need protection. Monitoring protocols that investigate whether the management achieves its objectives can also use traits (*e.g.*, whether the intervention preserved or restored important traits over time).

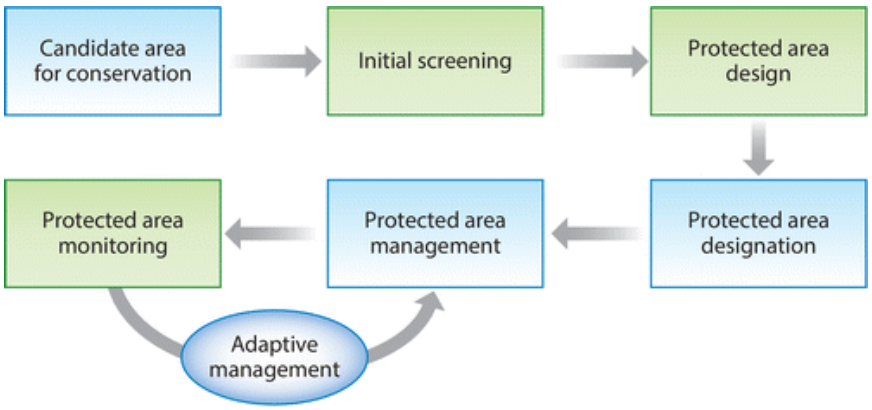

Figure 2.4: Key steps toward the creation of a new protected area. Green boxes highlight steps where the application of biological traits can enhance marine conservation outcomes.

Maximizing the benefits of using biological traits to inform PA design hinges on selecting the appropriate types and number of traits to include in the analysis, as well as the appropriate index or indices. We have four recommendations for how to do so.

First, traits and methodologies should be selected according to the key ecological questions that define conservation priorities. These questions might pertain to assemblage functioning, the presence and effects of anthropogenic impacts, or both (Bremner et al., 2006b). For example, feeding traits can be used as indicators of carbon transport between the pelagos and benthos (Frid et al., 2008). Similarly, some traits are more responsive than others to environmental change or anthropogenic impacts and can be used to detect community response signals (Coleman et al., 2015; Nock et al., 2016). Depending on the conservation objective, scientists can favour response traits, which determine the response of organisms to environmental conditions, or effect traits, which determine the effect of organisms on ecosystem processes and functions (Lavorel and Garnier, 2002). Finally, a useful trait generally varies among taxa, species, or individuals (McGill et al., 2006; Petchey and Gaston, 2006; Lefcheck et al., 2015; Nock et al., 2016). However, trait categories shared among individuals, species, or higher taxa provide a means to quantify the degree of redundancy within assemblages and identify vulnerable communities and ecosystem components (Lefcheck et al., 2015). Selecting traits representing specific ecosystem functions or those that respond to certain environmental stressors may be a good way to standardize protocols to relate communities to ecosystem functioning or to detect anthropogenic impacts (Bremner et al., 2006b). However, further studies are necessary to expand our understanding of the relationships between traits and functioning (Degen et al., 2018; Maureaud et al., 2020).

Second, analyses should include multiple biological traits and diverse taxa that represent a broader perspective on ecological functioning and the effects of natural and anthropogenic factors on biological communities (Bremner et al., 2006b; Lefcheck et al., 2015; Nock et al., 2016). Considering multiple traits leads to a more holistic representation of functional diversity, including both the tracking and prediction of trait responses of organisms and communities to different

scenarios and changes (Lefcheck et al., 2015). This benefit is particularly relevant for large-scale investigations, where a variety of factors typically affect traits (Lefcheck et al., 2015). Different traits may also drive different ecosystem processes, including potentially unidentified functions (Petchey and Gaston, 2006). Managing biodiversity based on multitrait diversity indices produces an inclusive framework that aims to support multiple ecosystem functions and associated goods and services (de Bello et al., 2010; Lefcheck et al., 2015). However, redundant traits should be avoided because similar traits add no new information and can combine to empirically overcontribute to diversity indices, hence disguising functional diversity effects (Nock et al., 2016). Ultimately, the selection of biological traits for analysis should be based on a trade-off between the information each trait provides and the time and effort required to gather such information (Bremner et al., 2006b). Sensitivity analyses, although not routinely used, can help users identifying how trait selection and uncertainties in trait information can affect the output of trait-based analysis (Degen et al., 2018).

Third, analyses should include intraspecific trait variability, if such an approach is warranted and possible. This goal may be achieved by measuring and including individual trait expressions in the analysis of the population considered and applying methodologies that incorporate intraspecific trait variability (*e.g.*, as proposed in de Bello et al., 2011). This need is particularly important for species whose traits vary considerably within or across populations and especially when management assessments span large scales, such as regional fishing assessments. Sometimes assessment of functional aspects of biodiversity can occur without assessing taxonomical diversity first. Some easily measured morphological traits (such as those for sponge or coral reefs, where taxa are hard to distinguish but morphological features relate directly to

habitat complexity) can provide a relatively inexpensive and time-saving solution for assessing diversity for conservation purposes (Vandewalle et al., 2010).

Finally, trait and methodology selection should be informative yet relatively simple to measure and interpret. The selection of traits and analytical tools should balance the sensitivity and power of the tool to describe assemblages, functions, and responses to impacts with the ease with which traits can be measured and results can be interpreted (Bremner et al., 2006b; Lefcheck et al., 2015). Thus, while we advocate for considering individual variability in traits and including as many taxa and traits as possible, analyses, by necessity, may need to focus on a subset of the available data, such as the most abundant or widespread species (Bremner, 2008). Moreover, indicators should also be appropriate for comparative studies across different communities, habitats, and ecoregions to enable applications in future monitoring protocols and comparisons with other locations (Vandewalle et al., 2010). Ultimately, establishing standardized methodologies for trait parameterization will offer a more accessible tool kit to advance marine conservation.

2.7 Conclusion

Trait-based indicators can complement, rather than replace, traditional biodiversity and habitat-based indicators in marine conservation. The specific added benefits include (*a*) identifying priorities for conservation by assessing and predicting the sensitivity and vulnerability of communities to anthropogenic impacts and climate change, predicting species extinction risk, and assessing community resilience; (*b*) implementing spatial planning by identifying new hot spots of diversity, identifying hot spots of function, and creating maps of functional diversity and functioning; and (*c*) informing monitoring programs that are designed to detect early recoveries after PA establishment and create functional diversity time series (**Figure 2.1**). For example, trait-

based approaches allow the identification of species responsible for key ecological processes, tracing function-related changes back to changes in the biota and hence back to impacting activities that management measures can control (Frid et al., 2008). Thus, trait-based approaches add a fundamental aspect to toolboxes available for conservation efforts and merit consideration in management decisions on ocean sustainability strategies. Such approaches can be relatively easy to estimate once they have been defined and standard methodologies have been established (Hodgson et al., 2005), and they therefore offer an excellent tool for managers (Vandewalle et al., 2010). However, advances in knowledge of marine species traits and how they respond to stressors and link to changes in ecosystem functions point to the need for ongoing development of pragmatic tools to apply trait-based approaches in marine conservation.

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CHAPTER 3 - SEA PENS AFFECT MACROFAUNAL COMMUNITIES IN DEEP-SEA SEDIMENTS: EVIDENCE FROM THE LAURENTIAN CHANNEL MARINE PROTECTED AREA*

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3.1 Abstract

Pennatulacean octocorals (sea pens), one of the primary conservation targets of the Laurentian Channel Marine Protected Area (MPA), in eastern Canada, occur at high densities in some deep-sea sedimentary habitats. Considered important habitat-forming organisms for many megafaunal organisms, the effect of sea pens on nearby sedimentary macrofaunal communities remains unexplored. During two cruises in September 2017 and July 2018, we sampled 9 stations within the MPA, including sea pen fields and bare sedimentary habitats (336-445 m depth), targeting individual sea pens in a subset of the cores to assess small-scale effects. We evaluated macrofaunal density, taxonomic diversity, vertical distribution, community composition, and biological trait expression and investigated variation between sea pen fields and other (bare) sedimentary habitats, as well as between cores with and without sea pen specimens. Using multivariate analyses, we identified abiotic and biotic drivers of macrofaunal community composition and biological trait expression. Enhancement of macrofaunal density and taxonomic diversity and higher percentages of organisms in the upper sediment layers characterized sea pen fields in autumn, with more variable results in summer. Community composition and biological trait expression consistently differed in sea pen fields compared to bare sedimentary habitats, with *Pennatula* sea pens density

as one of the primary drivers of variation especially for community composition, along with other environmental drivers (depth, grain size, and organic matter quantity and quality). We also detected small-scale enhancement of macrofaunal diversity in cores containing sea pens at stations characterized by predominantly bare sediments. Our results indicate that macrofaunal communities within sea pen fields differ from those in bare sediments and we propose sea pens play a role in influencing those patterns by increasing food availability, stability, and small-scale heterogeneity in sedimentary habitats. Characteristics of communities within sea pen fields also suggested potentially higher sensitivity to disturbance, which amplifies the need for protection of sea pen fields in deep-sea sedimentary environments.

3.2 Introduction

Though often seen as vast and monotonous expanses of mud and sand, most sedimentary habitats host high biomass and biodiversity of organisms and sustain important ecological processes, regulated by multiple factors at different scales (Snelgrove, 1998; Watling, 1998; Levin et al., 2011; Danovaro et al., 2014). For instance, past studies report effects on macrofaunal diversity by factors such as depth, organic matter input, and sediment type (Snelgrove, 1998; Levin et al., 2001). However, the influence of biogenic habitats (habitats created by structure-forming benthic organisms; Bruno and Bertness, 2001) on the macrofauna inhabiting marine sediments is generally less considered. Evidence shows that ecosystem engineers (organisms that cause physical change to the environment and impact the availability of resources to other species; Jones et al., 1994) affect and usually enhance macrofaunal diversity and ecological processes in intertidal and coastal sedimentary habitats. Such effect has been reported for seagrass (*e.g.*., Mills and Berkenbusch, 2009; Lundquist et al., 2018), tube-dwelling polychaetes (*e.g.*, Bolam and Fernandes, 2003), and oyster and mussel reefs (*e.g.*, Cranfield et al., 2004; Commito et al., 2008).

In deep-sea sediments, however, the role of biogenic habitats remains less understood (Meadows et al., 2012; Dunham et al., 2018; Ashford et al., 2019), mainly because of limited access to these habitats. Deep-sea corals, but also sponges and vent communities, form complex biogenic habitats in the deep sea and can sustain high levels of biodiversity (Buhl-Mortensen and Mortensen, 2005; Bell, 2008; Baillon et al., 2012; Baillon et al., 2014; Buhl-Mortensen et al., 2010; Levin et al., 2016; Dunham et al., 2018), even though only a few studies have explicitly explored their effect on sedimentary macrofauna (*e.g.*, Bett and Rice, 1992; Raes and Vanreusel, 2005; Gheerardyn et al., 2009; Barrio Froján et al., 2012; Ashford et al., 2019).

Sea pens (subclass Octocorallia, order Pennatulacea) are colonial soft corals with widespread distribution that primarily live on soft sediments, where most species use their muscular peduncle to anchor themselves in the sediment (Williams, 2011). These suspension feeders constitute one of the most poorly studied habitat-forming organisms, despite evidence that they play a fundamental ecological role in sedimentary habitats by adding structural complexity to an apparently homogeneous habitat, potentially representing biodiversity hotspots (Tissot et al., 2006; Buhl-Mortensen et al., 2010; De Clippele et al., 2015; Bastari et al., 2018). Unlike other megafaunal sessile organisms that require hard substrata for settlement and attachment, sea pens can inhabit unconsolidated sandy or muddy sediments, and therefore can cover extensive regions of the seafloor (Williams, 1992). Under suitable conditions (*e.g.*, relatively eutrophic conditions and moderately high energy environments; Williams, 2011) sea pens form dense aggregations known as sea pen fields (Buhl-Mortensen et al., 2010; Kenchington et al., 2014; Murillo et al., 2018), usually patchily distributed on the seafloor (Murillo et al., 2018). Sea pen fields provide important habitat for many species, including some of commercial importance (*e.g.*, redfish *Sebastes* spp.; Baillon et al., 2012), with many invertebrates and fish using sea pens as shelter,

substrate, refuge for their eggs (Baillon et al., 2012), and source of food (Garcia-Matucheski and Muniain, 2011). In addition, sea pens alter water current flow at the sediment-water interface, contributing to increase retention and sedimentation of nutrients and organic matter (Eckman, 1985; Tissot et al., 2006; Cerrano et al., 2010; Kenchington et al., 2011), and they are thought to contribute to sediment bioturbation and oxygenation. For instance, some sea pen genera (*e.g.*, *Pennatula*) exhibit withdrawal behaviour in response to disturbance that allows them to partially or completely burrow into the sediment (Hoare and Wilson, 1977; Chimienti et al., 2018). Such behaviour is unique among corals and may enhance mixing and oxygenation of the sediment, even though we acknowledge limited availability of information on this aspect of their biology. Given the important ecological role of sea pens, their vulnerability and slow recovery after bottom fishing or other disturbances (Heifetz et al., 2009; Malecha and Stone, 2009; Murillo et al., 2018; de Moura Neves et al., 2018), researchers globally consider sea pen fields as Vulnerable Marine Ecosystems (VME; FAO, 2009) that require special management consideration (OSPAR, 2008; Kenchington et al., 2014; DFO, 2017). In the Northwest Atlantic managers have already closed several areas to bottom-contact fishing in order to protect sea pen populations (NAFO, 2017), as well as prioritized their conservation through the creation of Marine Protected Areas (MPAs). One example includes the Laurentian Channel MPA, in Eastern Canada, designated by Fisheries and Oceans Canada in 2019 primarily to protect the sea pens that occur at high concentrations in the region (DFO, 2011; Murillo et al., 2018).

Despite recognition that sea pens provide habitat for many species of benthic fishes and mega-invertebrates, and affect sedimentary processes, no studies have explicitly investigated the role that sea pens may play in regulating macrofaunal densities and biodiversity in deep-sea sedimentary habitats. The presence of both surface and subsurface structures in sea pens

distinguish them from other deep-sea habitat-forming organisms such as scleractinian corals or sponges, whose structures occur exclusively above the sediment surface. In this sense, sea pens might functionally resemble seagrasses, and their peduncles could affect infaunal organisms in nearby sediments similarly to seagrass roots (*e.g.*, Lundquist et al., 2018). The role of sea pens for macrofauna remains an important knowledge gap.

Macrofauna - here defined as animals large enough to be retained on a 300 µm sieve (Snelgrove, 1998) - can occur in sediments in high abundances and biomass, and the ecological roles of macrofaunal organisms in critical processes such as bioturbation, sediment oxygenation and nutrient cycles, and as a food source for higher trophic levels is widely recognized (Meysman et al., 2006; Loreau, 2008; Braeckman et al., 2010; Baldrighi et al., 2017). Among macrofaunal organisms, polychaete worms (phylum Annelida, class Polychaeta) often dominate soft sediments, and usually exhibit higher taxonomic and functional diversity compared to other benthic organisms. For instance, they encompass a wide diversity of feeding modes and contribute to bioturbation processes in a variety of ways (Fauchald and Jumars, 1979; Queiros et al., 2013; Jumars et al., 2015), thereby providing a variety of ecosystem functions. For these reasons, many studies focus on polychaetes in environmental assessment and monitoring studies and to describe the diversity and functioning of benthic communities (Pocklington and Wells, 1992; Dauvin et al., 2003; Olsgard et al., 2003; Giangrande et al., 2005). Polychaetes and other macrofaunal taxa also offer the advantages of relatively well-developed quantitative sampling methods, and the availability of a more mature scientific literature compared to smaller sediment-dwelling organisms (Patricio et al., 2012).

Beyond consideration of taxonomic diversity and structure of biological communities, consideration of organisms' biological traits can complement information on the environmental

drivers determining communities' composition, dynamics, response to stressors, and functional role. In fact, biological traits (morphological, biochemical, physiological, structural, phenological, behavioural, and ecological characteristics of organisms; Diaz and Cabido, 2001) reflect interactions between organisms and their environment, and characterize the role organisms play in ecosystem processes (Violle et al., 2014; Lefcheck et al., 2015; Nock et al., 2016; Hajializadeh et al., 2020; Sutton et al., 2020). For these reasons, researchers have increasingly recognized the validity of incorporating trait-based approaches into marine spatial planning and conservation strategies to improve the outcomes of management actions (Bremner et al., 2008; Frid et al., 2008; Villnäs et al., 2019; Miatta et al., 2021). Biological Trait Analysis (Bremner et al., 2003) offers one way to quantify and investigate the expression of biological traits of biological communities. This approach uses multivariate ordination to describe patterns of biological trait composition over the entire biological community and quantifies the types of traits present and their relative frequency, thereby allowing exploration of patterns in assemblages' functional structure and functioning (Bremner et al., 2006). *A priori* selection of specific traits allows researchers to elucidate information on different aspects of the community (Bremner, 2008). For instance, "response" traits (*e.g.*, larval development) refer to traits that determine the response of organisms to environmental conditions and stressors, whereas "effect" traits (*e.g.*, bioturbation) determine the effect organisms have on ecosystem processes and functions (Lavorel and Garnier, 2002).

Our study evaluates spatial patterns of macrofaunal density, taxonomic diversity, vertical distribution, community composition and biological trait expression within the Laurentian Channel MPA, with a focus on distinguishing communities inhabiting sea pen fields from the ones occupying other (bare) sedimentary habitats. We also assess the small-scale effect of sea pens on macrofauna by comparing density, taxonomic diversity, taxa and biological trait structure in cores

targeting sea pen specimens and cores without sea pens. Finally, we examine a wide range of variables to identify drivers of variation in macrofaunal community composition and biological trait expression and help elucidate the effect of both abiotic and biotic (including sea pen densities) factors in shaping macrofaunal communities and selecting their biological traits. Our results not only provide additional information on the ecology of sedimentary habitats in the area, including sea pen fields, but also serve as a starting point for improving conservation strategies and for developing appropriate monitoring protocols for this MPA.

3.3 Materials and methods

3.3.1 Study location

Our study area is the Laurentian Channel Area of Interest (AOI), located on the edge of the continental shelf off the southwest coast of Newfoundland and Labrador, in Eastern Canada (**Figure 3.1**). This area was designated as an MPA under Canada's Oceans Act in 2019, and it currently represents the largest MPA in Canada, protecting 11,580 km^2 of deep-sea sediments. This MPA is considered important for a variety of marine species that live, mate, and/or feed in the area, including black dogfish, smooth skates, porbeagle and basking sharks, wolfish, and leatherback sea turtles (DFO, 2011). The main conservation target of this MPA, however, is the protection of corals, and particularly sea pens, that occur in the highest known concentrations of the entire Newfoundland and Labrador Shelves bioregion (DFO, 2011). To protect the fragile sea pen habitats, activities that disturb, damage, destroy or remove living marine organisms or any part of their habitat (*e.g.*, fishing, oil and gas activities) are prohibited within the MPA, with stricter regulations and higher levels of protection within the core protection management zones (Zones 1a and 1b as seen in **Figure 3.1**; DFO, 2019).

The Laurentian Channel MPA represented an ideal location for this study because our stations were characterized by spatial variability in mega-epifaunal densities (from almost totally bare sediments to dense sea pen fields dominated by *Pennatula* spp.; **Figure 3.2**), but homogeneous physico-chemical (see **Table 3.1**), sedimentary (see **Table 3.3** and **Table 3.4**) and morphological features, as revealed by our explorations and sampling. This environmental homogeneity allowed us to assess the role of sea pens for macrofauna without the confounding effect of other strong environmental gradients (*e.g.*, depth, bottom water properties, sediment type). From a conservation standpoint, this study provides baseline data on benthic communities that can be used to evaluate current management regulations and develop adequate monitoring programs.

3.3.2 Sampling design

Samples were collected in the Laurentian Channel AOI during two research cruises on board the *CCGS Martha L. Black* (September 2017) and the *CCGS Hudson* (July 2018). In total, we sampled 9 stations inside the MPA (**Figure 3.1**, **Table 3.1**). In September 2017, we sampled 7 stations in the central and southern part of the MPA using the Remotely Operated Vehicle (ROV) ROPOS. At each station, we collected 8 sediment push cores (internal diameter = 6.7 cm, length = 35 cm) at 2 different locations, and we used 2 cores per station to evaluate sedimentary properties and the remaining 6 cores to characterize macrofauna. In order to directly test the small-scale effect of sea pens on macrofauna, we collected a total of 10 cores (from St 2, St 14, St 4, and St 3) directly on sea pen specimens representing several different genera (*Pennatula* spp., *Anthoptilum* spp., *Funiculina* spp., *Kophobelemon* spp., *Protoptilum* spp.). In some instances, multiple attempts were necessary in order to collect the sea pens, because some individuals (*e.g*., specimens belonging to *Pennatula* and *Funiculina* spp.) retreated into the sediment following disturbance by the ROV. In

July 2018, we re-sampled two of the stations we sampled in 2017 (St 2 and St 5) to assess temporal variation, and we added 2 additional stations (St 25 and St 9) in the northern part of the MPA. In this instance, we used the Oktopus GmbH Mini-MultipleCorer to collect 9 sediment cores (internal diameter = 10 cm, length = 60 cm) at 3 different locations at each of the 4 stations, using 3 cores per station to evaluate sedimentary properties and 6 cores to characterize macrofauna (combining the data from cores from the same multicorer drop, resulting in 3 replicates per station). At every station, a ROPOS Seabird CTD 19plus profiler mounted on the ROV (in 2017) and a Seabird CTD SBE25 profiler deployed from the ship (in 2018) recorded bottom water physico-chemical properties at each station (**Table 3.1**).

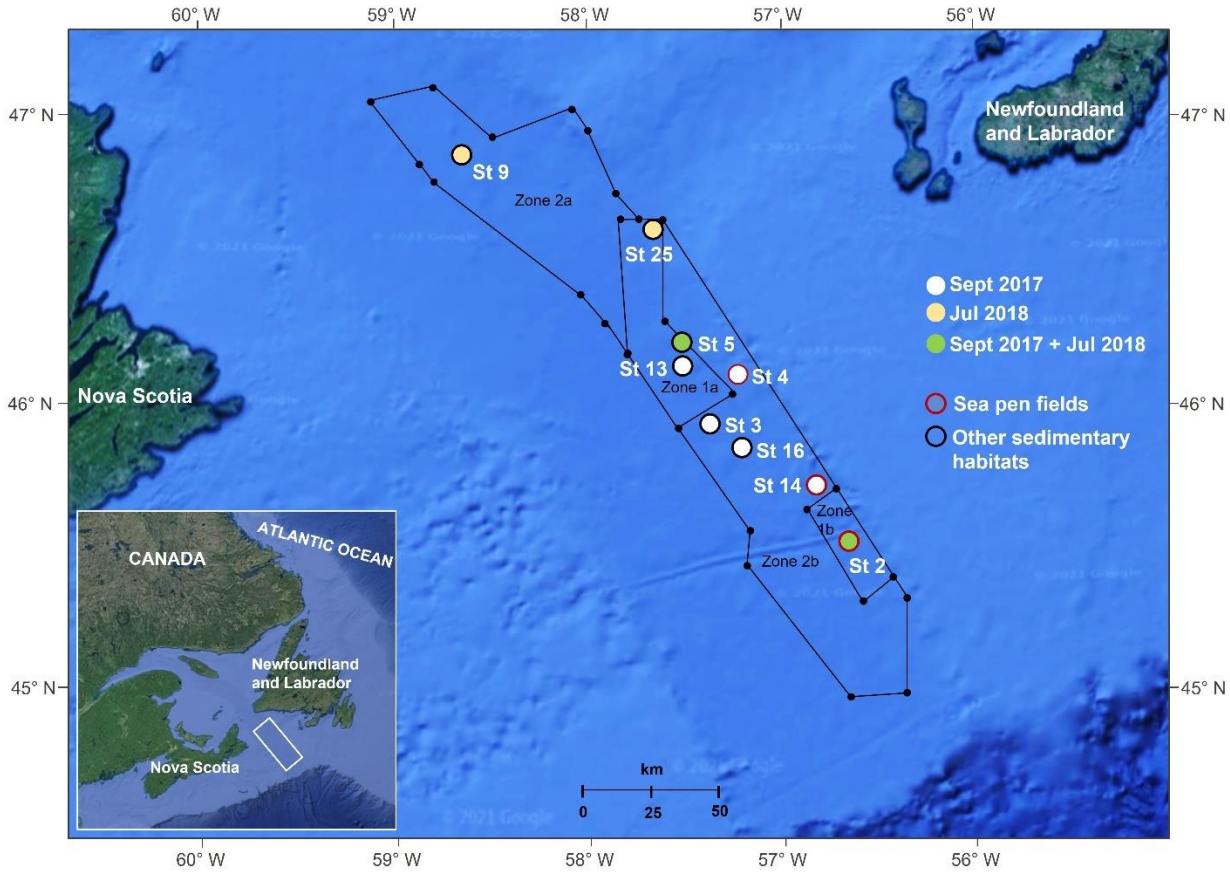

Figure 3.1: Map showing location, borders and zonation of the Laurentian Channel MPA and the 9 stations sampled in September 2017 and July 2018.

We classified the biogenic habitat at each station based on the observed presence of sea pens, because they constituted the dominant mega-epifauna. Stations with high densities of sea pens, especially *Pennatula* spp. (both juvenile and mature specimens) were classified as "sea pen fields" (**Figure 3.2, a**), whereas stations characterized by bare sediments or scattered presence of sea pens or other mega-epifauna were classified as "other sedimentary habitats" (**Figure 3.2, b**), as reported in **Figure 3.1** and **Table 3.1**. Aggregations of sea pigs (class Holothuroidea, order Elasipodida; **Figure 3.2, c**) occurred at some stations (St 13 and St 3 in 2017 and all the stations in 2018), but they were not considered in our classification of biogenic habitats because of the transient nature of their aggregations in response to high influx of fresh organic matter to the seafloor (Miller et al., 2000). Average sea pig coverage at each station (%), however, was used as a factor in our analyses to assess the effect of mega-epifauna on macrofaunal communities, together with total sea pen average density (ind · m^{-2}) and *Pennatula* spp. average density (ind · m^{-2}), all reported in **Table 3.1**. Other mega-epifauna (*e.g.*, sea anemones and other soft corals, echinoderms) occurred at some stations, but in much lower densities than sea pens and sea pigs, and in insufficient numbers to consider in our study. Data on average sea pen densities and sea pig coverage in the stations were derived from a parallel study (S. de Mendonça and A. Metaxas, unpublished), and were estimated from video transects collected using the ROV ROPOS in September 2017 and the Campod drop-camera in July 2018 (see de Mendonça and Metaxas (2021) for a detailed description of the methods). Importantly, the video transects that S. de Mendonça and A. Metaxas used to estimate average sea pen and *Pennatula* spp. densities and sea pig presence sampled much larger areas of the seafloor than our sediment sampling stations. Thus, for some stations that exhibited high intra-station variability in sea pen density (*e.g.*, St 3) the average values

of sea pen densities do not necessarily represent the densities observed at our sampling sites that constituted the basis for the habitat classification used in our study.

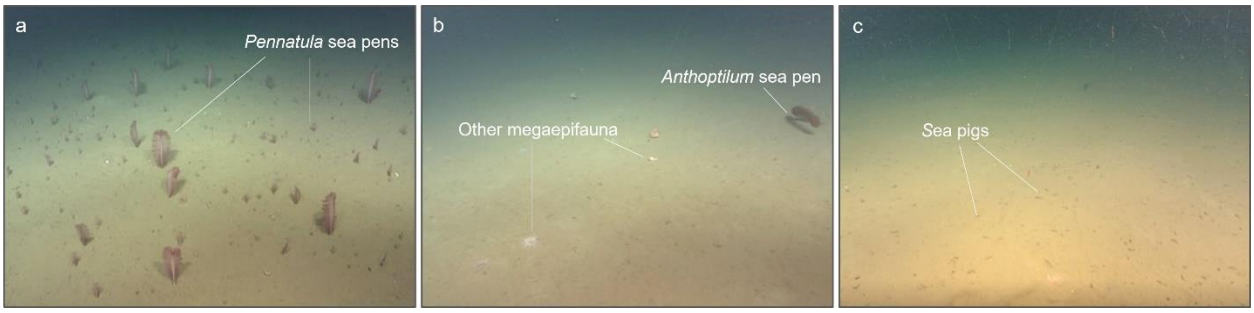

Figure 3.2: Biogenic habitats in the Laurentian Channel MPA stations: **a**) sea pen field (St 2), **b**) other sedimentary habitats with scattered presence of mega-epifauna (St 5), **c**) other sedimentary habitats with aggregation of sea pigs (St 3). Arrows highlight characteristic features. Photo credit CSSF ROPOS.

Table 3.1: Laurentian Channel MPA stations description. Data on average total sea pen density (Tot sea pen den.), *Pennatula* spp. density (Pennatula spp. den.), and sea pig coverage (Sea pig cov.) at each station are derived from de S. Mendonça and A. Metaxas (unpublished). Other sedim. hab.: other sedimentary habitats; B T: bottom water temperature; B [O2]: bottom water oxygen concentration; B S: bottom water salinity; *NA*: data not available.

Station	**Biogenic habitat**	**Sampling period**	**Latitude** (N)	**Longitude** (W)	**Depth** (m)	**B T** (℃)	**B [O2]** (ml · L^{-1})	**B S** (PSU)	**Tot sea pen den.** (ind · m^{-2})	***Pennatula* spp. den.** (ind · m^{-2})	**Sea pig cov.** (%)
St 2	Sea pen field	Sept 2017	45°32.09	56°40.07	354	6.2	7.93	35.00	3.63	3.56	0.00
St 4	Sea pen field	Sept 2017	46° 5.73	57° 14.73	336	6.5	7.93	35.01	0.67	0.66	0.00
St 14	Sea pen field	Sept 2017	45° 43.75	56° 51.16	351	6.3	7.91	35.01	0.59	0.54	0.00
St 3	Other sedim. hab	Sept 2017	45° 56.59	57° 22.28	445	5.8	7.94	35.00	0.45	0.01	0.02
St 16	Other sedim. hab	Sept 2017	46° 8.69	57° 30.99	442	5.9	7.94	35.00	0.30	0.00	0.00
St 5	Other sedim. hab	Sept 2017	46°13.28	57°31.45	440	5.7	7.93	34.99	0.07	0.01	0.01
St 13	Other sedim. hab	Sept 2017	45° 51.66	57° 12.23	436	5.8	7.92	35.00	0.03	0.00	0.07
St 2	Sea pen field	Jul 2018	45° 32.09	56° 40.07	356	*NA*	*NA*	34.50	6.65	6.63	0.27
St 5	Other sedim. hab	Jul 2018	46° 13.28	57° 31.45	448	5.7	*NA*	34.54	0.12	0.05	0.06
St 25	Other sedim. hab	Jul 2018	46° 37.81	57° 37.93	336	6.1	*NA*	34.51	0.02	0.01	0.07
St 9	Other sedim. hab	Jul 2018	46° 52.24	58° 38.14	422	5.9	*NA*	35.53	0.16	0.00	0.07

3.3.3 Macrofaunal identification and taxonomic diversity

Sediments from the cores dedicated to macrofaunal analysis were extruded and sectioned into 0-5 and 5-10 cm sediment layers using inert plastic spatulas, and subsequently transferred into 500- or 1000-ml plastic jars and fixed with 4% buffered formaldehyde seawater. In the laboratory, we processed samples over a 300-μm sieve and transferred sediments to 70% ethanol until we could complete microscopic taxonomic analysis. Before identification, samples were stained with a few drops of Rose Bengal (0.5 $g \cdot L^{-1}$) to facilitate sorting of the samples and identification of organisms (Eleftheriou and Holme, 1984).

For each sample, we sorted macrofaunal organisms and assessed the abundances of the major taxa (Classes: Polychaeta, Oligochaeta, Amphipoda, Isopoda, Copepoda, Ostracoda, Bivalvia, Gastropoda, Scapopoda, Sipunculidea, Ophiuroidea, Asteroidea, Holothuroidea, Echinoidea; Subclasses: Hexacorallia, Octocorallia). We further identified polychaetes to Family because they represented the most abundant taxa in most samples. The large number of samples and the time-consuming nature of taxonomic analysis precluded species identification. However, previous studies demonstrated the efficacy of assessing polychaete diversity at the family level as a valid alternative to species-level analysis when investigating patterns of community structure, functional diversity, species distribution and effects of environmental variables on biological communities (Fauchald and Jumars, 1979; Jumars et al., 2015; Checon and Amaral, 2017). For instance, biological traits such as feeding mode or reproductive strategies are usually conserved among polychaete species belonging to the same family, with just a few exceptions (Fauchald and Jumars, 1979; Jumars et al., 2015)

We calculated total macrofaunal densities (expressed as $ind \cdot m^{-2}$ to standardize results based on different sampling effort) and total number of taxa (including classes, subclasses and

polychaete families), as well as Simpson's index (d), Pielou's evenness (J'), Shannon-Wiener index (H') and expected number of taxa [ES(100)] for each sample combining the data from the entire 10 cm cores. Diversity indices were calculated in PRIMER 6+ using the DIVERSE routine. For each sample, we also calculated the proportion of macrofaunal organisms in the top 5-cm sediment layer (total number of organisms in the 0-5 cm layer / total number of organisms in the 0-10 cm core) as a measure of macrofaunal vertical distribution.

3.3.4 Macrofaunal biological trait expression

To evaluate the biological trait composition of the entire macrofaunal assemblages we used Biological Trait Analysis (Bremner et al., 2003). We selected 6 biological traits related to different aspects of life histories and ecosystem functioning, that were subdivided into 26 categories to characterize behaviour/strategies in more detail (**Table 3.2**). We included both "response" and "effect" traits that could provide insights into both the environmental drivers of community composition and the potential effects of macrofauna on ecosystem functioning (**Table 3.2**), noting that this approach cannot quantify ecosystem functioning *per se* (Bremner, 2008). We also partly based our selection on the availability of data on trait expression for the taxa collected. We used a fuzzy coding approach to assign trait categories to the taxa (classes, subclasses, or families), allowing each taxon to represent more than one trait modality and therefore capture inter- and intraspecific variation in trait expression. We adopted a scoring range of 0 to 5, with 0 reflecting no affinity for the given trait category, 1, 2, 3 or 4 reflecting partial, increasing affinity, and 5 denoting exclusive affinity. We derived information on trait expression for all the taxa from several published sources (Fauchald and Jumars, 1979; Highsmith and Coyle, 1991; Hyne, 2011; Queirós et al., 2013; Jumars et al., 2015; Polytraits, 2020) as well as from direct observations on our specimens. When information was unavailable for a given taxon, we obtained information from

one taxonomic rank higher (*e.g.*, from orders for polychaetes). In a few cases where no information was available for a given taxon, we distributed the 5 scores equally among all the plausible trait categories. For each taxon, we multiplied trait expression by the density of the taxon (ind · m^{-2}) in each sample and calculated the total trait expression across taxa to create a biological trait matrix for use in our multivariate analyses.

Table 3.2: Biological traits and categories used in trait analysis, with definitions and description of their ecological relevance.

Trait	Definition and ecological relevance	Modalities
Motility	Capability of an organism to move spontaneously and freely. Affects: habitat provisioning, reworking of sediments, communities' sensitivity and resilience (Hinchey et al., 2006; Bremner et al., 2006). Responds to: hydrodynamics, natural and anthropogenic disturbance, food availability and source (Harris, 2014; Pierdomenico et al., 2019.	Motile Discretely motile Sessile/sedentary
Feeding mode	Common feeding strategy of an organism (*e.g.*, food items that it is enzymatically and behaviourally capable of using). Affects: trophic structure, resource use and energy transfer, biogeochemical processes (Rosenberg, 1995; Norling et al., 2007). Responds to: hydrodynamics, resource availability, habitat stability and heterogeneity (Rosenberg, 1995; Simboura et al., 2000; Rossi et al., 2001; van der Zee et al., 2015).	Suspension/filter feeder Surface deposit feeder Subsurface deposit feeder Omnivore Predator Scavenger
Bioturbation	Capability of an organism to rework sediments through movement, feeding, and other activities. Affects: sediment biogeochemical properties, organic matter remineralization, nutrient recycling, carbon sequestration (Mermillod-Blondin et al., 2004; Hooper et al., 2005).	None Surface modifier Biodiffuser Upward conveyor Downward conveyor

Table 3.2 (continued)

Lifespan	Maximum length of time that an organism can be expected to live. Affects: susceptibility, resilience (de Juan et al., 2007). Responds to: environmental stability, resource availability (Pearson and Rosenberg, 1978; Beauchard et al., 2017).	<1 year 1-3 years 3-5 years >5 years
Larval development	Mode of development from the larval to the adult stage and habitat type of the larval settlement and early development after metamorphosis. Affects: recolonization potential, resilience, nutrient cycling (Thrush and Whitlatch, 2001; Degen et al., 2018; Bolam et al., 2020). Responds to: environmental stability and resource availability (Fauchald, 1983).	Direct Indirect Benthic Pelagic
Fecundity	The potential reproductive capacity of an organism (*e.g.*, number of gametes (eggs) or asexual propagules produced). Affects: recolonization potential, resilience (de Juan et al., 2007). Responds to: environmental stability, resource availability (Pearson and Rosenberg, 1978; Beauchard et al., 2017).	Low Medium High Very high

3.3.5 Sedimentary properties

We collected sediments for analysis of organic matter and grain size from the upper 0-2 cm of the dedicated 2-3 cores at each station, homogenizing the sediment and then placing it in Whirl-Pak bags prior to storage in the dark at -20 °C until analyzed (except for samples for lipid analysis, which were stored in pre-combusted aluminium tin foil at -80 °C). In this study, we use Total Organic Matter (TOM) and Total Organic Carbon (TOC) as a measure of food quantity. We use total nitrogen (TN) and total organic carbon to total nitrogen ratio (C: N) as a measure of organic matter quality over long time scales, with higher TN and lower C: N indicating fresher and higher quality organic matter (Godbold and Solan, 2009; Le Guitton et al., 2015; Campanyà-Llovet

et al., 2017). We use total lipids (TL) concentration as a measure of organic matter quality over intermediate-long time scales, with higher TL indicating higher nutritional value (Mayer, 1995; Parrish, 2013; Campanyà-Llovet et al., 2018). We use concentrations of chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), total pigments (Tot Pigm), as well as chlorophyll *a* to phaeopigments ratio (Chl *a*: Phaeo), and chlorophyll *a* to total organic carbon ratio (Chl *a*: TOC) as measures of phytodetritus input to the seafloor and short-term organic matter quality and freshness. In this sense higher concentrations of Chl *a* and higher Chl *a*: Phaeo and Chl *a*: TOC indicated higher inputs of fresh phytodetritus to the seafloor (Pusceddu et al., 2009; Le Guitton et al., 2015). We use % sand, % silt and % clay, and mean grain size of the sortable silt fraction (MGS), as a measure of sediment particle size and distribution.

Sediment total organic matter (TOM) was calculated as the difference between dry (desiccated at 60 ºC for 24 hours) and calcinated (muffle furnace at 450 ºC for 4 hours) weight, and expressed as mg · g DW^{-1} (Danovaro, 2010).

Total organic carbon (TOC) and total nitrogen (TN) were determined by drying a sediment subsample of 1-5 g (wet weight) at 60 °C for 24 h, grinding it to a fine powder, and then weighing and acidifying (with pure HCl fumes) for 24 h to eliminate inorganic carbon. Samples were dried again at 60 °C for 24 h before starting CHN analysis. We then weighed an aliquot of dried decarbonated sediments (15 mg) and folded it tightly into a tin capsule. A Carlo Erba NA1500 Series II elemental analyser (EA) determined the sediment concentration of TOC and TN, expressed as mg · g DW^{-1}.

Lipid samples were extracted (from sediment samples collected in September 2017 only) with a combination of chloroform and methanol according to Parrish (1999). We determined lipid class composition with a three-step chromatographic development method (Parrish, 1987).

Samples were analysed using an Iatroscan Mark VI TLC-FID (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan). We collected the data using Peak Simple software (ver 3.67, SRI Inc) and calibrated the Chromarods with standards prior to and during their use on our samples. The concentration of total lipids was estimated by summing all the lipid classes and expressed as mg · g WW^{-1}.

Sedimentary concentrations of chloroplastic pigments (chlorophyll *a* and phaeopigments) were determined using a spectrophotometer following Danovaro (2010). Pigments were extracted with 90% acetone (24 h in the dark at 4 °C). After centrifugation (800 x g), we used the supernatant to determine the functional chlorophyll *a* and acidified with 0.1 N HCl to estimate the concentration of phaeopigments. We then dried the sediment at 60 ºC for 24 h prior to weighing. Sediment concentrations of pigments were expressed as μg · g DW^{-1}. Total phytopigment concentrations were defined as the sum of chlorophyll *a* and phaeopigment concentrations, and utilized as an estimate of the organic material of algal origin, including the living (chlorophyll *a*) and senescent/detrital (phaeopigment) fractions (Pusceddu et al., 2009).

To determine granulometric properties, we digested a subsample of sediment with hydrogen peroxide to eliminate any organic material present and then freeze-dried sediments before analysis with a Beckman Coulter LD13-320 laser diffraction analyzer. Sieving was performed prior to analysis to ensure the elimination of large particles (gravel fraction > 2 mm), which however were not present in our sediment samples. For each sample, we determined the percentages of sand, silt and clay (with silt and clay together representing the mud fraction), as well as the mean grain size of the sortable silt fraction (MGS, μm). Sediments at each station were classified following the sediment classification scheme based on the percentage of sand, silt, and clay (Shepard, 1954).

3.3.6 Statistical analysis

Because preliminary results indicated variation in macrofaunal communities and sedimentary properties between the two sampling periods (t-tests and PERMANOVA, $p < 0.05$) and particularly given differences in sampling tools, we analyzed the data from September 2017 and July 2018 separately. This also allowed us to better identify any effects of biogenic habitat on macrofauna and the variables that contributed to spatial variation. For the stations sampled in July 2018, we averaged macrofauna data from the two cores from the same multicorer drop and used the resulting three values per station as replicates in the analysis to avoid pseudoreplication.

To investigate variation in total macrofaunal density, total number of taxa, Simpson's index, Pielou's evenness, Shannon-Wiener index, expected number of taxa, and vertical distribution between sea pen fields and other sedimentary habitats, and among stations within each biogenic habitat, we used two-way type III univariate analysis of variance (ANOVA). We used a nested design with the fixed factors "biogenic habitat" (2 levels) and "station (biogenic habitat)" (3 levels within sea pen fields and 4 levels within other sedimentary habitats in September 2017; 1 level within sea pen fields and 3 levels within other sedimentary habitats in July 2018). We verified that our data met homogeneity of variance (with Levene's tests) and normality (with Q-Q plots of residuals) assumptions prior to analysis. We ran post hoc pair-wise analyses whenever we found significant differences ($p < 0.05$) for the factor station (biogenic habitat). We additionally used species accumulation curves showing total number of macrofaunal taxa as a function of sampling effort (m^2) at each station to evaluate the adequacy of our sampling to describe diversity.

We used multivariate statistics to identify differences in macrofaunal community composition and biological trait expression (separately) between sea pen fields and other sedimentary habitats and among stations within each biogenic habitat. After applying a square-

root transformation to density and biological trait data, we generated Bray-Curtis similarity matrices (suitable for datasets that include a large number of zeros) and tested for significant differences between biogenic habitats (fixed factor "biogenic habitat" with 2 levels) and among stations nested in biogenic habitats (fixed factors "station (biogenic habitat)" with 3 levels within sea pen fields and 4 levels within other sedimentary habitats in September 2017; 1 level within sea pen fields and 3 levels within other sedimentary habitats in July 2018) with Permutational Multivariate Analysis of Variance (PERMANOVA), performed with 9999 random permutations. We ran the pair-wise comparisons as post hoc analyses whenever we found significant differences among station (biogenic habitat) ($p < 0.05$). We used a percent similarity procedure analysis (SIMPER; Clarke and Gorley, 2006) to identify the taxa and trait categories that distinguished assemblages in sea pen fields versus other sedimentary habitats. Non-metric multidimensional scaling (nMDS) ordinations of similarity matrices provided visualizations of multivariate patterns. To assess dispersion in macrofaunal community composition and biological trait expression between stations and biogenic habitats we ran a Permutational Analysis of Multivariate Dispersion (PERMDISP; Anderson et al., 2008).

To assess the localized, small-scale effect of the presence of sea pens in the cores (for 4 of the stations sampled in September 2017) on macrofaunal total density, all taxonomic diversity indices, and vertical distribution, we used independent Student's or Welch's (for not homogeneous data) t-tests, after assessing our data for homogeneity of variance (with Levene's tests) and normality (with Q-Q plots of residuals). We also assessed the localized, small-scale effect of the presence of sea pens in the cores on macrofaunal community composition and biological trait expression (separately) using Permutational Multivariate Analysis of Variance (PERMANOVA), performed with 9999 random permutations. For both these analyses, we tested differences between

cores containing sea pens and cores without (fixed factor with 2 levels) and analysed the 4 stations separately to better discern larger- from smaller-scale effects. The low number of replicates did not allow us to test the effect of different genera of sea pens on macrofauna.

To identify the best predictors of macrofaunal community composition and biological trait expression (separately), we used distance-based linear models (DistLM; McArdle and Anderson, 2001). Because of the large number of explanatory variables used in our analyses, we first tested the effect of each group of variables separately: physico-chemical characteristics (depth, bottom water temperature, salinity, and oxygen concentration), biogenic features (average total sea pen density, average *Pennatula* spp. density, and average sea pig coverage), sediment grain size (% sand, % mud, % silt, % clay, and MGS), organic matter quantity (TOC and TOM), and organic matter quality (TL, TN, C: N, Chl *a*, Phaeo, Tot Pig, Chl *a*: Phaeo, and Chl *a*: TOC). We then selected the variables from each group that correlated best with macrofaunal community composition and biological trait expression (separately) and combined all these selected variables in a final analysis to determine which variable(s) best explained total variation. We used "best" and "step-wise" selection procedures based on AIC (Akaike's information criterion) to identify the best model and we used resemblance matrices based on Bray-Curtis similarity calculated from square-root transformed community composition and biological trait expression data as a measure of between-samples similarity. All environmental variables were standardized to mean 0 and standard deviation 1 prior analyses. We explored relationships among environmental variables and data skewness using Draftsman plots and ensured that highly correlated variables did not simultaneously appear in the final models. We examined R^2 to identify the best models and determine the proportion of the variation explained by the models. We used distance-based

redundancy analysis (dbRDA) to visualize the best environmental models explaining variation in macrofaunal community composition and biological trait expression.

All PERMANOVA, PERMDISP, SIMPER, nMDS, DistLM and dbRDA analyses were performed in PRIMER v6 with the PERMANOVA+ add on (Anderson et al., 2008).

3.4 Results

3.4.1 Macrofaunal density, taxonomic diversity, and vertical distribution

We sorted over 15,000 macrofaunal organisms, representing 56 different taxa. Densities varied from 28,991 to 66,381 ind · m^{-2} (at St 16 and St 2, respectively). Polychaetes were the dominant taxon in most samples (averaging 34% of total macrofauna), followed by ostracods (averaging 29% of total macrofauna) and amphipods (averaging 20% of total macrofauna). Among the polychaetes, the families Paraonidae, Opheliidae and Cirratulidae dominated across the stations, representing averages of 27%, 14%, and 9% of total polychaetes, respectively.

For the stations sampled in September 2017 (**Figure 3.3, left side of panels**), we found significantly higher macrofaunal density and taxonomic diversity (all indices considered) in sea pen fields compared to other sedimentary habitats (ANOVA, $p < 0.05$, **Appendix 3A**). We found no significant differences in macrofaunal density and taxonomic diversity among stations nested in biogenic habitat (ANOVA, $p > 0.05$, **Appendix 3A**). Species accumulation curves (**Figure 3.4**) confirmed higher number of taxa in two of the sea pen fields (St 2 and St 14) when compared to other biogenic habitats, also confirming sufficient sampling effort as curves started reaching saturation at all stations.

For the stations sampled in July 2018 (**Figure 3.3, right side of panels**), we found significantly higher total macrofaunal density and lower Shannon-Wiener index and expected

number of taxa in sea pen fields compared to other sedimentary habitats (ANOVA, $p < 0.05$, **Appendix 3A**). We found no significant differences between biogenic habitats for the other diversity indices, nor significant differences in macrofaunal density and taxonomic diversity among stations nested in biogenic habitat (ANOVA, $p > 0.05$, **Appendix 3A**). Species accumulation curves (**Figure 3.4**) confirmed these results and all curves reached saturation, indicating sufficient sampling effort.

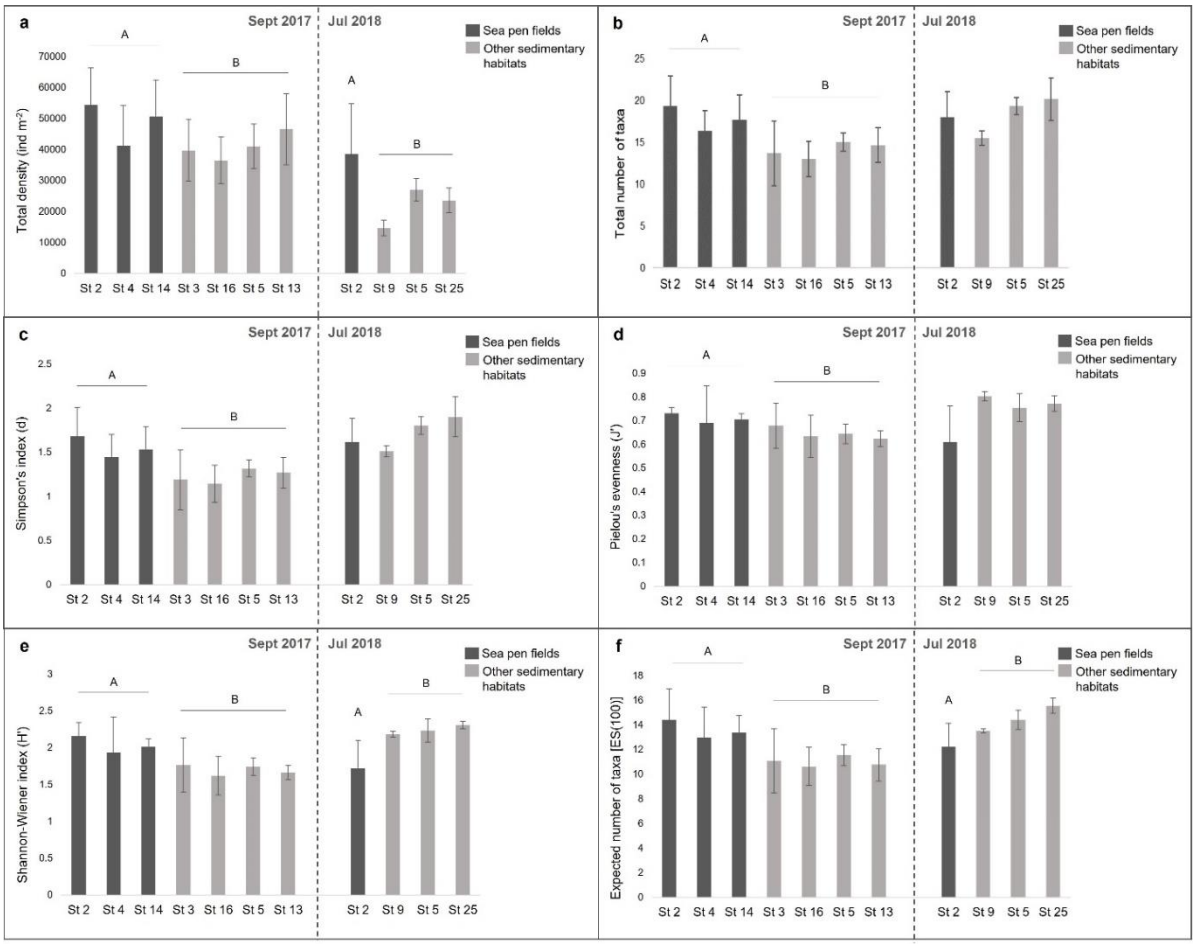

Figure 3.3: Macrofaunal density and taxonomic diversity indices at stations sampled in September 2017 (left sides) and July 2018 (right sides) expressed as average ± standard deviation. **a)** Total macrofaunal density expressed as ind·m^2; **b)** Total number of taxa; **c)** Simpson's index (d); **d)** Pielou's evenness (J'); **e)** Shannon-Wiener index (H'); **f)** Expected number of taxa [ES(100)]. Within each sampling period, stations are ordered on the x-axis from highest to lowest average total sea pen density. Letters highlight significant differences between biogenic habitats within each sampling period, when present.

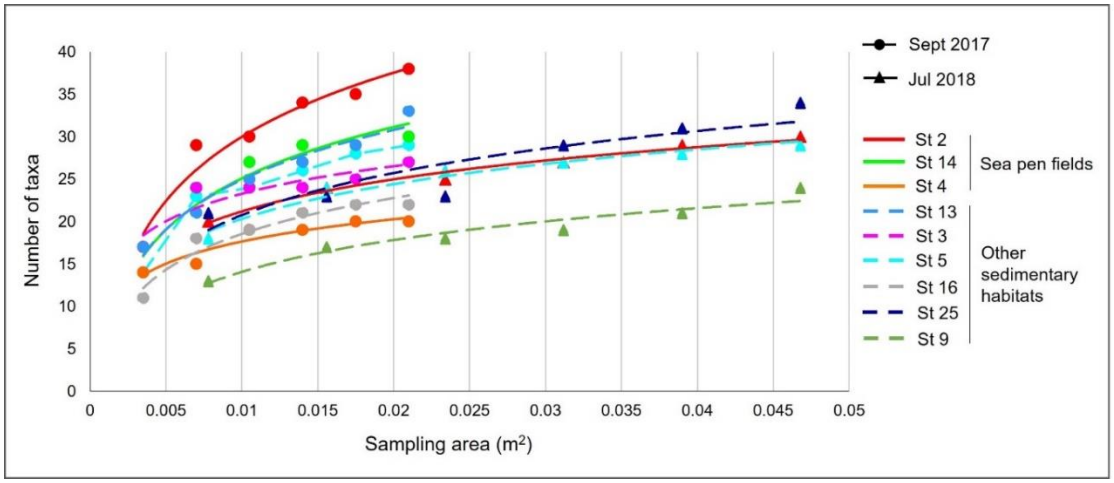

Figure 3.4: Species accumulation curves showing number of macrofaunal taxa per sampling effort (m^2) at each of the stations sampled in September 2017 and July 2018.

For the stations sampled in September 2017, we found a significantly higher proportion of organisms in top 5-cm layer in sea pen fields compared to other sedimentary habitats, as well as significant differences among stations nested in biogenic habitat (ANOVA, $p < 0.05$, **Appendix 3B**, **Figure 3.5, left side**), with higher proportion of organisms in the 0-5 cm layer at St 2 compared to St 14 and St 4 within sea pen fields, according to post hoc pair-wise tests. For the stations sampled in July 2018, we found no significant differences in the vertical distribution of macrofauna between biogenic habitats nor among stations nested in biogenic habitat (ANOVA, $p > 0.05$, **Appendix 3B**, **Figure 3.5, right side**).

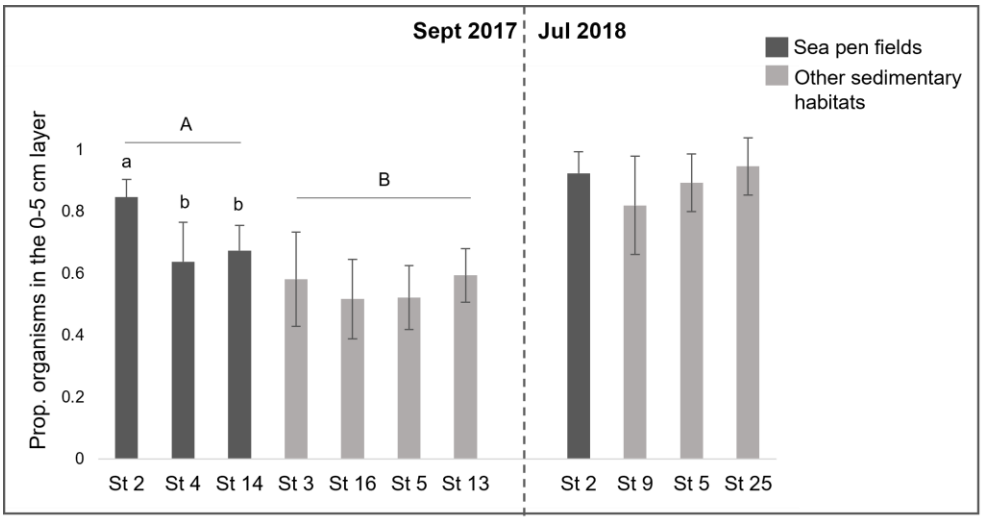

Figure 3.5: Relative vertical distribution of macrofauna in the cores at stations sampled in September 2017 (left side) and July 2018 (right side) expressed as average ± standard deviation of the proportion of macrofaunal organisms in the upper 5-cm layer. Letters highlight significant differences among stations (a,b; ANOVA, $p < 0.05$) and among biogenic habitats (A,B; ANOVA $p < 0.05$) within each sampling period, when present.

3.4.2 Macrofaunal community composition

For the stations sampled in September 2017, we found significant differences in macrofaunal community composition between sea pen fields and other sedimentary habitats, as well as among stations nested in biogenic habitat (PERMANOVA, $p < 0.05$, **Appendix 3C**). Post hoc pair-wise tests revealed that within sea pen fields, St 2, St 14, and St 4 differed significantly from each other (PERMANOVA, $p < 0.05$, **Appendix 3C)**. Stations and habitats also clearly separated in ordination space according to our nMDS analysis (**Figure 3.6, a**), where we consider the stress level of 0.18 to be acceptable (Clarke, 1993), particularly given the confirmatory PERMANOVA results. Data were homogeneously dispersed among stations and habitats (PERMDISP, $p > 0.05$). SIMPER analysis revealed greater abundance of cirratulid, cossurid, nerellidid and paraonid polychaetes, and amphipods in sea pen fields, in contrast to higher abundance of opheliid polychaetes, ostracods, bivalves, and scaphopods in other sedimentary

habitats. These taxa alone explained 59% of the dissimilarities between sea pen fields and other sedimentary habitats, whose average dissimilarity was 38%.

For the stations sampled in July 2018, we found significant differences in macrofaunal community composition between sea pen fields and other sedimentary habitats and among stations nested in biogenic habitat (PERMANOVA, $p < 0.05$, **Appendix 3C**). Post hoc pair-wise tests revealed that within other sedimentary habitats, St 25 and St 9 differed significantly from each other (PERMANOVA, $p < 0.05$, **Appendix 3C)**. Stations and habitats also clearly separated in ordination space according to our nMDS analysis (**Figure 3.6, b**). Data were homogeneously dispersed among stations and habitats (PERMDISP, $p > 0.05$). SIMPER analysis revealed that amphipods, bivalves, scaphopods and cirratulid polychaetes were more abundant in sea pen fields, whereas ostracods and opheliid polychaetes were more abundant in other sedimentary habitats. These taxa alone explained 52% of the dissimilarities between sea pen fields and other sedimentary habitats, whose average dissimilarity was 30%.

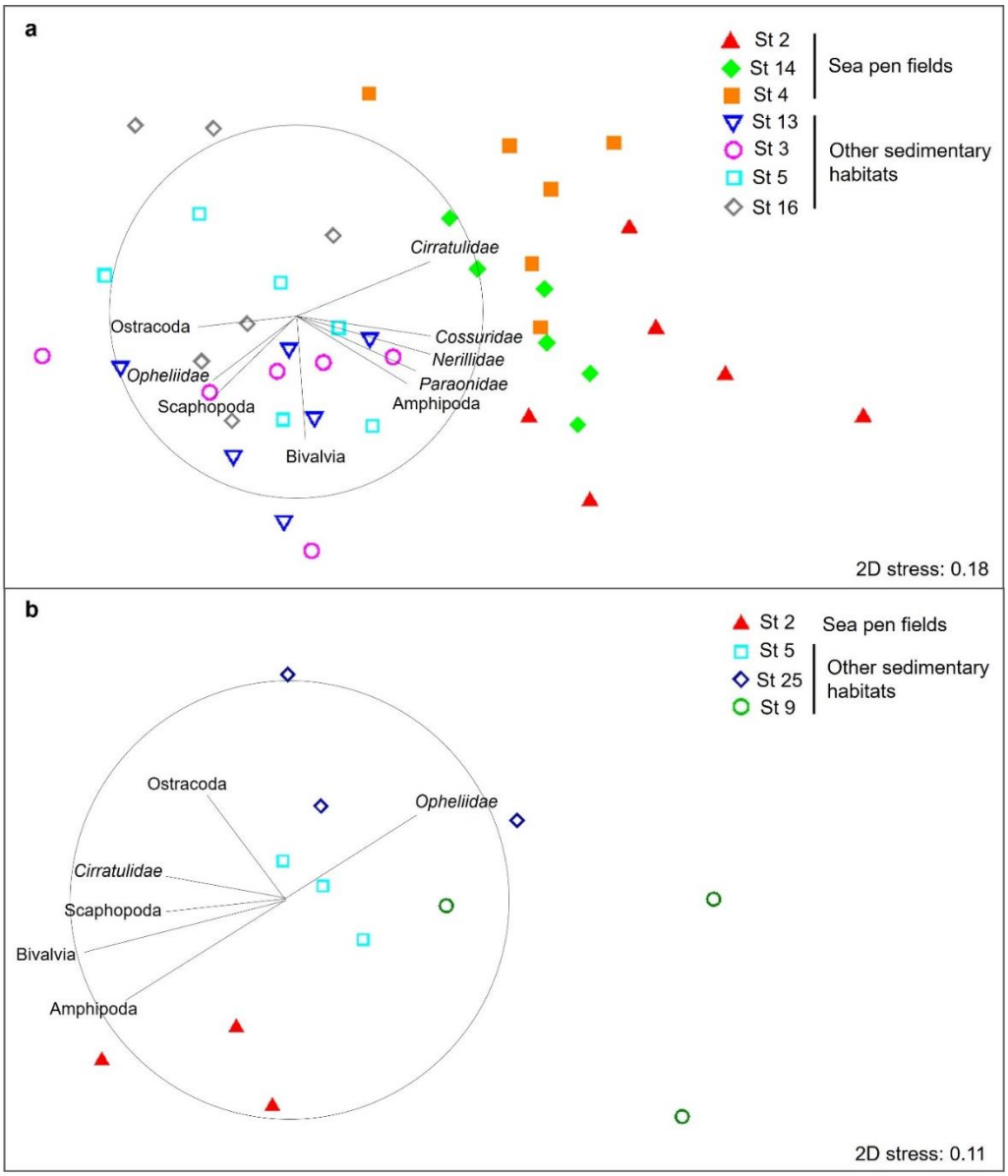

Figure 3.6: Nonmetric multidimensional scaling (nMDS) of the macrofaunal communities in the Laurentian Channel MPA, based on Bray-Curtis similarity of square root transformed density data. Vectors show the taxa and polychaete families (italic font) mostly contributing to the differences between sea pen fields and other sedimentary habitats (selected based on SIMPER results). **a**) Stations sampled in September 2017; **b**) Stations sampled in July 2018.

3.4.3 Macrofaunal biological trait expression

Macrofaunal communities in the Laurentian Channel MPA were dominated overall by motile deposit feeders with indirect development, pelagic larvae, low or medium fecundity,

lifespans between 1 and 5 years, surface modifiers and biodiffusers as bioturbation mode. Within these general patterns, our analysis discerned some differences among biogenic habitats and stations.

For the stations sampled in September 2017, we found significant differences in macrofaunal biological trait expression between sea pen fields and other sedimentary habitats (PERMANOVA, $p < 0.05$, **Appendix 3C**), but no significant differences among stations nested in biogenic habitat (PERMANOVA, $p > 0.05$, **Appendix 3C**). Biogenic habitats also clearly separated in ordination space according to our nMDS analysis (**Figure 3.7, a**). Data were homogeneously dispersed among stations and habitats (PERMDISP, $p > 0.05$). SIMPER analysis identified the trait modalities that contributed most to differences among biogenic habitats. In order of decreasing importance, they included: "direct larval development", "benthic larval development", "high fecundity", "surface modifiers", "motile", "lifespan 3-5 years", which all showed higher expression in sea pen fields compared to other sedimentary habitats, and "indirect larval development" and "low fecundity", which displayed higher expression in other sedimentary habitats. These trait modalities alone explained up to 53% of the dissimilarities between sea pen fields and other sedimentary habitats, whose average dissimilarity was 12%.

For the stations sampled in July 2018, we found significant differences in macrofaunal biological trait expression between sea pen fields and other sedimentary habitats, as well as among stations nested in biogenic habitat (PERMANOVA, $p < 0.05$, **Appendix 3C**). Post hoc, pair-wise tests revealed that within other sedimentary habitats, St 5 differed significantly from St 25 and St 9 (PERMANOVA, $p < 0.05$, **Appendix 3C**). Stations and habitats also clearly separated in ordination space according to our nMDS analysis (**Figure 3.7, b**). Data were homogeneously dispersed among stations and habitats (PERMDISP, $p > 0.05$). SIMPER analysis identified the

trait modalities that contributed most to differences among biogenic habitats. In order of decreasing importance, they included: “indirect larval development”, “pelagic larval development”, “high fecundity”, “motile”, “lifespan 3-5 years”, “omnivore”, “suspension/filter feeder”, and “no bioturbation”, which all displayed higher expression in sea pen fields compared to other sedimentary habitats. These trait modalities alone explained 52% of the dissimilarities between sea pen fields and other sedimentary habitats, whose average dissimilarity was 17%.

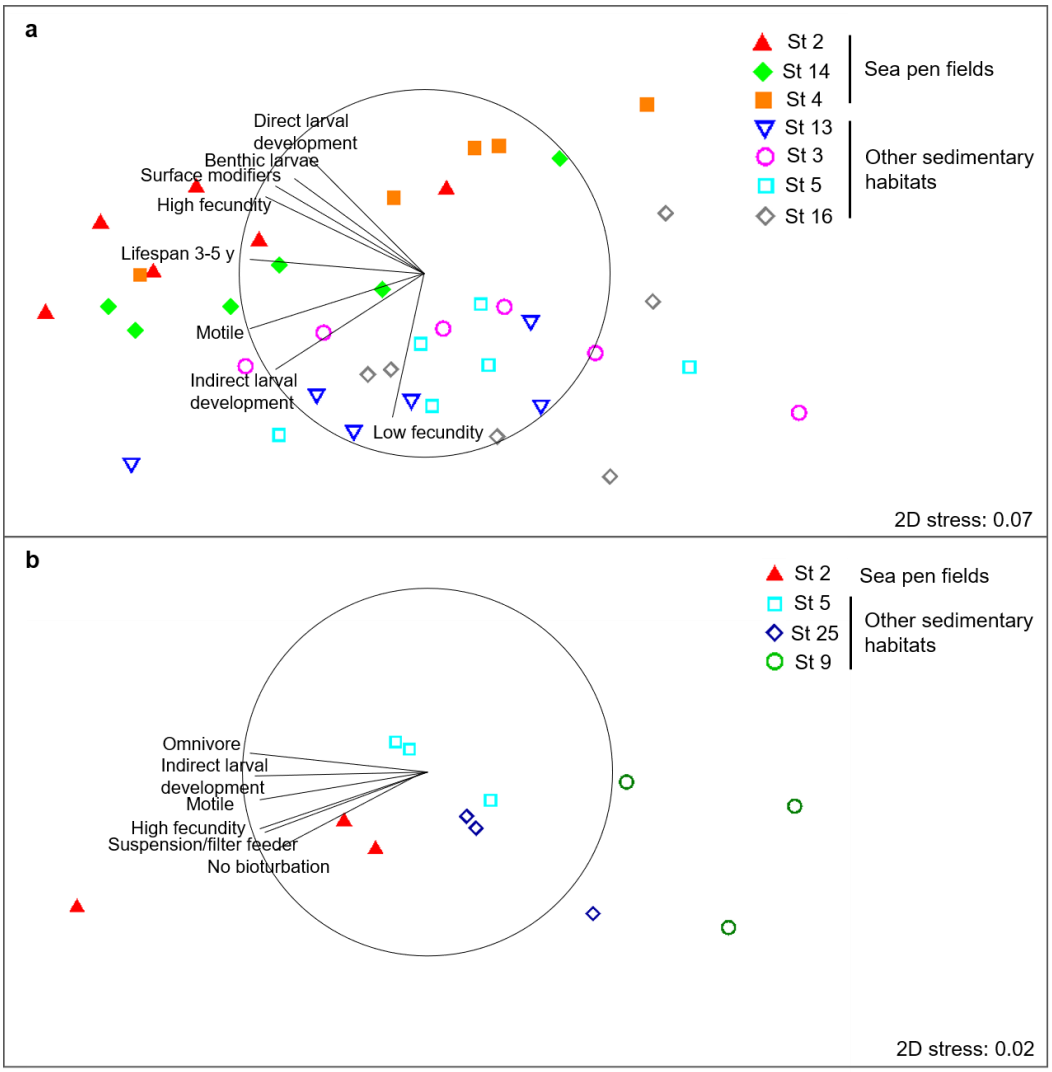

Figure 3.7: Nonmetric multidimensional scaling (nMDS) of the macrofaunal biological trait expression in the Laurentian Channel MPA, based on Bray-Curtis similarity of square root transformed trait expression data. Vectors show the trait categories mostly contributing to the differences between sea pen fields and other sedimentary habitats (selected based on SIMPER results). **a**) Stations sampled in September 2017; **b)** Stations sampled in July 2018.

3.4.4 Small-scale effect of sea pens in the cores on macrofauna

Among the 4 stations sampled in September 2017, we found significantly higher (t-tests, $p < 0.05$, **Appendix 3D**) total macrofaunal density (**Figure 3.8, a**), number of taxa (**Figure 3.8, b**), Simpson's index, and expected number of taxa in cores with sea pens than those with no sea pens at St 3 only (other sedimentary habitat). Here, Pielou's evenness and Shannon-Wiener index did not vary significantly between cores with and without sea pens (t-tests, $p > 0.05$, **Appendix 3D**). In contrast, at St 2, St 14, and St 4 (all sea pen fields), we found no significant small-scale effect of sea pens in cores on macrofaunal density and taxonomic diversity (t-test, $p > 0.05$, **Appendix 3D**).

Our analysis detected no significant differences in vertical distribution of organisms (ANOVA, $p > 0.05$, **Appendix 3E**), nor in macrofaunal community composition and biological trait expression (PERMANOVA, $p > 0.05$, **Appendix 3F**) between cores containing sea pens and cores with no sea pens, within any of the stations considered.

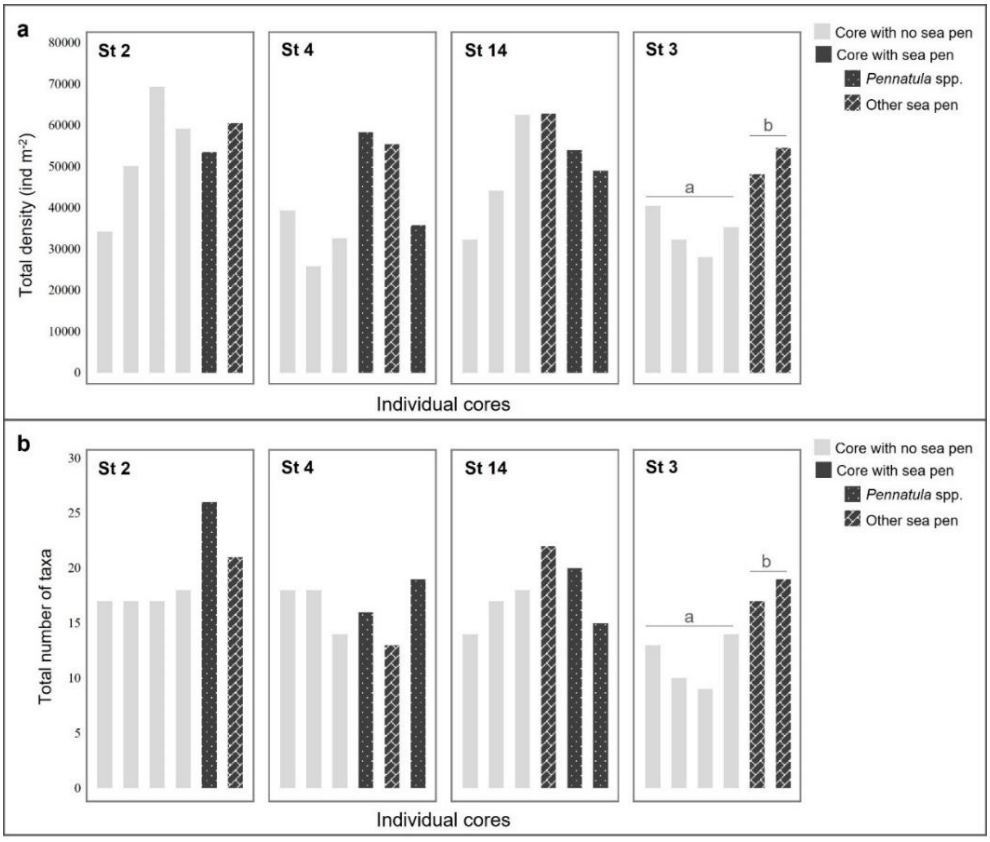

Figure 3.8: Total macrofaunal density (**a**) and total macrofaunal taxa richness (**b**) in sea pen vs no sea pen cores in the 4 stations sampled in September 2017. The analysis only assessed differences between cores with sea pens and cores without, however, the figures also differentiate cores containing sea pens belonging to the order *Pennatula* from cores containing other sea pens (which included *Funiculina* spp., *Anthoptilum* spp., *Protoptilum* spp., and *Kophobelemnon* spp.). Letters indicate significant differences between cores with and without sea pens (ANOVA, $p < 0.05$).

3.4.5 Sedimentary properties

Table 3.3 summarizes organic matter quantity and quality for all the stations sampled. In September 2017, we found overall higher organic matter quantity (TOM and TOC) and long-term quality (*e.g.*, higher TN, lower C: N) of sedimentary organic matter in two of our sea pen field stations (St 2 and St 14) compared to other stations, which showed intermediate quantity and quality. The other sea pen field station (St 4) was instead characterized by lower quantity and the lowest long-term quality of organic matter (*e.g.*, lowest TN and highest C: N). Different patterns

were observed for variables related to input of phytodetritus (*e.g.*, Chl *a*, Chl *a*: TOC) and short-term organic matter quality (*e.g.*, Chl *a*: Phaeo), with highest values at St 5 and St 13, indicative of fresh input of phytodetritus. In July 2018, St 2 was again characterized by higher organic matter quantity and long-term quality compared to other station, even though St 9 had the lowest C: N, which suggests higher long-term quality. Higher Chl *a* in the stations sampled in July 2018 compared to stations sampled in September 2017, suggests higher input of phytodetritus in the summer, consistent with the seasonality of phytoplanktonic blooms in the area. Higher input of phytodetritus was observed at St 2 and St 5 in July 2018. **Table 3.4** summarizes granulometric properties of the stations, which all had clayey-silt sediments, except for St 4 and St 25, both characterized by coarser sediments (sandy silt).

Table 3.3: Summary of main organic matter quantity and quality parameters measured in the Laurentian Channel stations sampled in September 2017 and July 2018 (average ± standard deviation derived from 2 or 3 replicate samples per station). TOM: total organic matter; TOC: total organic carbon; TN: total nitrogen; C: N: carbon to nitrogen ratio; Chl *a*: chlorophyll *a*; Chl *a*: Phaeo: chlorophyll *a* to phaeopigments ratio; Chl *a*: TOC: chlorophyll *a* to total organic carbon ratio. *values derived from only one replicate sample per station.

Station	Sampling period	Biogenic habitat	TOM (mg · g DW^{-1})	TOC (mg · g DW^{-1})	TN (mg · g DW^{-1})	C: N	Chl *a* (µg · g DW^{-1})	Chl *a*: Phaeo	Chl *a*: TOC
St 2	Sept 2017	Sea pen field	106.8 ± 10.2	36.0 ± 2.1	3.8 ± 0.05	9.6 ± 1.3	2.7 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
St 4	Sept 2017	Sea pen field	79.1 ± 14.3	33.7 ± 2.9	1.7 ± 0.02	20.4 ± 2.7	1.8 ± 0.8	0.1 ± 0.0	0.0 ± 0.0
St 14	Sept 2017	Sea pen field	114.7 ± 7.2	39.8 ± 0.9	3.6 ± 0.36	11.1 ± 0.9	2.0 ± 0.0*	0.2 ± 0.0*	0.0 ± 0.0*
St 3	Sept 2017	Other sed. hab.	82.7 ± 13.5	25.7 ± 5.3	2.1 ± 0.02	12.0 ± 0.2	1.7 ± 1.0	0.2 ± 0.0	0.1 ± 0.0
St 16	Sept 2017	Other sed. hab.	100.1 ± 10.3	30.7 ± 4.8	3.2 ± 0.05	9.7 ± 0.2	1.9 ± 0.3	0.1 ± 0.1	0.1 ± 0.0
St 5	Sept 2017	Other sed. hab.	73.9 ± 10.0	31.1 ± 0.2	2.9 ± 0.03	11.0 ± 0.7	2.6 ± 0.2	0.2 ± 0.0	0.1 ± 0.0
St 13	Sept 2017	Other sed. hab.	111.2 ± 43.7	30.0 ± 1.2	2.6 ± 0.02	11.7 ± 2.1	2.9 ± 1.3	0.3 ± 0.0	0.1 ± 0.0
St 2	Jul 2018	Sea pen field	85.6 ± 25.4	47.9 ± 0.5	4.2 ± 0.1	11.5 ± 0.4	5.5 ± 2.7	0.1 ± 0.1	0.1 ± 0.1
St 5	Jul 2018	Other sed. hab.	63.4 ± 15.3	40.1 ± 0.3	3.2 ± 0.1	12.5 ± 0.7	5.2 ± 0.0*	0.2 ± 0.0*	0.1 ± 0.0*
St 25	Jul 2018	Other sed. hab.	44.4 ± 17.2	42.1 ± 0.6	2.3 ± 0.1	18.4 ± 1.1	3.6 ± 1.1	0.2 ± 0.0	0.1 ± 0.0
St 9	Jul 2018	Other sed. hab.	48.0 ± 7.1	26.0 ± 1.1	3.1 ± 0.3	8.5 ± 0.6	1.9 ± 1.0	0.1 ± 0.1	0.1 ± 0.0

Table 3.4: Summary of granulometric properties of the sediments the Laurentian Channel stations sampled in September 2017 and July 2018, based on one sample per station. MGS: mean grain size of the sortable silt fraction. Sedim. Class.: sediment type classification following the Shepard (1954) scheme based on relative percentages of sand, silt, and clay.

Station	Sampling period	Biogenic habitat	% sand	% silt	% clay	MGS (μm)	Sedim. class.
St 2	Sept 2017	Sea pen field	1.9	64.3	33.8	21.3	Clavey silt
St 4	Sept 2017	Sea pen field	34.5	52.9	12.5	29.7	Sandy silt
St 14	Sept 2017	Sea pen field	10.2	62.6	27.3	24.7	Clavey silt
St 3	Sept 2017	Other sed. hab.	1.8	63.2	35.0	18.7	Clavey silt
St 16	Sept 2017	Other sed. hab.	3.6	60.1	36.3	20.3	Clavey silt
St 5	Sept 2017	Other sed. hab.	3.5	61.9	34.6	21.9	Clavey silt
St 13	Sept 2017	Other sed. hab.	2.1	62.3	35.7	20.3	Clavey silt
St 2	Jul 2018	Sea pen field	9.3	64.0	26.7	24.2	Clavey silt
St 5	Jul 2018	Other sed. hab.	9.7	59.5	30.9	24.6	Clavey silt
St 25	Jul 2018	Other sed. hab.	29.1	52.0	18.9	30.0	Sandy silt
St 9	Jul 2018	Other sed. hab.	2.6	70.6	26.8	16.4	Clavey silt

3.4.6 Drivers of variation of macrofaunal community composition

For the stations sampled in September 2017, the best distance-based linear model (DistLM, **Table 3.5**) explained 40% of the variation of macrofaunal community composition and included the variables depth, % clay, and *Pennatula* spp. average density. Stations were separated in space based on redundancy (dbRDA) analysis from the best distance-based linear model (DistLM, **Figure 3.9, a**). The first axis of the dbRDA explained 26% of the total variation and correlated with depth and average *Pennatula* spp. density, and separated St 2, St 14 and St 4 (sea pen field stations, shallower and with higher *Pennatula* spp. densities) from the others. The second axis of the dbRDA explained 12% of the total variation and correlated mostly with % clay, separating St 2 (highest % clay) and St 4 (lowest % clay) from each other and all the other stations.

For the stations sampled in July 2018, the best distance-based linear model (DistLM, **Table 3.5**) explained 57% of variation of macrofaunal community composition and included the variables sedimentary concentration of TOC, *Pennatula* spp. average density, and MGS. Stations separated in space based on redundancy (dbRDA) analysis from the best distance-based linear model (DistLM, **Figure 3.9, b**). The first axis of the dbRDA explained 29% of the total variation and mostly correlated with sedimentary concentrations of TOC, separating St 2 (highest TOC) from St 25 and St 5 (intermediate TOC) and from St 9 (lowest TOC). The second axis of the dbRDA explained 16% of the total variation and mostly correlated with average *Pennatula* spp. density and MGS, separating St 2 from the others because of its higher density of *Pennatula* sea pens, as well as St 9 from the others because of its lower grain size.

Table 3.5: Statistical results of DistLM analysis (final models) for fitting environmental factors to macrofaunal community composition in the stations sampled in September 2017 and July 2018 (separately). Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AIC (Akaike Information Criteria), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model. TOC: concentration of total organic carbon; MGS: mean grain size.

Macrofaunal community composition							**Sept 2017**
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
Depth	6362.7	13.706	0.001	0.2552	254.48	0.404	14858
Pennatula spp density	4565.2	8.966	0.001	0.18311			
% Clay	4302.2	8.3417	0.001	0.17256			
Macrofaunal community composition							**Jul 2018**
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
TOC	1154.8	3.6726	0.001	0.26861	68.519	0.567	1859.6
Pennatula spp density	980.77	2.9556	0.013	0.22813			
MGS	820.99	2.3605	0.011	0.19097			

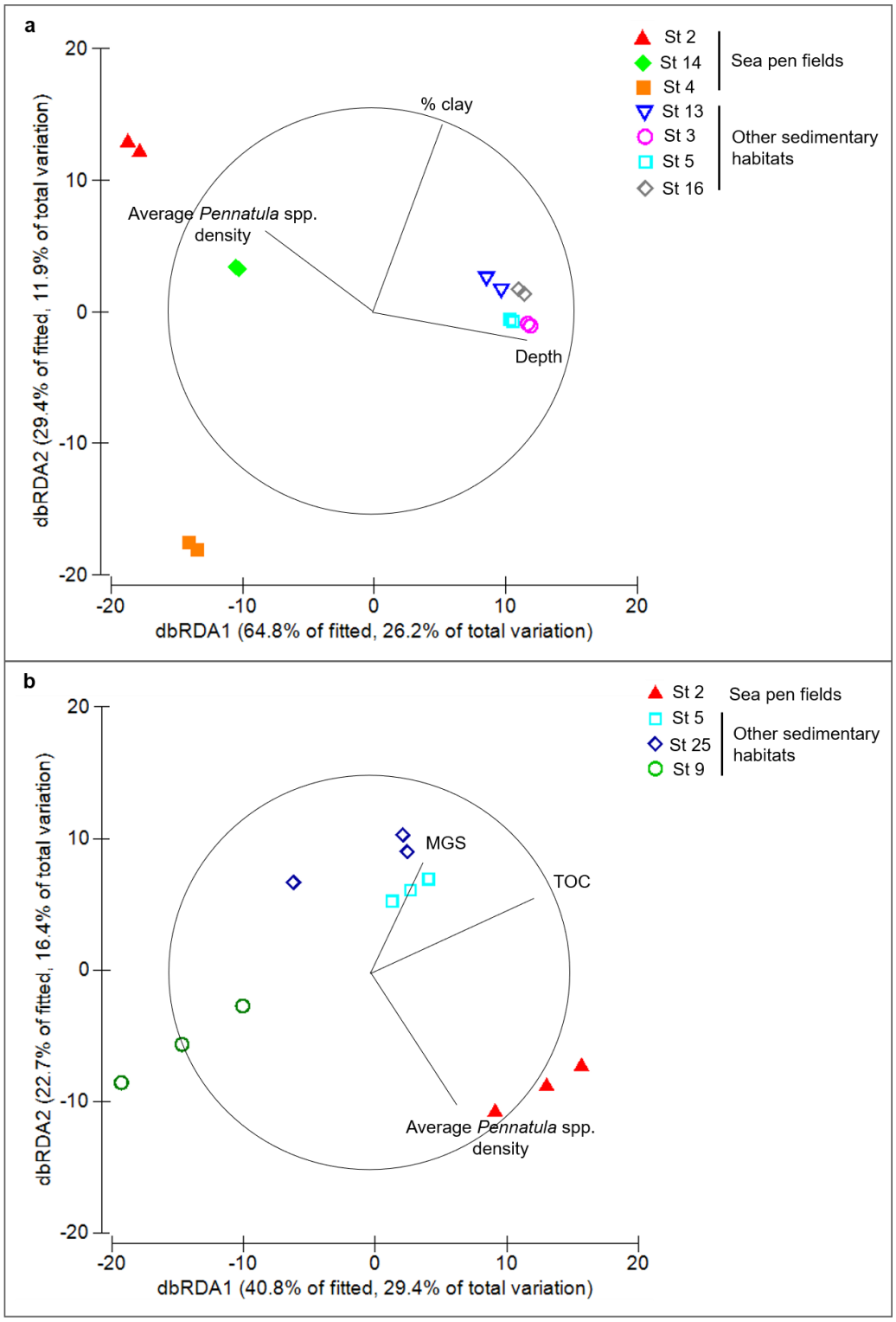

Figure 3.9: Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of macrofaunal community composition and all selected environmental variables for the stations sampled in September 2017 (**a**) and July 20108 (**b**). Vectors show direction and strength of environmental variables contributing to variation in macrofaunal community composition. TOC: concentration of total organic carbon; MGS: mean grain size.

3.4.7 Drivers of variation of macrofaunal biological trait expression

For the stations sampled in September 2017, the best distance-based linear model (DistLM, **Table 3.6**) explained 41% of variation of macrofaunal biological trait expression and included depth, sediment concentration of Chl *a*, and MGS as key explanatory variables. The 7 stations separated in space based on redundancy (dbRDA) analysis from the best distance-based linear model (DistLM, **Figure 3.10, a**). The first axis of the dbRDA explained 33% of the total variation and correlated best with depth and concentrations of Chl *a*, separating St 2 (shallower, with higher Chl *a*), from St 14 and St 4 (shallower but with lower Chl *a*) and from all the others (deeper, with variable concentrations of Chl *a*). The second axis of the dbRDA explained 7% of the total variation and correlated best with concentrations of Chl *a*.

For the stations sampled in July 2018, the best distance-based linear model (DistLM, **Table 3.6**) explained 82% of variation of macrofaunal biological trait expression and included sediment concentration of TOC and Chl *a*, *Pennatula* spp. average density, and MGS as key explanatory variables. The 4 stations separated in space based on redundancy (dbRDA) analysis from the best distance-based linear model (DistLM, **Figure 3.10, b**). The first axis of the dbRDA explained 67% of total variation and correlated best with concentrations of TOC and Chl *a*, separating St 2 (with higher concentrations of TOC and Chl *a*), from St 5 and St 25 (intermediate concentrations of TOC and Chl *a*) and from St 9 (lower concentrations of TOC and Chl *a*). The second axis of the dbRDA explained 10% of the total variation and correlated best with *Pennatula* spp. average density and concentrations of Chl *a*.

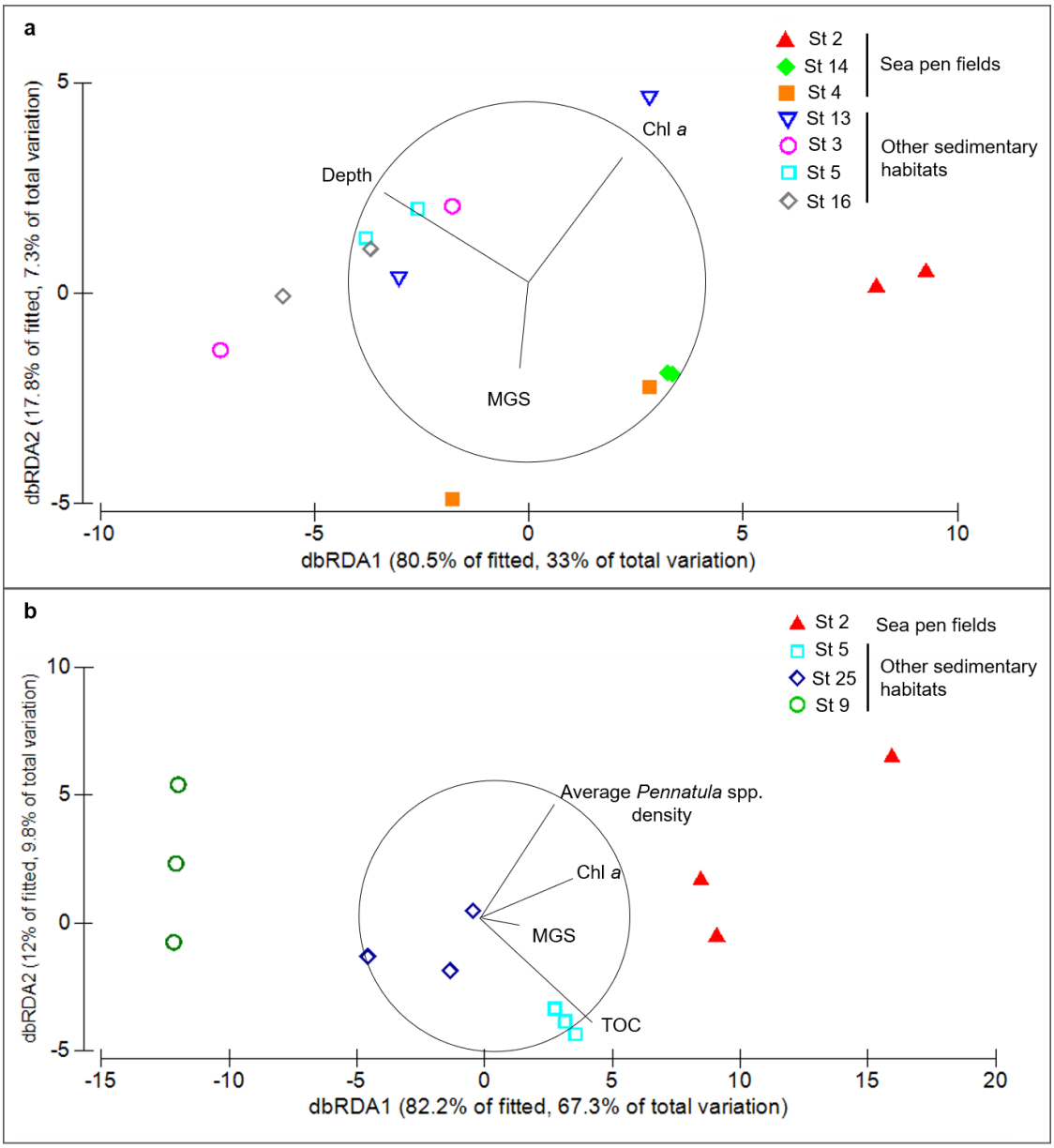

Figure 3.10: Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of macrofaunal biological trait expression and all selected environmental variables for the stations sampled in September 2017 (**a**) and July 20108 (**b**). Vectors show direction and strength of environmental variables contributing to variation in macrofaunal biological trait expression. Chl *a*: concentration of chlorophyll *a*; MGS: mean grain size; TOC: concentration of total organic carbon.

Table 3.6: Statistical results of DistLM analysis (final models) for fitting environmental factors to macrofaunal biological trait expression in the stations sampled in September 2017 and July 2018 (separately). Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AIC (Akaike Information Criteria), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model. Chl *a*: concentration of chlorophyll *a*; MGS: mean grain size; TOC: concentration of total organic carbon.

Macrofaunal biological trait expression							**Sept 2017**
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
Depth	617.88	10.611	0.001	0.20965	164.41	0.409	1740.2
Chl *a*	420.97	6.6657	0.001	0.14284			
MGS	185.24	2.6828	0.068	0.062853			
Macrofaunal biological trait expression							**Jul 2018**
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
TOC	753.4	12.947	0.001	0.5642	46.062	0.819	242.27
MGS	144.35	2.969	0.023	0.1081			
Pennatula spp density	100.88	2.3968	0.111	0.075543			
Chl *a*	94.432	2.7285	0.07	0.070718			

3.5 Discussion

Ours is the first study to explicitly test the potential role of sea pen octocorals in shaping macrofaunal communities inhabiting surrounding sediments. The collaborative nature of this research program and the availability of a remotely operated vehicle (ROV) for sampling created an unusual opportunity to gain a more comprehensive understanding of the structure and ecology of benthic communities in the area, and to assess the influence of sea pens at different scales. Finally, the relatively homogeneous environmental conditions within the MPA (as evident in **Table 3.1**, **Table 3.3**, and **Table 3.4**) allowed us to evaluate the influence of sea pens on macrofauna without the potential confounding effects of strong environmental gradients. Our results suggest that macrofaunal communities within sea pen fields differ from those in mostly

bare sediments in the Laurentian Channel MPA. Such effect was particularly evident for the stations sampled in September 2017, where higher densities of *Pennatula* sea pens were associated with higher density and diversity of macrofauna, distinct communities and different vertical distributions within sediments (**Figure 3.11**). When discussing possible mechanisms that make sea pen fields biogenic habitats for macrofauna, we acknowledge the possibility that environmental filtering processes might in reality determine the occurrence of sea pens and simultaneously shape macrofauna. For instance, environmental factors such as the input of Particulate Organic Carbon (POC), sediment granulometry, and bottom-water currents have been documented to affect both mega-epifauna and macro-infauna in deep-sea sediments (MacDonald et al., 2010; Williams, 2011; Barrio Froján et al., 2012; Greathead et al., 2014; Lauria et al., 2017).

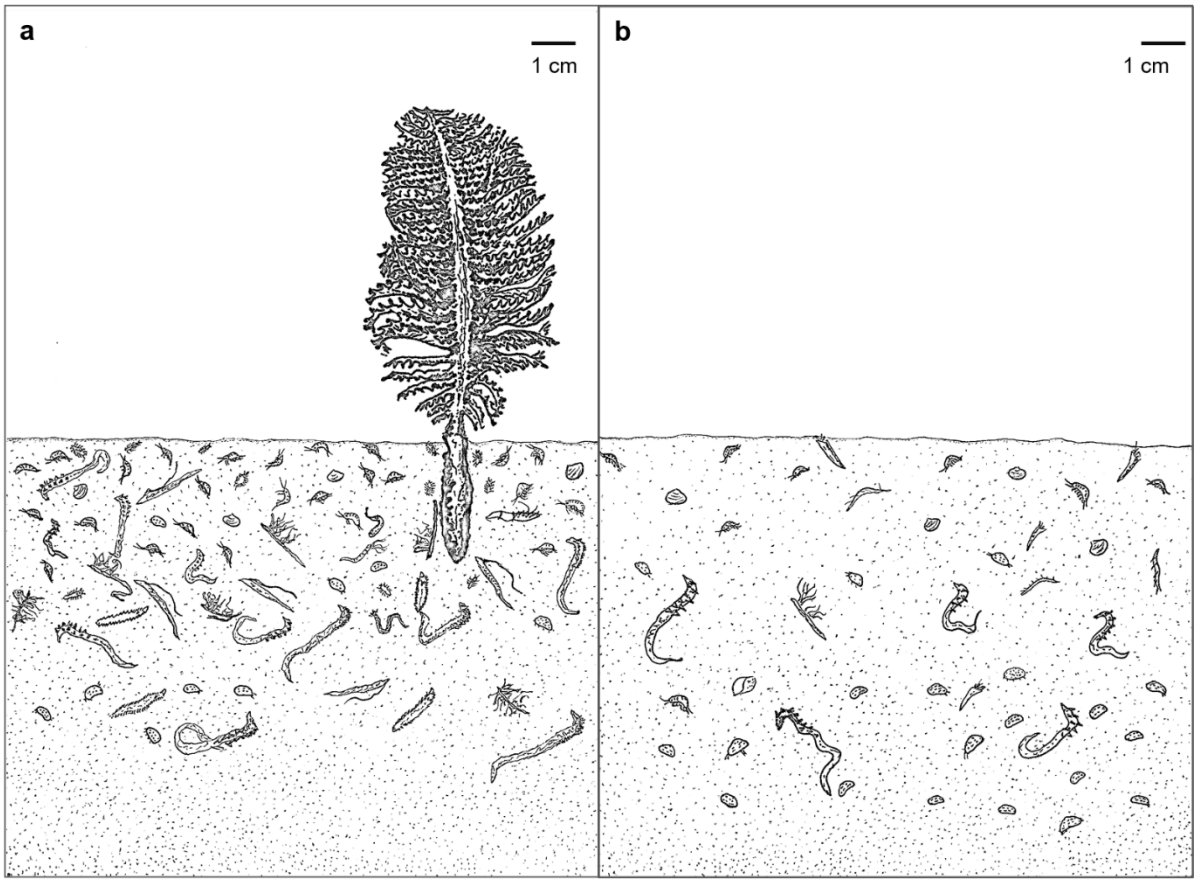

Figure 3.11: Schematic drawing of the macrofaunal communities in the Laurentian Channel MPA (in September 2017) in **a)** sea pen field and **b)** sediment with no visible mega-epifauna. Relative abundances of organisms were derived from real counts in 2 cores per panel: **a)** one randomly selected core (left) and one core containing a *Pennatula* sea pen (right) from station 2 and **b)** two randomly selected cores from station 16. Relative abundances in each core were reduced by 5 times to adjust for bi-dimensionality in the drawing. Drawings represent the top 10 cm of sediment.

3.5.1 Macrofaunal communities within and outside sea pen fields in the Laurentian Channel MPA

3.5.1.1 Macrofaunal density and taxonomic diversity

The higher density of macrofauna within sea pen fields found in both sampling periods may be a consequence of the higher availability of organic matter associated with biogenic habitats, which is known to contribute to supporting higher macrofaunal biomasses in marine sediments (Levin et al., 2001). Enhanced settlement and mixing of food particles into the upper sediment layers (Eckman, 1985) caused by increased retention of particulate organic matter at the

sediment-water interface often occurs in the presence of erect structures on the seafloor, such as sea pens or other sessile organisms (Tissot et al., 2006; Cerrano et al., 2010). In our study, higher levels of organic matter quantity and long-term quality found at St 2, the densest sea pen field, support the enhancement of organic matter deposition near sea pens. Direct measurement of organic matter quantity and quality in cores containing sea pens would have provided a clearer indication of smaller-scale sedimentary processes.

Variation of food availability can also partially explain patterns of taxonomic diversity. In particular, high food availability can support high densities of a few opportunistic species that can rapidly take advantage of fresh food input (Levin et al.,1991, 2001), which explains the lower or comparable diversity found within and outside sea pen fields in July 2018. However, in September 2017 we detected higher taxonomic diversity and evenness within sea pen fields, pointing to other mechanisms controlling macrofaunal dynamics. For example, we suggest that sea pens enhance local food availability through other mechanisms. For example, most species of sea pens continuously produce a protective mucus layer, typically comprised of glycoproteins, proteins that contain oligosaccharide (carbohydrate) chains (Brown and Bythell, 2005) and might represent a readily available food source for some macrofauna. Moreover, sea pens might increase the prokaryotic biomass in the sediments surrounding their peduncle. Although investigations comparing microbial communities in the sediments directly surrounding sea pens to those in bare sediments are lacking, previous studies reported distinct, species-specific bacterial communities associated with tissue and mucus from some sea pen species, indicative of symbiotic relationships between sea pens and bacterial communities (Porporato et al., 2013). Presumably some portion of microorganisms that live on sea pens ends up in the contiguous sediments, representing a source of food for bacterivorous macrofauna. Alternative food resources such as glycoproteins and

bacteria perhaps contribute to increasing food availability and creating new niches that promote the coexistence of different taxa and ecological strategies, increasing total biodiversity (Levin et al., 2001). Finally, sea pens increase small-scale heterogeneity and complexity in sedimentary habitats, which can contribute to the coexistence of multiple ecological niches that increase total biodiversity (Simboura et al., 2000; Buhl-Mortensen et al., 2010; McLain and Barry, 2010; Hasemann and Soltwedel, 2011). This effect is also suggested by the generally higher variability between replicates at the sea pen field stations (*e.g.*, higher standard deviation of diversity indices, higher dispersion in the nMDS relative to macrofaunal community composition and biological trait expression).

Interesting insights can also be gained by observing temporal patterns of macrofaunal diversity, that might reflect seasonality, even though this aspect goes beyond the scope of this study and explicit patterns are not identifiable because of limited temporal replication and differences in sampling tools and design. Acknowledging such limitations, we measured comparable density and diversity between sampling periods at St 2 (the densest sea pen field), in contrast to lower density and higher biodiversity in July 2018 compared to September 2017 at St 5 (bare sediments). These findings support our hypothesis that sea pen fields offer a more stable environment. Here, more persistent resource availability can sustain dense and diverse macrofaunal communities over time, whereas in bare sediments density and diversity of macrofauna is subjected to temporal variations associated with changing environmental conditions and resource availability (*e.g.*, higher biodiversity following input of fresh phytodetritus to the seafloor in the summer). More studies are necessary to confirm our hypothesis and verify the persistence of macrofaunal density and diversity in periods of extremely low resource input (*e.g.*, over winter).

3.5.1.2 Macrofaunal vertical distribution in the sediments

We detected significantly higher (in September 2017) or comparable (in July 2018) proportions of macrofaunal organisms in upper sediment layers in sea pen fields compared to other sedimentary habitats, with the highest proportion of organisms in the upper sediment layers at St 2, the station with the highest density of *Pennatula* sea pens. These findings were unexpected, in that we had hypothesized that burrowing by some sea pen species (*e.g., Pennatula, Funiculina*), which we observed on several occasions during our sampling as well as in the sea pens collected in our cores, would increase oxygen and nutrients penetration into deeper sediment layers and therefore increase the penetration of organisms into sediments. Even though we were unable to assess vertical penetration of oxygen or sedimentary organic matter in the sediments, we noted no signs of anoxia or severe hypoxia in cores (no darker sediment layers; Parisi et al., 1987), suggesting that oxygen did not limit biota within the sediment layers we considered. Moreover, because many species of sea pens contract during withdrawal, consistently reducing the apparent size of the colony (as reported for *Pennatula rubra* by Chimienti et al., 2018), relatively small sea pens, such as those considered in our study, likely did not affect sedimentary processes deeper than a few centimetres into the sediments.

We suggest that the more abundant and reliable food supply within sea pen fields might contribute to higher presence of macrofauna close to the sediment-water interface by reducing competition for resources. For example, Witte (2000) studied macrofaunal vertical distribution in deep-sea sediments in the Arabian Sea, reporting higher concentrations of fauna in deeper sediment layers (below 5 cm) at stations that experienced food limitation and highly seasonal food input. She relates these findings to stronger competition at the sediment-water interface (where many organisms utilize organic matter) in these stations, pressuring larger macrofaunal organisms

to live deeper in the sediment and transfer fresh material quickly to modest depths in the sediment to minimize competition (Jumars et al., 1990; Witte, 2000). Comparably, according to our organic matter data, our stations with lower densities of sea pens experience lower and less reliable organic matter input, which might increase the proportion of macrofauna living deeper in sediments to escape competition. Such effect was more pronounced in autumn, when resources are more limiting than in summer, also supporting this hypothesis. Additionally, we infer that some macrofaunal organisms might be advantaged by occupying the sediments adjacent to sea pens' peduncles, located in the upper sediment layers. Here, these organisms could directly take advantage of alternative food sources such as sea pens' mucus and associated bacteria. Finally, sea pens provide protection from pelagic and epibenthic predators by limiting accessibility to sediments (Baillon et al., 2012), which might also reduce burrowing by some macrofauna that normally avoids predators by occupying deeper sediment layers.

3.5.1.3 Macrofaunal community composition and biological trait expression

Our results revealed different macrofaunal community composition and biological trait expression in sea pen fields compared to other sedimentary habitats for both sampling periods. Analysis of biological trait expression proved less sensitive in detecting differences among communities than community composition analysis, potentially reflecting environmental filtering processes that cause convergence of biological traits in communities exposed to relatively similar environmental conditions and stressors, despite diverse species composition (Perronne et al., 2017), as reported by other studies (*e.g.*, Henseler et al., 2019; Rand et al., 2017; Törnroos et al., 2013, Bremner et al., 2006). Biological trait analysis (Bremner et al., 2003) offered interesting insights into the functional structure of macrofaunal communities, complementing information gained from community composition analysis. For instance, different expression of "response"

traits associated with life histories and lifestyle pointed to the presence of contrasting environmental (biotic and abiotic) factors within and outside sea pen fields (see Díaz and Cabido, 2001; Bremner et al., 2006), as well as possible different sensitivity to natural or anthropogenic changes (Bolam et al., 2014; Beauchard et al., 2017). The relatively lower importance of "effect" traits in differentiating communities suggests that differences in macrofaunal diversity and community composition may not translate into differences in ecosystem functioning (Queirós et al., 2013; Beauchard et al., 2017). A parallel study confirms this hypothesis in finding overall comparable organic matter remineralization and nutrient regeneration in sea pen fields and other sedimentary habitats in some of the 2017 stations considered here (Miatta and Snelgrove, 2021).

The characteristics of macrofaunal communities inhabiting sea pen fields, such as higher presence of less resilient taxa that prefer relatively stable and pristine environments (*e.g.*, amphipods; Bellan-Santini, 1980; Ré at al., 2009; de-la-Ossa Carretero et al., 2012) and traits usually considered fragile, such as longer lifespan (Beauchard et al., 2017), suggest higher environmental stability in sea pen fields and potentially higher susceptibility to disturbance of associated communities (Fjeldså and Lovett, 1996). Greater presence of deposit and suspension/filter feeders within sea pen fields likely relates to higher quantity and quality of sedimentary organic matter that favours organisms directly feeding on it (Rossi et al., 2001). However, higher densities of predatory taxa (*e.g.*, hesionid polychaetes) and other higher trophic levels (*e.g.*, omnivores) within sea pen fields reflects higher trophic diversity and might relate to the stabilizing effect of sea pens, as also observed in the presence of other ecosystem engineers such as mussels and tube worms (van der Zee et al., 2015). The higher small-scale heterogeneity and complexity created by sea pens also contributes to sustaining higher trophic diversity by generating new ecological niches for different taxa with contrasting feeding strategies (Simboura

et al., 2000). Inter-station variability between the three sea pen field stations sampled in September 2017 also points to higher heterogeneity of sea pen fields compared to bare sedimentary habitats.

Interestingly, other taxa and biological traits showed contradictory patterns between the two sampling periods. For instance, higher densities of nerillid polychaetes, and lower densities of bivalves and scaphopods, coupled with higher expression of the trait categories direct, benthic larval development characterized sea pen fields in September 2017, but the same taxa and trait categories dominated other sedimentary habitats in July 2018. Although our study does not provide a comprehensive understanding of these temporal patterns, we hypothesize that seasonal variation in environmental conditions might play a role. For example, the ecological advantage some macrofaunal organisms gain from the enhanced food quantity and quality found in sea pen fields might be more pronounced during periods of more limiting resources (*e.g.*, autumn). In this case, benthic larval development (typifying organisms living in relatively stable and advantageous environments) would ensure that offspring settle nearby within sea pen fields rather than undergoing transport away by currents as would tend to occur with pelagic larvae (Fauchald, 1983; Jablonski and Lutz, 1983; Pechenik, 1999). When resources are broadly plentiful, such as following the spring phytoplankton bloom, this strategy might lose its advantage and pelagic larvae might become more common because that strategy helps to maintain maximum genetic flexibility (Fauchald, 1983) and offers greater recovery potential following impacts (Bolam et al., 2020). However, these changes could also reflect other factors, such as interannual differences in food supply, environmental conditions, or disturbance regimes.

Even when accounting for a wide range of environmental variables, *Pennatula* spp. density was one of the best predictors of variation in macrofaunal community composition, explaining up to 23% of total variation. This supports the role of sea pen fields as biogenic habitat for

macrofauna. Even though other factors resulted important in shaping macrofaunal communities (*e.g.*, depth, granulometric properties, and organic matter quantity) and might have contributed to favour sea pen as well, the effect of *Pennatula* density had overriding role in separating St 2 (with the highest *Pennatula* spp. density) from St 14, despite almost identical environmental conditions. Notably, a recent study by Ashford et al. (2019) explored correlation between several habitat-forming megafauna and peracarid crustaceans in a Northwest Atlantic Fisheries Organisation (NAFO) regulatory area relatively close to our study area and found significant even if weak correlation between pennatulacean biomass and peracarid crustaceans community structure.

In contrast, sea pen density had only a minor role in determining variation in biological trait expression, which was mostly driven by environmental variables. For example, concentrations of total organic carbon explained up to 56 % of the variation in biological trait expression, and concentrations of chlorophyll *a* up to 14%. This strong influence of food quantity and quality on biological trait expression suggests different adaptations of organisms based on resource availability, confirming previous findings (*e.g.*, Käß et al., 2021). Slighter influence of biogenic habitat in selecting macrofaunal biological traits might also partially explain comparable rates of benthic nutrient fluxes in sea pen fields and bare sediments found by our parallel study (Miatta and Snelgrove, 2021).

Interestingly, *Pennatula* spp. density predicted variation in macrofaunal community composition better than total sea pen density, which we suggest relates to the fact that *Pennatula* forms the densest sea pen fields in the Laurentian Channel MPA. Other studies have reported dense fields dominated by *Pennatula* in our study region (*e.g.*, Langton et al., 1990; Murillo et al., 2011; Baker et al., 2012; Murillo et al., 2018), as well as in other geographical areas (*e.g.*, Chimienti et al., 2018a). For example, Baker et al. (2012) reported densities of *Pennatula* spp. up to 622

colonies per 10-m video segment in deep-sea habitats off Newfoundland. We do note, however, a need for further studies to better characterize species-specific relationships between sea pens and macrofauna, which the results of our study alone cannot clearly delineate.

Up to 60 % of the variation of macrofaunal community composition and biological trait expression could not be explained by the variables considered in our study, pointing to the potential contributions of other drivers. For example, smaller-scale sediment heterogeneity, geomorphic features, hydrodynamic regimes, anthropogenic and natural disturbances, as well as ecological aspects such as predation, competition, and resource partitioning are all known to affect macrofaunal communities (Levin et al., 2001; Rand et al., 2017; Pisareva et al., 2015; Bolam et al., 2020). For example, a previous study exploring benthic communities in the Laurentian Channel detected a correlation between the presence of iceberg scours and pockmarks on the seafloor and differences in macrofaunal biodiversity and community composition (Lacharité et al., 2020).

3.5.2 Small-scale effect of sea pens on macrofauna

We detected a significant enhancement of macrofaunal density and taxonomic diversity in cores containing sea pens at St 3, which was not classified as a sea pen field because of the scattered presence of sea pens. This result suggests potential localized effects of sea pens on macrofaunal density and diversity in the immediately adjacent sediment in otherwise bare sedimentary habitats, where larger-scale effects (*e.g.*, sea pen fields) do not conceal small-scale patterns (*e.g.*, sea pen specimens). Results from sea pen field stations were less clear, with no statistically significant effect of sea pen specimen on macrofaunal diversity, even though we observed some enhancement of diversity. These results could also derive from biases related to sampling strategy. For example, we did not account for the physical volume occupied by sea pens in comparing density and diversity among cores. Some of the sea pens we collected (especially *Pennatula* spp.) were large,

potentially resulting in underestimates of macrofaunal densities and biodiversity. In addition, we were unable to collect sufficient replicates to test the effects of different genera of sea pens on macrofauna, which could have led to contrasting findings at St 3, where sea pen specimens belonged to different genera than at the other stations (*e.g.*, *Kophobelemon* was only sampled at St 3, whereas *Pennatula* dominated the other stations).

Additionally, even though differences were not statistically significant, we observed some variations in community composition in cores containing sea pens, such as higher density of nerillid polychaetes. These small, interstitial polychaetes commonly found in muddy sediments (Worsaae and Kristensen, 2005) are considered selective deposit feeders that feed on bacteria (Fauchald and Jumars, 1979), acknowledging limited availability of information for this family. We hypothesize that this family favours sediments near sea pens, where they can feed on the bacteria associated with individual sea pens. A clearer understanding of the small-scale, localized and species-specific effects of sea pens on macrofauna in the surrounding sediment requires further study.

3.6 Conclusions

Our study provides the first evidence of the role of sea pen fields as biogenic habitats for macrofaunal organisms in the Laurentian Channel MPA. We hypothesize that sea pens affect macrofauna mostly by increasing food availability, environmental stability, and small-scale heterogeneity in deep-sea sediments, even though we do not exclude other environmental filtering effects that simultaneously drive the observed patterns in sea pen distribution and macrofaunal communities in the area. We acknowledge the need for more investigations to clarify the specific mechanisms behind our findings. Nevertheless, our findings confirm the importance of sea pens as key habitat-forming organisms in deep-sea sedimentary habitats and highlight the importance

of implementing targeted conservation efforts. In addition, the composition and biological traits of the communities that inhabit sea pen fields suggest higher susceptibility of macrofaunal taxa to change and impact, in addition to the well-known high vulnerability and slow recovery potential of sea pens. We therefore recommend that in the Laurentian Channel MPA protection prioritizes regions with high densities of sea pens (*e.g.*, St 2) and prohibit all damaging activities, including fishing, oil and gas exploration, anchoring and laying of submarine cables. The observed variability of macrofaunal communities over time, possibly between seasons, also highlights the importance of designing monitoring protocols that take natural variation and seasonality of biological communities into consideration.

3.7 Acknowledgements

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3.8 Appendices

Appendix 3A. Statistical results of ANOVA main tests for all the diversity indices considered in our study between biogenic habitats and among stations nested in biogenic habitats for the stations sampled in September 2017 and July 2018, separately. * indicates significant p-values (< 0.05). df: degrees of freedom; F: F-statistic; p: p-value.

Total macrofaunal density					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	6.322e +8	1	63216608	5.621	0.0234*
Station (Biog. hab.)	8.806e +8	5	17611635	1.566	0.1953
Res.	3.936e +9	35	11246229		

Total number of taxa					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	140.39	1	140.39	18.56	0.0001*
Station (Biog. hab.)	42.28	5	8.46	1.118	0.3687
Res.	264.67	35	7.56		

Simpson's index (d)					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	1.091	1	1.0909	17.62	0.00018*
Station (Biog. hab.)	0.274	5	0.0548	0.885	0.3687
Res.	2.167	35	0.0691		

Pielou's evenness (J')					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	0.04275	1	0.04275	6.516	0.0152*
Station (Biog. hab.)	0.01562	5	0.00312	0.476	0.7916
Res.	0.22965	35	0.00656		

Shannon-Wiener index (H')					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	1.1824	1	1.1824	16.33	0.00028*
Station (Biog. hab.)	0.2348	5	0.047	0.649	0.66423
Res.	2.5335	35	0.0724		

Expected number taxa [ES(100)]					**Sept 2017**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	68.49	1	68.49	18.497	0.0001*
Station (Biog. hab.)	9.46	5	1.89	0.511	0.76609
Res.	129.59	35	3.7		

Total macrofaunal density					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	62924468	1	62924468	8.387	0.02*
Station (Biog. hab.)	24043637	2	12021818	1.602	0.26

Total number of taxa					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	0.25	1	0.25	0.057	0.8167
Station (Biog. hab.)	37.17	2	18.583	4.268	0.0548

Res.	60021784	8	75027230		

Simpson's index (d)					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	0.03258	1	0.03258	0.975	0.3524
Station (Biog. hab.)	0.24914	2	0.12457	3.727	0.0718
Res.	0.26742	8	0.03343		

Shannon-Wiener index (H')					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**P**
Biog. hab.	0.603	1	0.603	14.51	0.00517*
Station (Biog. hab.)	0.0239	2	0.0119	0.287	0.75764
Res.	0.3325	8	0.0416		

Res.	34.83	8	4.354		

Pielou's evenness (J')					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	0.06261	1	0.06261	8.803	0.018*
Station (Biog. hab.)	0.00365	2	0.00183	0.257	0.78
Res.	0.0569	8	0.00711		

Expected number taxa [ES(100)]					**Jul 2018**
Source	**Sum of**	**df**	**Mean**	**F**	**p**
Biog. hab.	11.481	1	11.481	9.901	0.0137*
Station (Biog. hab.)	6.316	2	3.158	2.723	0.1253
Res.	9.276	8	1.16		

Appendix 3B. Statistical results of ANOVA main tests for vertical distribution of macrofauna (expressed as proportion of organisms in the 0-5 cm layer) between biogenic habitats and among stations nested in biogenic habitats for the stations sampled in September 2017 and July 2018, separately. * indicates significant p-values (< 0.05). df: degrees of freedom; F: F-statistic; p: p-value.

Vertical distribution					**Sept 2017**
Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Biog. hab.	0.2838	1	0.2838	23.578	0.00025*
Station (Biog. hab.)	0.1775	5	0.03549	2.949	0.0253*
Res.	0.4213	35	0.01204		
Vertical distribution					**Jul 2018**
Source	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Biog. hab.	0.00323	1	0.003234	0.274	0.615
Station (Biog. hab.)	0.02405	2	0.012023	1.018	0.404
Res.	0.09448	8	0.01181		

Appendix 3C. Statistical results of PERMANOVA main tests of macrofaunal community composition between biogenic habitats and among stations nested in biogenic habitats for the stations sampled in September 2017 and July 2018, separately. * indicates significant p-values (< 0.05). df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Macrofaunal community composition						**Sept 2017**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Biog. hab.	1	6631.3	6631.3	17.221	0.0001*	9918
Station (Biog. hab.)	5	3189.6	637.91	1.6566	0.0207*	9893
Res	35	13477	385.06			
Macrofaunal biological trait expression						**Sept 2017**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Biog. hab.	2	699.54	349.77	6.4508	0.0003*	9952
Station (Biog. hab.)	4	349.86	87.466	1.6131	0.1307	9946
Res	35	1897.7	54.221			
Macrofaunal community composition						**Jul 2018**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Biog. hab.	1	977.96	977.96	4.0432	0.0002*	9183

Appendix 3C (continued)

Station (Biog. hab.)	2	1386.1	693.04	2.8653	0.0011*	9115
Res	8	1935	241.88			

Macrofaunal biological trait expression						**Jul 2018**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Biog. hab.	1	525.99	525.99	11.653	0.0002*	9156
Station (Biog. hab.)	2	448.24	224.12	4.9654	0.0092*	9231
Res	8	361.1	45.137			

Appendix 3D. Statistical results of t-tests for all the diversity indices considered in our study between cores containing sea pens and cores without in each of the 4 stations sampled in September 2017 separately. * indicates significant p-values (< 0.05). t: t-statistic; df: degrees of freedom; p: p-value.

Total macrofaunal density				**St 2**	**Total number of taxa**				**St 2**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-0.337	4	0.753	Sea pen	t-test	-2.488	1.02	0.239
Total macrofaunal density				**St 4**	**Total number of taxa**				**St 4**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-2.094	4	0.104	Sea pen	t-test	0.452	4	0.675
Total macrofaunal density				**St 14**	**Total number of taxa**				**St 14**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-0.925	4	0.407	Sea pen	t-test	-1.109	4	0.329
Total macrofaunal density				**St 3**	**Total number of taxa**				**St 3**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-3.927	4	0.017*	Sea pen	t-test	-3.444	4	0.026*
Simpson's index (d)				**St 2**	**Pielou's evenness (J')**				**St 2**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test (Welch)	-2.312	1.023	0.255	Sea pen	t-test	-0.672	1.16	0.61
Simpson's index (d)				**St 4**	**Pielou's evenness (J')**				**St 4**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	0.61	4	0.575	Sea pen	t-test	1.691	2.104	0.277
Simpson's index (d)				**St 14**	**Pielou's evenness (J')**				**St 14**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-1.079	4	0.341	Sea pen	t-test	-0.438	4	0.684

Appendix 3D (continued)

Simpson's index (d)				**St 3**	**Pielou's evenness (J')**				**St 3**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-3.302	4	0.030*	Sea pen	t-test	-0.341	4	0.75
Shannon-Wiener index (H')				**St 2**	**Expected number of taxa [ES(100)]**				**St 2**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test (Welch)	-1.673	1.023	0.339	Sea pen	t-test	-1.419	1.055	0.382
Shannon-Wiener index (H')				**St 4**	**Expected number of taxa [ES(100)]**				**St 4**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test (Welch)	1.382	2.012	0.3	Sea pen	t-test	-0.846	4	0.445
Shannon-Wiener index (H')				**St 14**	**Expected number of taxa [ES(100)]**				**St 14**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-1.794	4	0.147	Sea pen	t-test	-0.846	4	0.445
Shannon-Wiener index (H')				**St 3**	**Expected number of taxa [ES(100)]**				**St 3**
Source	**Test**	**t**	**df**	**p**	**Source**	**Test**	**T**	**df**	**p**
Sea pen	t-test	-1.292	4	0.266	Sea pen	t-test	-3.5	3.698	0.028*

Appendix 3E. Statistical results of t-tests tests for vertical distribution of macrofauna (expressed as proportion of organisms in the 0-5 cm layer) between cores containing sea pens and cores without in each of the 4 stations sampled in September 2017, separately. t: t-statistic; df: degrees of freedom; p: p-value.

Vertical distribution				**St 2**
Source	**Test**	**t**	**df**	**p**
Sea pen	t-test (Student)	-2.108	4	0.103
Vertical distribution				**St 4**
Source	**Test**	**t**	**df**	**p**
Sea pen	t-test (Student)	0.805	4	0.466
Vertical distribution				**St 14**
Source	**Test**	**t**	**df**	**p**
Sea pen	t-test (Welch)	-0.371	2.043	0.746
Vertical distribution				**St 3**
Source	**Test**	**t**	**df**	**p**
Sea pen	t-test (Student)	0.366	4	0.733

Appendix 3F. Statistical results of PERMANOVA main tests for macrofaunal community composition and biological trait expression between cores containing sea pens and cores without in each of the 4 stations sampled in September 2017, separately. df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Macrofaunal community composition						**St 2**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	566.56	566.56	1.4995	0.0626	15
Res	4	1511.3	377.83			
Macrofaunal community composition						**St 4**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	244.14	244.14	0.69344	0.9014	10
Res	4	1408.3	352.08			
Macrofaunal community composition						**St 14**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	407.47	407.47	1.2828	0.3025	10
Res	4	1270.6	317.65			
Macrofaunal community composition						**St 3**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	684.97	684.97	2.3418	0.1343	15
Res	4	1170	292.5			
Macrofaunal biological trait expression						**St 2**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	24.328	24.328	0.51726	0.6566	15
Res	4	188.13	47.032			
Macrofaunal biological trait expression						**St 4**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	144.71	144.71	1.8741	0.1913	10
Res	4	308.87	77.218			
Macrofaunal biological trait expression						**St 14**
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	54.693	54.693	1.1457	0.4025	10
Res	4	190.96	47.739			

Appendix 3F (continued)

Macrofaunal biological trait expression						St 3
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Sea pen	1	193.37	193.37	5.0472	0.1371	15
Res	4	153.25	38.312			

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CHAPTER 4 - BENTHIC NUTRIENT FLUXES IN DEEP-SEA SEDIMENTS WITHIN THE LAURENTIAN CHANNEL MPA (EASTERN CANADA): THE RELATIVE ROLES OF MACROFAUNA, ENVIRONMENT AND SEA PEN OCTOCORALS*

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4.1 Abstract

In order to characterize spatial patterns and environmental and biological drivers of organic matter remineralization and nutrient regeneration in deep-sea sedimentary habitats, we measured fluxes of nitrate, nitrite, ammonium, phosphate, and silicate at the sediment-water interface during 48-h e*x situ* incubation of sediment cores. We sampled a total of 6 stations (351–445 m depth) inside the Laurentian Channel Marine Protected Area (MPA), on the outer continental shelf of Newfoundland (Canada). We assessed the potential effect of octocoral sea pens on uni- and multivariate benthic nutrient fluxes at large- and small-scale by comparing sea pen fields and other sedimentary habitats, and cores with and without sea pens. For each station, we evaluated a wide range of environmental variables, including physico-chemical and sedimentary factors, and we identified macrofaunal organisms inhabiting the cores, assessed their taxonomic diversity, community composition, and bio-logical trait expression. Our analysis identified macrofaunal species richness and the density of a few key taxa, as well as environmental factors such as the quantity of sedimentary organic matter as the primary drivers of variation of multivariate benthic fluxes. Macrofauna explained up to 41% of the variation in benthic fluxes, whereas environmental variables only explained up to 19%, highlighting the importance of biodiversity for ecosystem

functioning. Uni- and multivariate analysis of fluxes did not reveal clear spatial patterns within the MPA habitats and stations, and fluxes showed high small-scale variability. We found enhanced ammonium efflux rates associated with the presence of sea pens at both small- and large-scale, likely reflecting both direct and indirect effects of these soft corals on organic matter deposition and sedimentary biogeochemical processes. Sea pens, however, did not appear to influence other fluxes, leaving their role for organic matter remineralization unclear. The extreme complexity and small-scale heterogeneity of benthic processes, particularly within what appears to be a relatively homogeneous environment, underscores the need for further studies to facilitate generalizations of patterns and drivers of benthic nutrient fluxes at larger scales, which will ultimately enable the effective integration of ecosystem functioning into conservation strategies.

4.2 Introduction

A portion of the organic matter produced by photosynthesis slowly sinks through the water column, ultimately reaching the seafloor to provide the dominant source of food that many organisms rely on for their survival. In marine sediments, mega-, macro-, and meiofauna, in tandem with microbes, degrade and transform labile organic matter (*e.g.*, phytodetritus) into simpler inorganic nutrients, a process known as “organic matter remineralization”. The release of inorganic nutrients into the water column can ultimately lead to their return to the surface layer of the ocean through currents and upwelling, where phytoplankton re-utilize them to sustain primary productivity (Nixon, 1981). By releasing up to 80% of the essential nutrients that stimulate primary productivity in the shallow ocean (Aller, 2014), organic matter remineralization in marine sediments plays a fundamental role in the functioning of marine ecosystems (Jahnke, 1996; Nixon, 1981; Snelgrove et al., 2014; Griffiths et al., 2017). Many studies use oxygen consumption to estimate rates of organic matter remineralization in marine sediments. However, understanding

biogeochemical cycles requires measurements of inorganic nutrient fluxes at the sediment-water interface (Bourgeois et al., 2017), which oxygen consumption does not necessarily represent accurately (Berelson et al., 2003; Link et al., 2013a; Morata et al., 2020). Benthic nutrient fluxes provide a recognized approach for quantifying organic matter remineralization in marine sediments (Giller et al., 2004), and can be measured with in situ chambers deposited on the seafloor (Archer and Devol 1992; Devol and Christensen, 1993; Forja and Gómez-Parra, 1998; Berelson et al., 1996, 2003, 2013), or *ex situ* incubations of sediment cores (Rowe and Phoel, 1992; Link et al., 2013a,b; Belley et al., 2016; Belley and Snelgrove, 2016). Nevertheless, relatively few studies provide such measurements, particularly in deep-sea environments.

Multiple environmental and biological variables can influence organic matter remineralization rates in marine sediments. At larger scales, the quantity and quality of the organic matter reaching the seafloor play a central role in driving organic matter remineralization (Berelson et al., 1996; Jahnke, 1996). The density, composition, and activity of sedimentary microbial communities that play a primary role in converting organic matter into inorganic forms (Jorgensen, 2006) also strongly affect the fate of the organic matter that reaches the seafloor (remineralization vs burial and sequestration). Factors such as temperature and dissolved oxygen concentration can also influence remineralization rates (Link et al., 2013a; Alonso-Pérez and Castro, 2014; Belley et al., 2016), likely by affecting microbial metabolic activity, enzymatic, and stoichiometric reactions (López-Urrutia et al., 2006; Jorgensen, 2006). On a finer scale of millimetres or centimetres, the laws of thermodynamics influence organic matter remineralization and nutrient cycles because nutrients partly move by molecular diffusion following gradients of concentration within the porewater and between the sediment and the overlying water (Schulz, 2000).

Organisms living in the sediment, such as macroinfauna, can affect the rates and efficiency of organic matter remineralization and the release (or uptake) of nutrients, both directly and indirectly. Directly, macrofaunal organisms can generate pore-water pressure gradients that drive advective pore-water flow through the sediment, enhancing the movement and release of nutrients (Huettel and Gust, 1992). Furthermore, the direct flushing of some macrofaunal burrows and tubes, a process known as bioirrigation, can markedly increase nutrient ex-change between pore water and the overlying water column (Aller, 1988; Kristensen and Andersen, 1987; Kristensen and Holmer, 2000; Heilskov et al., 2006; Meysman et al., 2006). Indirectly, macrofaunal activities such as burrowing, feeding, excretion, and ventilation (Aller, 2001; Welsh, 2003; Lohrer et al., 2004; Meysman et al., 2006) can alter sediment properties (Murray et al., 2002; Solan et al., 2004), in turn influencing how effectively microbes remineralize organic matter (Laverock et al., 2011). Similarly, organisms living on the sediment, such as mega-epifauna, can affect the rates of organic matter deposition and processing (Tissot et al., 2006; Cerrano et al., 2010) altering nutrient fluxes, even though fewer studies have assessed this aspect (Khripounoff et al., 2014; Cathalot et al., 2015; Pierrejean et al., 2020). Specifically, no studies have assessed the effect of sea pens (subclass Octocorallia, order Pennatulacea) on benthic nutrient fluxes in deep-sea sediments, despite recognition of their fundamental ecological role in sedimentary habitats (Buhl-Mortensen et al., 2010; De Clippele et al., 2015; Bastari et al., 2018).

Changes in biodiversity caused by increasing human impacts and global change have pushed researchers to increasingly explore relationships between biodiversity and ecosystem functioning (BEF), and the potential implications of biodiversity loss for ecosystem processes and the provisioning of goods and services required to support human life (Loreau et al., 2001; Solan et al., 2004; Loreau, 2010; Cardinale et al., 2012; Snelgrove et al., 2014). For example, recent

studies emphasize the importance of biological traits and functional diversity for ecosystem functioning, as opposed to taxonomic diversity (Hooper et al., 2005; Danovaro et al., 2008; Belley and Snelgrove, 2016; Thrush et al., 2017). However, the limited number of studies exploring BEF in natural environments, their relatively small spatial scale, and the extreme complexity of natural ecosystems, currently limit any generalizations on how biodiversity affects ecosystem functioning at broader, basin scales (Snelgrove et al., 2014; Thompson et al., 2018). These constraints limit the inclusion of aspects of ecological functioning in conservation planning, a factor considered vital to improving marine conservation (*e.g.*, MPA design; Frid et al., 2008; Miatta et al., 2021), thereby exacerbating other constraints that reduce the effectiveness of conservation efforts (Davies et al., 2007), especially for remote and under-studied environments such as the deep sea (Glover et al., 2018; Danovaro et al., 2020).

In this study, we use fluxes of nitrate, nitrite, ammonium, silicate, and phosphate at the sediment-water interface, measured from *ex situ* incubation of sediment cores, to estimate organic matter remineralization in deep-sea sedimentary habitats. Specifically, we focus on the Laurentian Channel Marine Protected Area (MPA) on the outer continental shelf of Newfoundland (Canada). This recently established MPA represents the largest existing MPA in Canada (11,580 km2; DFO, 2011) and mainly encompasses sedimentary habitats considered important for several priority marine species. Sea pens (soft corals: subclass Octocorallia, order Pennatulacea), one of the main conservation foci of this MPA, occur in the highest known concentrations for the entire Newfoundland and Labrador shelf bioregion (DFO, 2011). Here, we explore spatial patterns of uni- and multivariate benthic nutrient fluxes, as well as the potential role of sea pens in affecting benthic processes at both small- and large-scale. We determine the overall role of sediments in regenerating essential nutrients and, based on a wide array of potential explanatory variables,

including multiple measures of macro-faunal diversity, taxa, and biological trait composition, we investigate both environmental and biological drivers of benthic nutrient flux variation. In our study, the relative environmental homogeneity of the area offered an opportunity to evaluate the role played by varying biodiversity (*e.g.*, macrofaunal and mega-epifaunal communities) in regulating benthic ecosystem functioning.

4.3 Materials and methods

4.3.1 Sampling location and strategy

We collected sediment push cores in the Laurentian Channel MPA (which was still an Area of Interest (AOI) at the time of sampling) during a two-week research cruise on board the *CCGS Martha L. Black* in September 2017. We used the Remotely Operated Vehicle (ROV) ROPOS to collect replicate cores (internal diameter = 6.7 cm, length = 35 cm) from 6 different stations inside the MPA (**Figure 4.1**; **Table 4.1**), taking care not to disturb the sediment surface within the cores. At each station, we collected 8 sediment push cores at 2 different sites and used 2 cores to evaluate sediment properties and 6 cores for incubations to evaluate benthic nutrient fluxes and subsequently quantify macrofauna. To directly test the small-scale effect of sea pens on benthic nutrient fluxes, we collected a total of 8 cores (from St 2, St 14, St 3, and St 16) containing sea pens representing several different genera (*Pennatula*, *Anthoptilum*, *Funiculina*, and *Kophobelemon*). In some instances, multiple attempts were necessary in order to collect the sea pens, because some individuals (*e.g.*, specimens of *Pennatula* and *Funiculina* spp.) retreated into the sediment following disturbance by the ROV. At every station, a ROPOS CTD Seabird 19plus mounted on the ROV recorded near-bottom temperature, pH, salinity, and dissolved oxygen (**Table 4.1**). Niskin bottles attached to the ROV collected bottom water that we used to exchange

with water sampled in the incubations, and to quantify dissolved inorganic nutrient concentrations in bottom water.

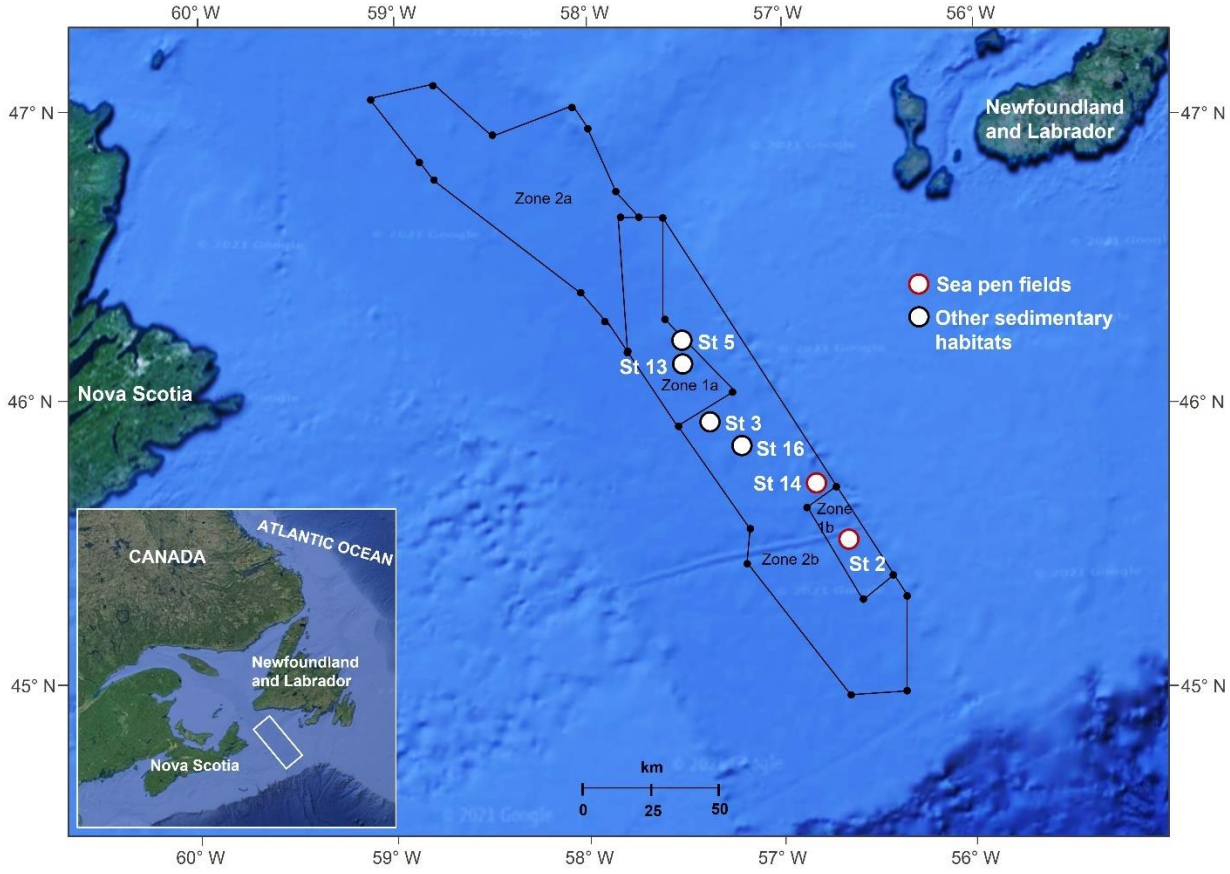

Figure 4.1: Map showing location, borders, and zonation of the Laurentian Channel MPA and the 6 stations sampled in September 2017 to evaluate benthic nutrient fluxes at the sediment-water interface, within sea pen fields and in other sedimentary habitats.

Despite generally similar sedimentary composition, morphological features, and physico-chemical properties (**Table 4.1**), the mega-epifauna differed markedly among the stations. We classified the biogenic habitat at each station based on the presence of sea pens, because they were the dominant mega-epifauna. Stations with high densities of sea pens, especially *Pennatula* spp. (both juvenile and mature specimens) were classified as "sea pen fields" (**Figure 4.2, a**), whereas stations characterized by bare sediments or scattered presence of sea pens or other mega-epifauna were classified as "other sedimentary habitats", **Figure 4.2, b**), as shown in **Figure 4.1** and **Table**

4.1. Aggregations of sea pigs (class Holothuroidea, order Elasipodida; **Figure 4.2, c**) occurred at some stations (St 13 and St 3). Sea pigs were not considered in our classification of biogenic habitats because these mobile deposit feeders only form transient (hours to days; Miller et al., 2000), aggregations on the seafloor in response to high influx of fresh organic matter. However, we used their average coverage at each station (%) as a factor in our analysis to assess the effect of mega-epifauna on benthic nutrient fluxes, together with total sea pen average density (ind · m^{-2}) and *Pennatula* spp. average density (ind · m^{-2}), which are shown in **Table 4.1**. Other mega-epifauna (*e.g.*, sea anemones and other soft corals, echinoderms) occurred at some stations, but in much lower densities than sea pens and sea pigs, and in insufficient numbers to consider in our study. Data on average sea pen densities and sea pig coverage in the stations were derived from a parallel study (de Mendonça and Metaxas, unpublished). Sea pen total average densities and densities of common genera (*e.g.*, *Pennatula* spp., *Anthoptilum* spp., *Protoptilum* spp., and *Kophobelemnon* spp.) were estimated from video transects collected using the ROV ROPOS in September 2017 and the Campod drop-camera in July 2018 (see de Mendonça and Metaxas (2021) for a detailed description of the methods).

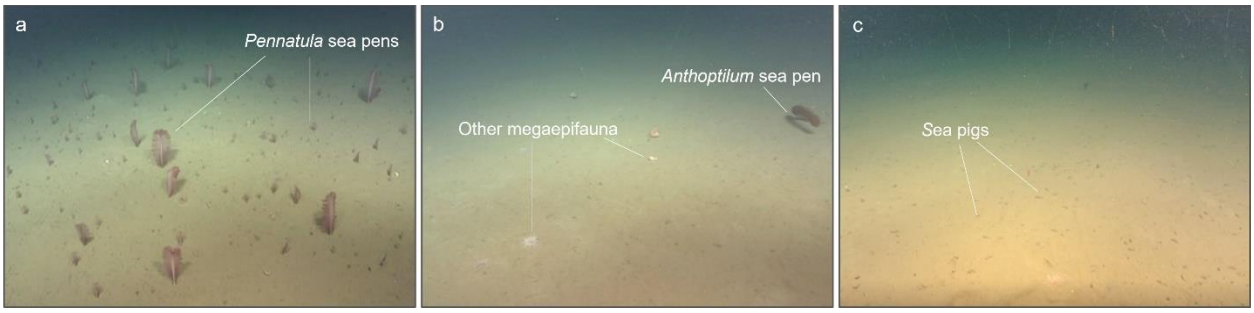

Figure 4.2: Biogenic habitats in the Laurentian Channel MPA stations: **a**) sea pen field (St 2), **b**) other sedimentary habitats with scatter presence of mega-epifauna (St 5), **c**) other sedimentary habitats with aggregation of sea pigs (St 3). Arrows highlight characteristic features. Photo credit CSSF ROPOS.

Table 4.1: Description of the stations sampled within the Laurentian Channel MPA in September 2017 to evaluate nutrient fluxes at the sediment-water interface. B T: bottom water temperature; B [O2]: bottom water oxygen concentration; B S: bottom water salinity; Tot sea pen den.: average total sea pen density; *Pennatula* spp. den.: average *Pennatula* spp. density; Sea pig cov.: average sea pig coverage; Other sedim. hab.: other sedimentary habitats.

Station	**Biogenic habitat**	**Latitude** (N)	**Longitude** (W)	**Depth** (m)	**B T** (℃)	**B [O2]** (ml · L^{-1})	**B S** (PSU)	**B pH**	**Tot sea pen den.** (ind · m^{-2})	***Pennatula* spp. den.** (ind · m^{-2})	**Sea pig cov.** (%)
St 2	Sea pen field	45°32.09	56°40.07	354	6.2	7.93	35.00	7.87	3.63	3.56	0.00
St 14	Sea pen field	45° 43.75	56° 51.16	351	6.3	7.91	35.01	7.91	0.59	0.54	0.00
St 3	Other sedim. hab	45° 56.59	57° 22.28	445	5.8	7.94	35.00	7.93	0.45	0.01	0.02
St 16	Other sedim. hab	46° 8.69	57° 30.99	442	5.9	7.94	35.00	7.94	0.30	0.00	0.00
St 5	Other sedim. hab	46°13.28	57°31.45	440	5.7	7.93	34.99	7.90	0.07	0.01	0.01
St 13	Other sedim. hab	45° 51.66	57° 12.23	436	5.8	7.92	35.00	7.92	0.03	0.00	0.07

4.3.2 Fluxes of inorganic nutrients at the sediment-water interface

In order to evaluate fluxes of nitrate, nitrite, phosphate, ammonium, and silicate at the sediment-water interface, we incubated a total of 36 sediment cores (sediment volume 0.73 ± 0.08 L, water volume 0.51 ± 0.08 L) and overlying water for ~48 hours and removed water samples for dissolved inorganic nutrients analysis at regular intervals during the incubation.

After collection, we acclimated sediment cores for ~12 hours, allowing any sediment particles resuspended during experiment preparation to settle on the sediment surface. We only used cores with intact surface layers for our incubations. Several hours before the beginning of the experiment, we carefully (without resuspending the sediment) exchanged the overlying water with fresh, oxygenated bottom seawater collected *in situ*, allowing it to overflow in the surrounding water bath. This step prevented hypoxia in the incubated cores and removed any metabolites produced by community metabolism. To start the incubation, we removed all visible bubbles from the surface, sealed the cores with acrylic caps equipped with magnetic stirrers, and incubated the sediment cores in a refrigerator at in situ temperature (~4.5 °C) and in the dark for ~48 hours. Stirrers were working for the entire experimental period at approximately 3 revolutions per minute to maintain oxygenation of the sediment but without resuspending or disturbing it. Each 48-h experiment comprised three ~ 12-hour incubation segments. At the beginning of every incubation, we extracted ~30 ml of water using a 60-ml acid-rinsed plastic syringe, rinsing the syringe and sample bottle with ~ 5 ml of water. We stored an additional ~25 ml of water in upright, acid-rinsed HDPE plastic 30-ml bottles at -80 °C for successive analysis of dissolved inorganic nutrients. At the end of every 12-h incubation segment, we removed lids and resampled the water for nutrient analysis. During the following 1-6 hours, we carefully exchanged overlying water in the cores with

fresh, oxygenated bottom seawater collected at each station to prevent hypoxia and remove toxic metabolites produced by the community.

The concentrations of dissolved inorganic nutrients (nitrate, nitrite, ammonium, phosphate, and silicate) in the water sampled from the incubations as well from the bottom water samples were determined using a continuous segmented flow analyzer (Seal AutoAnalyzer 3) at the Bedford Institute of Oceanography (Darmouth, NS). Analyses were performed following Industrial Method 186-72W (adapted from Strickland and Parsons, 1968) for silicates, Industrial Method 158-71W (adapted from Armstrong et al., 1967; Grasshoff, 1969) for nitrate and nitrite, Industrial Method 155-71W (adapted from Murphy and Riley, 1962; Aoyama et al., 2012) for orthophosphate and the fluorometric method developed by Aminot and Kérouel (1997) for ammonium. Nutrient fluxes, expressed as $\mu mol \cdot m^{-2} \cdot d^{-1}$, were determined from the measured concentration changes in the overlying water as a function of time, water volume, and sediment area, summed over the three ~12-hour incubation segments.

4.3.3 Macrofaunal identification and taxonomic diversity

At the end of each ~48-h incubation, we removed all cores from the water bath and immediately processed them by extruding the cores and sectioning them into 0-5 and 5-10 cm sediment layers using inert plastic spatulas. We then fixed the sediment in 500- or 1000-ml plastic jars with 4% buffered formaldehyde seawater for later sorting and faunal identification. In the laboratory, we processed the samples over a 300-μm sieve and transferred them to 70% ethanol until we could complete microscopic analysis. Before identification, samples were stained with a few drops of Rose Bengal ($0.5 g \cdot L^{-1}$) to facilitate sorting and identification of organisms.

For each sample, we sorted macrofaunal organisms and assessed abundances of the major taxa (Classes: Polychaeta, Oligochaeta, Amphipoda, Isopoda, Copepoda, Ostracoda, Bivalvia,

Gastropoda, Scapopoda, Sipunculidea, Ophiuroidea, Asteroidea, Holothuroidea, Echinoidea, and Subclasses: Hexacorallia, Octocorallia). We further identified polychaetes to the family level because they represented the most abundant taxa in most samples and their high taxonomic and functional diversity make them good indicators of environmental quality, as well as effective surrogates for total biodiversity in ecological studies (Pocklington and Wells, 1992; Dauvin et al., 2003; Olsgard et al., 2003).

We calculated total macrofaunal densities (ind · m^{-2}) and total taxon richness (including classes, subclasses, and polychaete families), as well as Simpson's index (d), Pielou's evenness (J'), Shannon-Wiener index (H') and expected number of taxa [ES(100)] for each sample, combining the vertical sections of the entire 10 cm cores. Diversity indices were calculated in PRIMER 6+ using the DIVERSE routine. We also determined the proportion of macrofaunal organisms in the top 0-5 cm layer of the cores (total number of organisms in the 0-5 cm layer / total number of organisms in the 0-10 cm core) to assess differences in vertical distributions within sediments.

4.3.4 Biological trait expression

To evaluate the biological trait composition of the assemblages, we used Biological Trait Analysis (Bremner et al., 2003), which utilizes multivariate ordination to describe patterns of biological trait composition over the entire macrofaunal assemblage. The analysis also quantifies the types of traits present in assemblages and the relative frequency with which they occur, therefore providing a way to explore patterns in benthic assemblage functioning, noting that this approach cannot quantify ecosystem functioning per se (Bremner et al., 2006, 2008). We selected 3 biological traits, based on their presumed influence on organic matter remineralization: motility, feeding mode, and bioturbation (**Table 4.2**). We subdivided the 3 traits into 14 categories that characterized behaviour/strategies in more detail (**Table 4.2**). We used a fuzzy coding approach to

assign trait categories to the taxa (classes, subclasses, or families) that allowed each taxon to represent more than one trait category, and therefore capture inter- and intraspecific variation in trait expression. We adopted a scoring range of 0 to 5, with 0 reflecting no affinity for the given trait category, 1, 2, 3, or 4 reflecting partial, increasing affinity, and 5 denoting exclusive affinity. We derived information on trait expression for all taxa from several published sources (Highsmith and Coyle, 1991; Fauchald and Jumars, 1979; Hyne, 2011; Queirós et al., 2013; Jumars et al., 2015; Polytraits Team, 2020), as well as from direct observations on our specimens. When information was unavailable for a given taxon, we obtained information from one taxonomic rank higher (*e.g.*, from orders of polychaetes). In a few cases where no information was available for a given taxon, we distributed the 5 scores equally among all the plausible trait categories. To obtain the community trait expression in each sample, we multiplied trait categories for each taxon present in a sample by its density (ind · m^{-2}) in that sample, and then summed over all taxa present at each station to obtain a single value for each trait category in each sample (Bremner et al., 2006).

Table 4.2: Biological traits and categories used in trait analysis, with definitions and description of their ecological relevance in terms of affected ecosystem functions.

Trait	Definition and ecological relevance	Modalities
Motility	Capability of an organism to move spontaneously and freely. Affects: Habitat provisioning, reworking of sediments, communities' sensitivity and resilience (Hinchey et al., 2006; Bremner et al., 2006). Responds to: Hydrodynamics, natural and anthropogenic disturbance, food availability and source (Harris, 2014; Pierdomenico et al., 2019).	Motile Discretely motile Sessile/sedentary

Table 4.2 (continued)

Feeding mode	Common feeding strategy of an organism (*e.g.*, food items that it is enzymatically and behaviourally capable of using). Affects: Trophic structure, resource use and energy transfer, biogeochemical processes (Rosenberg, 1995; Norling et al., 2007). Responds to: Hydrodynamics, resource availability, habitat stability and heterogeneity (Rosenberg, 1995; Simboura et al., 2000; Rossi et al., 2001; van der Zee et al., 2015).	Suspension/filter feeder Surface deposit feeder Subsurface deposit feeder Omnivore Predator Scavenger
Bioturbation	Capability of an organism to rework sediments through movement, feeding, and other activities. Affects: Sediment biogeochemical properties, organic matter remineralization, nutrient recycling, carbon sequestration (Mermillod-Blondin et al., 2004; Hooper et al., 2005).	None Surface modifier Biodiffuser Upward conveyor Downward conveyor

4.3.5 Sedimentary properties

We collected sediment for analysis of organic matter and grain size from the 0-2 cm top layer of the dedicated 1 or 2 cores at each station, homogenizing the sediment and then placing it in Whirl-Pak bags prior to storage in the dark at -20 °C (except for samples for lipid analysis, which were stored in pre-combusted aluminium tin foil at -80 °C) until analyzed. In this study, we use Total Organic Matter (TOM) and Total Organic Carbon (TOC) as a measure of food quantity. We use total nitrogen (TN) and total organic carbon to total nitrogen ratio (C: N) as a measure of organic matter quality over longer time scales, with higher TN and lower C: N indicating fresher and higher quality organic matter (Godbold and Solan, 2009; Le Guitton et al., 2015; Campanyà-Llovet et al., 2017). We use total lipids concentration (TL) as a measure of organic matter quality over intermediate-long time scales, with higher TL indicating higher nutritional value (Parrish, 2013; Campanyà-Llovet et al., 2018). We use concentrations of chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), total pigments (Tot Pigm), as well as chlorophyll *a* to phaeopigments ratio

(Chl *a*: Phaeo), and chlorophyll *a* to total organic carbon ratio (Chl *a*: TOC) as a measure of phytodetritus input to the seafloor and short-term organic matter quality and freshness, with higher concentrations of pigments and higher Chl *a*: Phaeo and Chl *a*: TOC indicating higher inputs of fresh phytodetritus to the seafloor (Pusceddu et al., 2009; Le Guitton et al., 2015). We use % sand, % silt and % clay, and mean grain size of the sortable silt fraction (MGS), as a measure of sediment particle size and distribution.

Sediment total organic matter (TOM) was calculated as the difference between dry (desiccated at 60 ºC for 24 hours) and calcinated (muffle furnace at 450 ºC for 4 hours) weight, expressed as mg · g DW^{-1} (Danovaro, 2010).

Total organic carbon (TOC) and total nitrogen (TN) were determined by drying a sediment subsample of 1-5 g (wet weight) at 60 °C for 24 h, grinding it to a fine powder, and then weighing and acidifying (with pure HCl fumes) for 24 h to eliminate inorganic carbon. Samples were dried again at 60 °C for 24 h before starting CHN analysis. We then weighed an aliquot of dried decarbonated sediments (15 mg) and folded it tightly into a tin capsule. A Carlo Erba NA1500 Series II elemental analyser (EA) determined TOC (%) and TN (%). The instrument averaged ± 0.1 precision for nitrogen and ± 0.17 precision for carbon. TOC and TN were expressed as mg · g DW^{-1}.

We extracted lipid samples with a combination of chloroform and methanol according to Parrish (1999) and we determined lipid class composition with a three-step chromatographic development method (Parrish, 1987). Total lipid (TL) concentration in the sediments was calculated by summing lipid classes and expressed as μg · g WW^{-1}.

Sedimentary concentrations of chloroplastic pigments (chlorophyll *a* and phaeopigments) were determined using a spectrophotometer following Danovaro (2010). Pigments were extracted

with 90% acetone (24 h in the dark at 4 °C). After centrifugation (800 x g), the supernatant was used to determine the functional chlorophyll *a* and acidified with 0.1 N HCl to estimate the amount of phaeopigments. We then dried the sediment at 60 ºC for 24 h prior to weighing. Total phytopigment concentrations were defined as the sum of chlorophyll *a* and phaeopigment concentrations, and utilized as an estimate of the organic material of algal origin, including the living (chlorophyll *a*) and senescent/detrital (phaeopigment) fractions (Pusceddu et al., 2009). All chloroplastic pigment concentrations were expressed as $\mu g \cdot g\ DW^{-1}$.

We digested a subsample of sediment with hydrogen peroxide to eliminate any organic material present and then freeze-dried sediments before analysis with a laser diffraction analyzer to determine granulometric properties. Sieving was performed prior to analysis to ensure the elimination of large particles (gravel fraction), which were not present in our sediment samples. For each sample, we determined % sand, % silt and % clay (with silt and clay together representing the mud fraction). We also determined mean grain size of the sortable silt fraction (MGS, μm).

4.3.6 Statistical analysis

We measured a single negative value for silicate fluxes (from St 16), which we considered an outlier and therefore removed it from the analysis. In order to investigate variation in benthic flux of each nutrient (nitrate, nitrite, phosphate, silicate, and ammonium) across stations we used one-way type III univariate analysis of variance (ANOVA) with the factor “station” (fixed with 6 levels). To test for differences in single nutrient fluxes between sea pen fields and other sedimentary habitats, we used Student’s or Welch’s (for non-normally distributed data) in-dependent t-tests with the factor “biogenic habitat” (fixed with 2 levels). To assess the direct effect of the presence of sea pens in the cores on each nutrient flux, we ran independent Student’s or Welch’s (for not homo-geneous data) t-tests with the factor “sea pen” (fixed with 2 levels). In this

case, because fluxes did not significantly differ among stations, we compared cores with and without sea pen specimens across all stations instead of within single stations to discriminate patterns better. Data were assessed for homogeneity of variance (with Levene's tests) and normality (with Q-Q plots of residuals) prior to all analyses and non- homogeneously dispersed variables were corrected using a Brown- Forsythe correction before ANOVA analyses. We also investigated variation in multivariate benthic nutrient fluxes (based on fluxes of the 5 inorganic nutrients considered) across the stations (with the fixed factor "station", 6 levels), between sea pen fields and other sedimentary habitats (with the fixed factor "biogenic habitat", 2 levels), and between cores containing sea pens and cores without (with the fixed factor "sea pen", 2 levels) using multivariate analysis of variance (PERMANOVA) performed with 9999 random permutations of appropriate units using the PERMANOVA+ add on in PRIMER v6 (Anderson et al., 2008). We calculated the resemblance matrix from Euclidean distances of standardized to mean 0 and standard deviation 1 [using the "normalise" routine in PRIMER v6 (Clarke and Gorley, 2006)] benthic flux data and verified homogeneity of multivariate dispersions using the PERMDISP routine. Principal Component Analysis (PCA) provided visualizations of the ordination of samples in multidimensional space based on the 5 measured nutrient fluxes. We used a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001) to identify which set of environmental variables best predicted variation of multivariate benthic nutrient fluxes. We used a resemblance matrix of multivariate benthic nutrient flux data (based on Euclidean distances of standardized to mean 0 and standard deviation 1 flux data) as a measure of between-samples similarities and we allowed the following predictive variables to enter the analysis: depth, bottom water temperature, salinity, oxygen con-centration, oxygen saturation, concentrations of nitrate, silicate, phosphate, and ammonium, TOM, TOC, TN, C: N, Chl *a*: Phaeo,

total lipids, Chl *a*: TOC, % sand, % mud, % silt, % clay, and MGS, average sea pen density, average Pennatula spp. density, and average sea pig coverage. Variables were standardized to mean 0 and standard deviation 1 prior to analysis. In order to represent natural variation as much as possible in the analysis, we attributed each environmental sample from a sampling site within each station to each replicate flux or faunal sample from the same site, whenever available. In instances where only one replicate of environmental parameters was available per station, we assigned the same values for each replicate flux sample from that station. We assessed normality and collinearity of predictor variables using Draftsman's plots, ensuring that highly correlated variables did not appear simultaneously in the final models. The stepwise routines were run employing 9999 permutations and AICc (Akaike's information criterion corrected) selection criterion, which is recommended for analyses with a small number of samples relative to the number of predictor variables (Anderson et al., 2008). We examined R2 to identify the best model and determine the proportion of the variation explained by that model, visualizing results with distance-based redundancy analysis (dbRDA; Anderson et al., 2008). We also used stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001) to identify which set of biological variables (macrofaunal taxonomic diversity, taxa composition, and biological trait expression) predicted variation in multivariate benthic nutrient fluxes and to assess underlying biodiversity and ecosystem functioning (BEF) relationships. The use of matrices of community composition and biological trait expression in this analysis allowed testing of the relevance of each taxon density and trait category expression, respectively, on multivariate flux variation. We used the resemblance matrix of multivariate benthic nutrient flux data (based on Euclidean distances of standardized to mean 0 and standard deviation 1 flux data) as a measure of between-samples similarities. Because in this case the number of predictor variables greatly

exceeded the number of samples, we first tested the influence of each group of biological variables (macrofaunal density, taxonomic diversity indices, and proportion of organisms in the 0–5 cm layer; macrofaunal community composition; macrofaunal biological trait expression) on benthic nutrient fluxes separately. Taxa that appeared in fewer than three core samples and whose relative density did not exceed 2 specimens per core (including gastropods, octocorals, and the polychaetes Amphinomidae, Apistobranchidae, Capitellidae, Glyceridae, Nephtyidae, Pectinaridae, Phyllodocidaes, Poecilochaetidae, Polynoidae, Sabellaridae, Scalibregmidae, Serpulidae, Sphaerodoridae, and Trichobranchidae) were removed from the community composition matrix to further reduce the number of predictor variables and the number of zeros. Taxonomic diversity indices were log (X +1) transformed and standardised to mean 0 and standard deviation 1 prior to analysis. For each group, the variable(s) that correlated best with benthic nutrient fluxes were selected and combined in a final analysis to determine the best biological and functional model explaining variation in benthic nutrient fluxes. The stepwise routine was run employing 9999 permutations and using AICc (Akaike's information criterion corrected) selection criterion (Anderson et al., 2008). We examined R^2 to identify the best model and determine the proportion of the variation explained by that model, visualizing results with distance-based redundancy analysis (dbRDA; Anderson et al., 2008). All DistLM and dbRDA analyses were performed in PRIMER v6.

4.4 Results

4.4.1 Patterns of individual nutrient fluxes

Nitrate flux (**Figure 4.3, a**) ranged from -2145.4 μmol · m^{-2} · d^{-1} to 3079.3 μmol · m^{-2} · d^{-1} across our replicate cores and the average values were directed into the sediment (nitrate uptake) at all stations except at St 5 and St 3. Nitrite flux (**Figure 4.3, b**) ranged from -114.4 μmol · m^{-2} ·

d^{-1} to 36.9 μmol · m^{-2} · d^{-1} in the cores and the average values were directed into the sediment (nitrite uptake) at all stations except at St 5. Ammonium flux (**Figure 4.3, c**) ranged from -350.3 μmol · m^{-2} · d^{-1} to 391.9 μmol · m^{-2} · d^{-1} in the cores and the average values were into the sediment (ammonium uptake) at all stations except at St 14. Phosphate flux (**Figure 4.3, d**) ranged from -264 μmol · m^{-2} · d^{-1} to 160.9 μmol · m^{-2} · d^{-1} in the cores and the average values were into the sediment (phosphate uptake) at all stations except at St 13. Silicate flux (**Figure 4.3, e**) ranged from 52.14 μmol · m^{-2} · d^{-1} to 14,170.2 μmol · m^{-2} · d^{-1} and was consistently into the water column (release of silicate).

Overall, the intra-station variability of fluxes between replicate cores exceeded the inter-station average variability up to 3.5 times, as is also evident in the large standard deviation values in **Figure 4.3**. Our analysis detected no significant differences across stations for any of the fluxes considered (ANOVA, $p > 0.05$, **Appendix 4A**). We detected significant higher effluxes or lower uptakes of ammonium in sea pen fields compared to other sedimentary habitats (t-test, $p < 0.05$, **Figure 4.3, c** and **Appendix 4A**), but no significant differences between biogenic habitats for any of the other fluxes (t-tests, $p > 0.05$, **Appendix 4A**). We also detected significantly higher effluxes of ammonium in cores containing sea pens than in cores without (t-test, $p < 0.05$, **Figure 4.3, f** and **Appendix 4A**), but no differences in any of the other nutrient fluxes between cores containing sea pens and cores without (t-tests, $p > 0.05$, **Appendix 4A**).

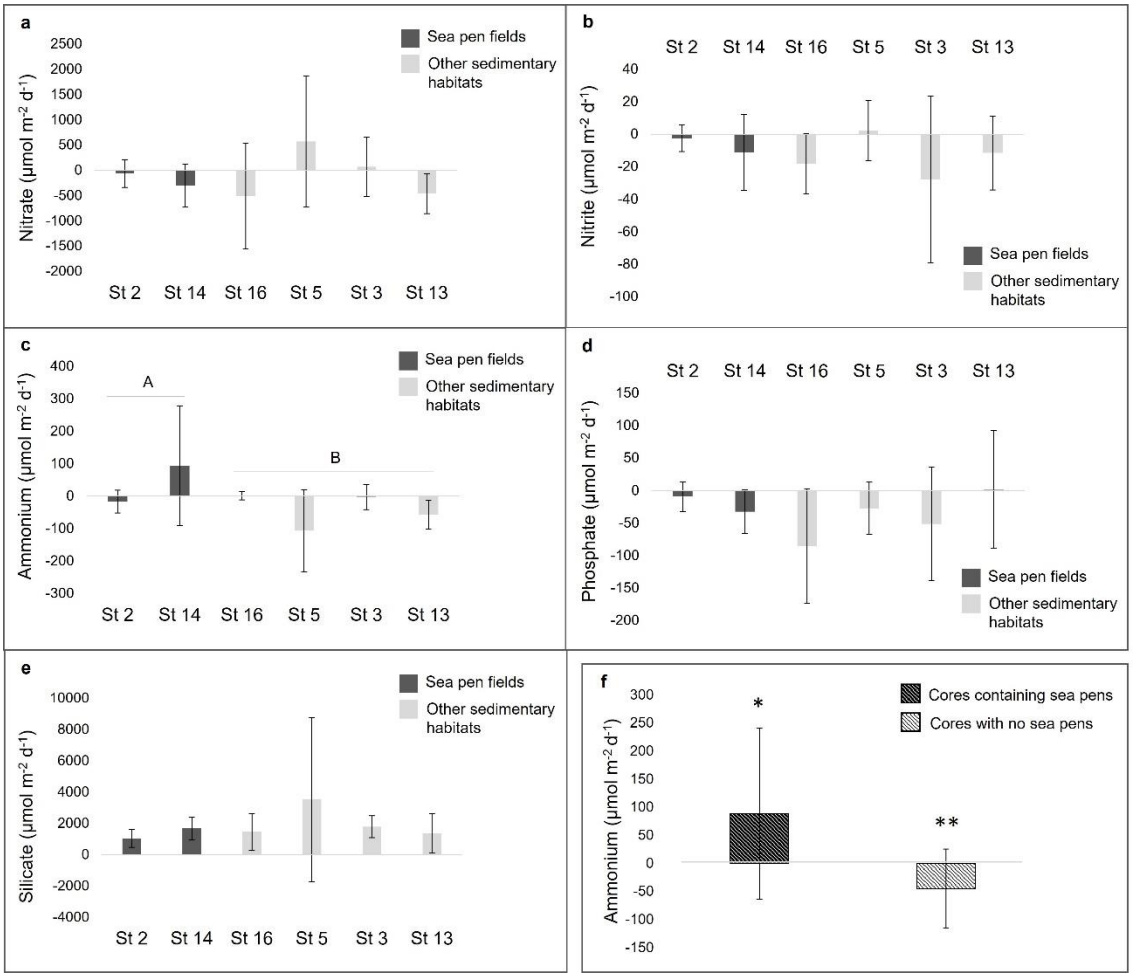

Figure 4.3: Inorganic nutrient fluxes at the sediment-water interface in the 6 stations sampled in September 2017 in the Laurentian Channel MPA. **a)** Nitrate flux, **b)** Nitrite flux, **c)** Ammonium flux, **d)** Phosphate flux, **e)** Silicate flux. For each station, we show fluxes average and standard deviation (based on 6 replicates). All fluxes are expressed as $\mu mol \cdot m^{-2} \cdot d^{-1}$. Positive values indicate release from sediments, negative values indicate uptake by sediment. Letters highlight significant differences between biogenic habitats (t-tests, $p < 0.05$), when present. **f)** Ammonium fluxes in cores containing sea pens ($n = 8$) versus cores without ($n = 28$) showed as average ± standard deviation, with symbols (*) highlighting significant differences (t-tests, $p < 0.05$).

4.4.2 Patterns of multivariate benthic nutrient fluxes

Multivariate analysis of benthic nutrient fluxes provides an estimation of the overall remineralisation function of the sediments. Our analysis detected no significant differences in multivariate benthic fluxes across stations, between sea pen fields and other sedimentary habitats, nor between cores containing sea pens and cores without (PERMANOVA, $p > 0.05$, **Appendix**

4B). Data were homogeneously dispersed (PERMDISP $p > 0.05$), and stations and biogenic habitats were not clearly separated in ordination space, as seen in the PCA plot, where the first two PC axes explained 77% of variation of benthic fluxes (**Figure 4.4**). Analysis of the eigenvectors showed that no single flux dominated the multivariate similarity pattern among samples, with silicate, nitrate, phosphate, and nitrite fluxes correlating most strongly with the first PC axis, and nitrite and phosphate fluxes also relating to the second PC axis.

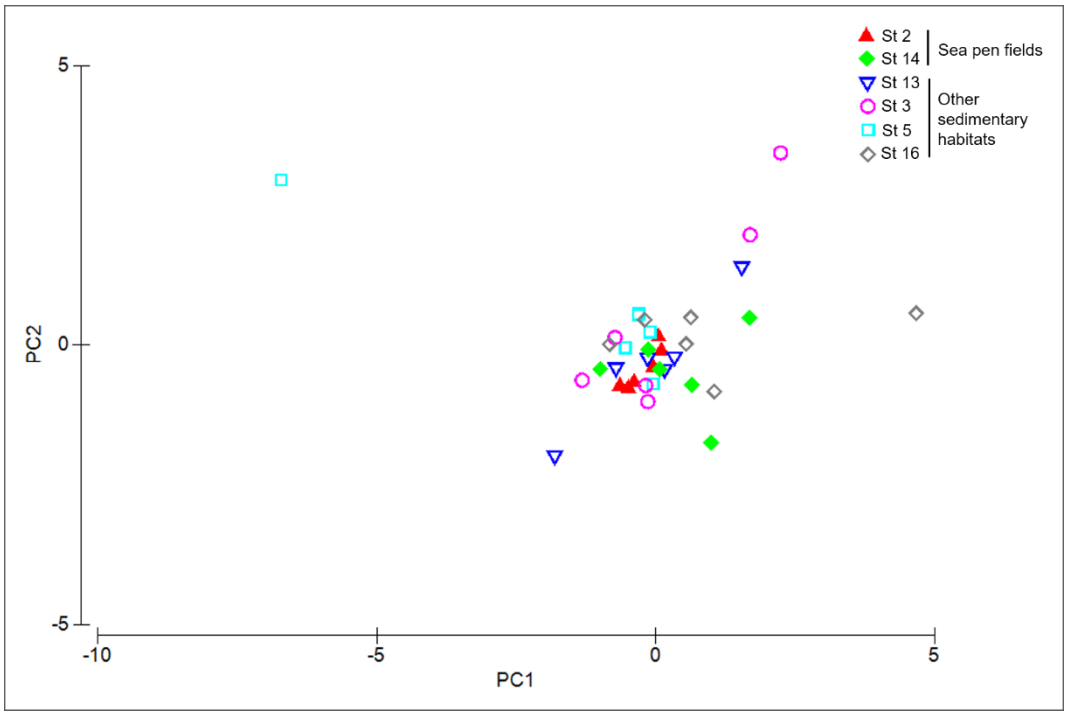

Figure 4.4: Principal Component Analysis (PCA) showing the nonmetric multivariate similarity among replicate samples for each station in terms of benthic nutrient fluxes, based on Euclidean distance matrix of standardized flux data.

4.4.3 Environmental drivers of multivariate benthic nutrient flux variation

Table 4.3 and **Table 4.4** summarize the main sedimentary variables used in the analysis and their variation across stations, compared to the physico-chemical variables described in **Table 4.1**. The final best DistLM model (**Table 4.5**) based on all the environmental variables considered explained 19% of the total variation of benthic nutrient fluxes and included bottom salinity (R^2 =

0.13), and sedimentary concentration of total organic carbon (R^2 = 0.06). The first axes of the dbRDA explained 15% of the variation and correlated mostly with bottom water salinity, whereas the second dbRDA axes explained 4% of the variation and correlated mostly with sedimentary concentration of TOC (**Figure 4.5**).

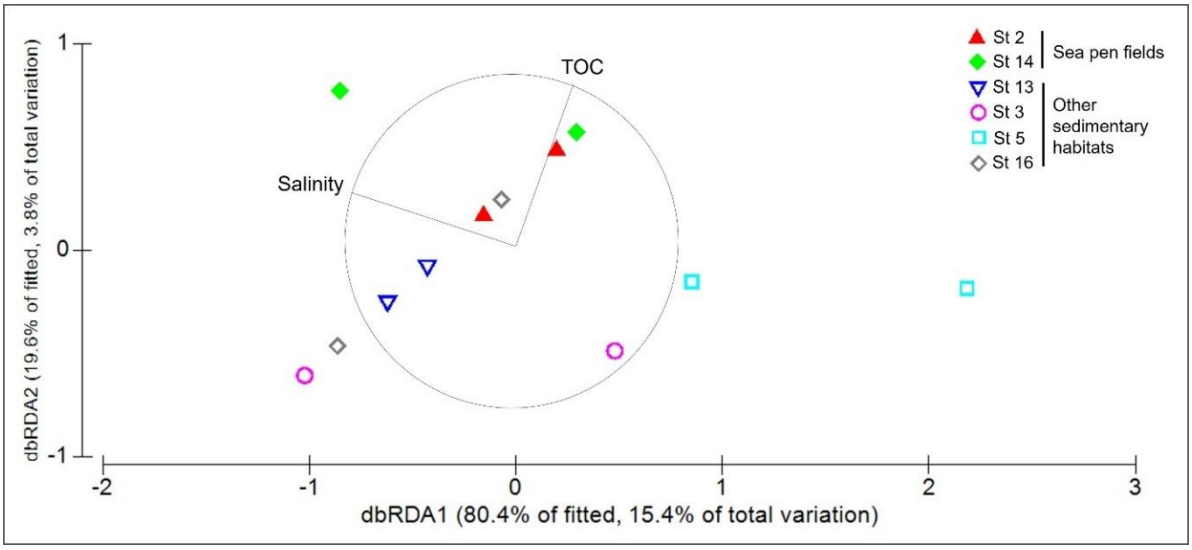

Figure 4.5: Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes (based on Euclidean distance matrix of standardized flux data) and all selected environmental variables (based on standardized data) for the Laurentian Channel MPA stations. Vectors show direction and strength of the environmental variables explaining variation in benthic nutrient fluxes. TOC: sedimentary concentration of total organic carbon.

Table 4.3: Summary of the main sedimentary properties in the Laurentian Channel MPA stations (average ± standard deviation based on analysis of two sediment cores at each station). * indicates values only based on one measurement. TOM: total organic matter; TOC: total organic carbon; TL: total lipid concentration; TN: total organic nitrogen concentration; Chl *a*: concentration of chlorophyll *a*; Chl *a*: Phaeo: chlorophyll *a* to phaeopigments ratio; C: N: carbon to nitrogen ratio; Chl *a*: TOC: chlorophyll *a* to total organic carbon ratio.

Station	TOM ($mg \cdot g\ DW^{-1}$)	TOC ($mg \cdot g\ DW^{-1}$)	TL ($\mu g \cdot g\ WW^{-1}$)	TN ($mg \cdot g\ DW^{-1}$)	Chl *a* ($\mu g \cdot g\ DW^{-1}$)	Chl *a*: Phaeo	C: N	Chl *a*: TOC
St 2	106.8 ± 10.2	36.0 ± 2.1	387.3 ±122.7	3.8 ± 0.05	2.72 ± 0.13	0.13 ± 0.06	9.6 ± 1.3	0.08 ± 0.01
St 14	114.7 ± 7.2	39.8 ± 0.9	631.1 ± 23.2	3.6 ± 0.36	2.02 ± 0.00*	0.18 ± 0.00*	11.1 ± 0.9	0.05 ± 0.00*
St 16	100.1 ± 10.3	30.7 ± 4.8	378.3 ± 305.6	3.2 ± 0.05	1.92 ± 0.35	0.14 ± 0.06	9.7 ± 0.2	0.06 ± 0.00
St 5	73.9 ± 10.0	31.1 ± 0.2	520.9 ± 586.5	2.9 ± 0.03	2.57 ± 0.21	0.16 ± 0.03	11.0 ± 0.7	0.08 ± 0.01
St 3	82.7 ± 13.5	25.7 ± 5.3	109.4 ± 323.2	2.1 ± 0.02	1.72 ± 1.03	0.17 ± 0.02	12.0 ± 0.2	0.06 ± 0.03
St 13	111.2 ± 43.7	30.0 ± 1.2	218.2 ± 171.8	2.6 ± 0.02	2.9 ± 1.26	0.29 ± 0.03	11.7 ± 2.1	0.1 ± 0.04

Table 4.4: Summary of the main granulometric properties in the Laurentian Channel MPA stations (average based on analysis of one sediment core at each station). MGS: mean grain size of the sortable silt fraction.

Station	% sand	% silt	% clay	MGS (μm)
St 2	1.9	64.3	33.8	21.3
St 14	10.2	62.6	27.3	24.7
St 3	1.8	63.2	35.0	18.7
St 16	3.6	60.1	36.3	20.3
St 5	3.5	61.9	34.6	21.9
St 13	2.1	62.3	35.7	20.3

Table 4.5: Statistical results of DistLM analysis (final model) for fitting environmental factors to benthic nutrient fluxes. Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AICc (Akaike Information Criteria corrected), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model. TOC: sedimentary concentration of total organic carbon.

Environmental drivers							
Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**	**AICc**	**R^2**	**RSS**
Salinity	22.156	4.9287	0.006	0.1266	56.03	0.191	141.52
TOC	11.327	2.641	0.0446	0.0647			

4.4.4 Biological drivers of multivariate benthic nutrient flux variation

Macrofaunal density, taxonomic diversity, vertical distribution, community composition, and biological trait expression all significantly differed in comparing sea pen fields to other (bare) sedimentary habitats and a complete description of macrofaunal diversity patterns across stations and biogenic habitats can be found in Chapter 3. The best DistLM model based on macrofaunal community composition explained 41% of the total variation in benthic nutrient fluxes and included the relative densities of 5 taxa: scaphopods (R^2 = 0.15), chrysopetalid polychaetes (R^2 = 0.05), amphipods (R^2 = 0.03), onuphid polychaetes (R^2 = 0.02), and asteroids (R^2 = 0.02). The best

DistLM model based on biological trait expression explained 24% of the total variation in benthic nutrient fluxes and included the trait categories discretely motile (R^2 = 0.15), and predator (R^2 = 0.1). The best DistLM model based on taxonomic diversity indices explained 13% of the total variation of benthic nutrient fluxes and included the total number of taxa. The best final DistLM model (**Table 4.6**) based on all biological variables explained 35% of the total variation in benthic nutrient fluxes and included the relative density of scaphopods, the total number of taxa, and the relative densities of onuphid polychaetes. The first axis of the dbRDA explained 24% of the total variation and mainly correlated with total number of taxa and relative density of scaphopods, whereas the second dbRDA axis explained 9% of the total variation and correlated mainly with the relative densities of scaphopods (**Figure 4.6**).

Table 4.6: Statistical results of DistLM analysis (final model) for fitting biological variables related to macrofaunal diversity, community composition, and biological trait expression to benthic nutrient fluxes. Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AICc (Akaike Information Criteria corrected), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model.

Biological drivers							
Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**	**AICc**	**R^2**	**RSS**
Scaphopoda density	26.309	6.0159	0.0019	0.15034	50.596	0.352	113.4
Total number of taxa	21.346	5.5314	0.0026	0.12197			
Onuphidae density	13.949	3.9362	0.0314	0.07971			

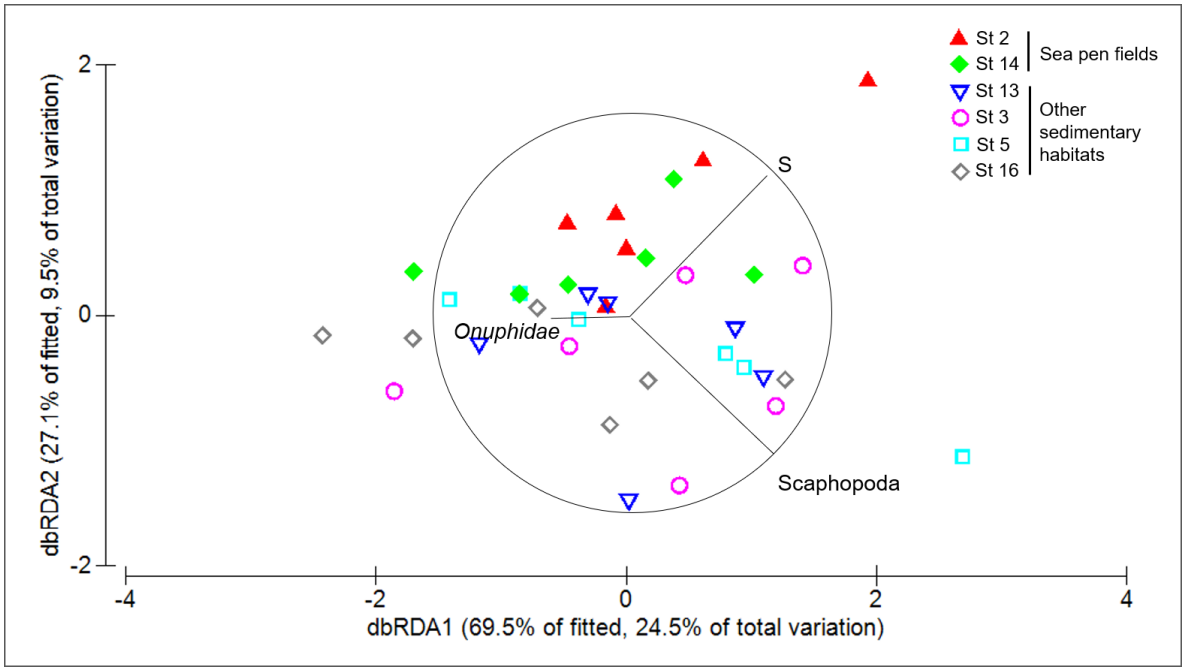

Figure 4.6: Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes (based on Euclidean distance matrix of standardized flux data) and all biological variables for the Laurentian Channel MPA stations (6 replicates per station). Vectors show direction and strength of the main biological variables explaining variation in benthic nutrient fluxes. S: total number of taxa.

4.5 Discussion

Our study evaluated fluxes of inorganic nutrients at the sediment- water interface in order to estimate organic matter remineralization in sedimentary habitats across 6 stations in the Laurentian Channel MPA. Overall, fluxes showed greater small- (between cores) than large-scale (between stations or biogenic habitats) variability in the stations sampled. Such small-scale heterogeneity in benthic nutrient fluxes emphasizes the complexity of sedimentary processes and the combined roles of different factors in determining flux rates. Despite the large array of environmental variables considered in this study, environment could not explain a large proportion of the variation in multivariate benthic fluxes, whereas biological factors related to macrofaunal biodiversity, and the presence of a few key taxa explained a greater portion of the variation in benthic fluxes. Sea pens appeared to enhance ammonium effluxes at different scales but did not

appear to affect other nutrient fluxes nor the overall organic matter remineralization function in Laurentian Channel sediments, leaving their role for organic matter remineralization unclear.

4.5.1 Overall role of sediments for organic matter remineralization and nutrient regeneration in the Laurentian Channel MPA

Our findings indicate that sediments in the Laurentian Channel MPA act both as sink and source for most of the inorganic nutrients considered (**Figure 4.7**). Only silicate was consistently released by the sediments and represented the highest flux rates of all the nutrients (**Figure 4.7, a**). This result points to high rates of remineralization of silica, a key element in the ocean, essential for the growth of many other organisms that sustain primary productivity, such as diatoms and radiolarians (Tréguer and De La Rocha, 2013). Remineralization of biogenic silica into inorganic silicate in marine sediments (regeneration in **Figure 4.7, a**), can be particularly high following the deposition of phytodetritus containing excess biogenic silica (usually from diatom-dominated phytoplanktonic blooms), which seems to be the case in our study, where the measured concentrations of chlorophyll *a* (between 1.72 and 2.9 $\mu g \cdot g\ DW^{-1}$) suggest fresh input of phytodetritus, likely following the fall phytoplanktonic bloom.

Phosphate was mostly taken up by the sediments at our stations (**Figure 4.7, a**), suggesting sequestration of phosphorus, an essential element for all living organisms that often limits primary productivity in marine ecosystems. In the ocean, phosphate incorporated into organic materials by photosynthetic organisms can sink to the seafloor and be remineralized by microorganisms or buried (Baturin, 2003; Paytan and McLaughlin, 2007). Previous studies have reported increased uptake of phosphate in oxic marine sediments, where the abundance of ferric iron [Fe(III)] and manganese phases take up large amounts of phosphate by adsorption and mineral formation (Ingall and Jahnke, 1994). Low oxygen environments, in contrast, often release large amounts of

phosphate into the water column, because of ferric iron depletion and sedimentary redox conditions that diminish the capacity of sediments to retain phosphate (Ingall and Jahnke, 1994; Paytan and McLaughlin, 2007). Similarly, Belley et al. (2016) reported strong correlations between fluxes of phosphate and bottom water dissolved oxygen concentration and oxygen penetration depth into sediments. We, therefore, attribute the relatively low release of phosphate in our sediments to the oxic conditions of the area, with bottom water dissolved oxygen concentrations ranging from 3.74 to 4.06 ml · L^{-1}, and no signs of hypoxia in the sediments.

Ammonium, nitrite, and nitrate are all major components of the nitrogen cycle (**Figure 4.7, b**), the most complex of all marine biogeochemical cycles, in that nitrogen occurs in many distinct chemical forms and undergoes myriad chemical transformations (Gruber, 2008). Microorganisms in marine sediments can remineralize organic nitrogen into its inorganic forms through ammonification and nitrification. In addition, anaerobic reactions such as nitrate reduction and denitrification can occur in sediments, contributing to nitrogen conversion (Gruber, 2008). The complexity of this cycle complicates the interpretation of fluxes beyond the scope of our study. In our samples, we detected both release and uptake of ammonium, nitrite, and nitrate from sediments, depending on location (**Figure 4.7, b**). The generally low flux of nitrite reflects its role as an intermediate product of nitrogen transformation. In general, higher rates of nitrite uptake were coupled with higher nitrate uptake or lower nitrate release, and higher ammonium intakes were also usually coupled with higher nitrate release (*e.g.*, at St 5), as reported in other studies (*e.g.*, Hall et al., 1996; Link et al., 2013a). The trends observed in our study point to the importance of both nitrification, which causes ammonium uptake from the water column to meet bacteria demand, and denitrification processes, which causes nitrite and nitrate consumption and associated fluxes into sediments (Hall et al., 1996). Ammonium release was higher in sea pen fields compared

to bare sediments, as well as in cores containing sea pens compared to cores without, suggesting a role for sea pens in enhancing ammonium release at both small- and larger scales. We further discuss this idea in section *4.5.3*.

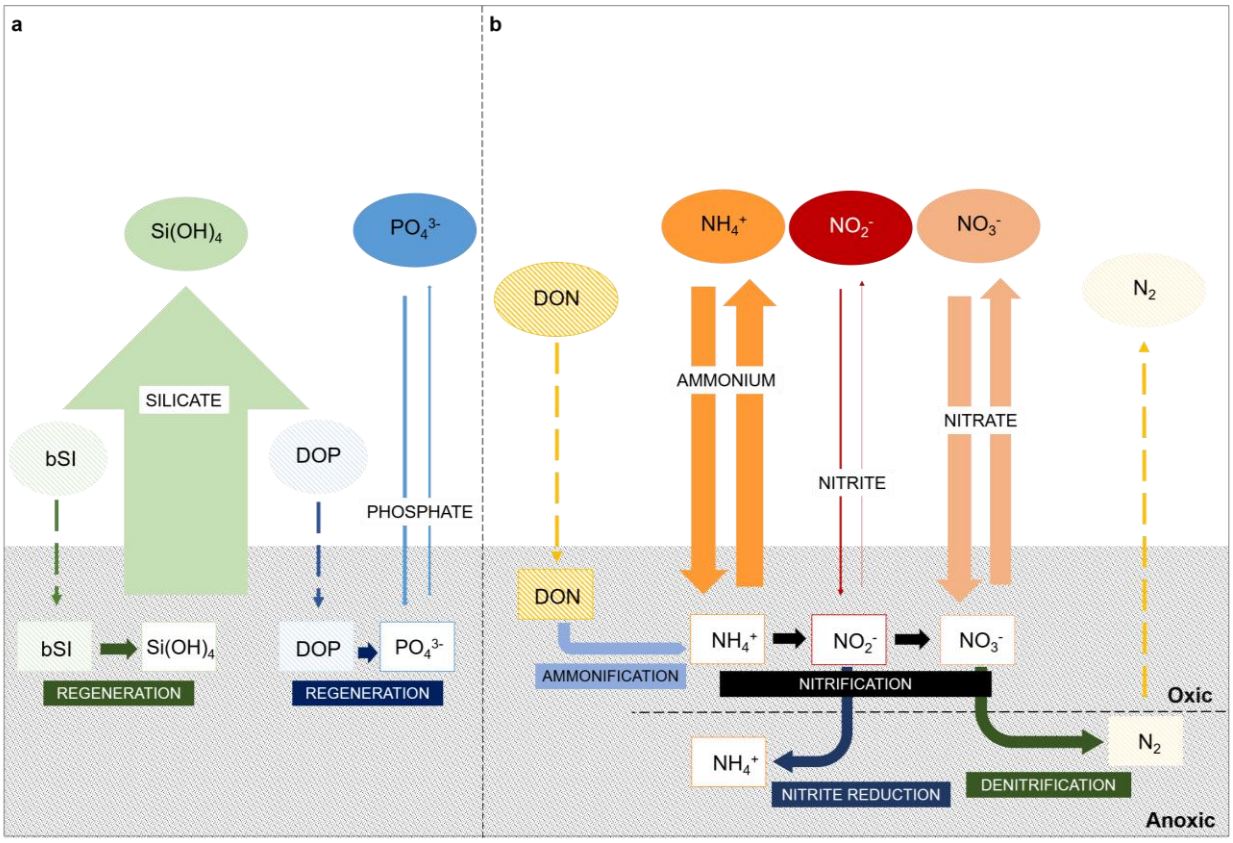

Figure 4.7: Schematic representation of benthic nutrient fluxes at the sediment-water interface measured in the Laurentian Channel MPA stations. Arrows entering and exiting the sediment on both sides of the figure indicate the direction and intensity of fluxes of silicate, phosphate, ammonium, nitrite, and nitrate, with arrow width proportional to the intensity of the fluxes (effluxes are based on average positive values of each flux measured across the 6 stations and intakes are based on average negative values of each flux measured across the 6 stations). **a)** Silicate [$Si(OH)_4$] and phosphate(PO_4^{3-}) cycles and fluxes (simplified), where bSI denotes biogenic silica and DOP denotes dissolved organic phosphorus **b)** Simplification of the nitrogen cycle based on Gruber (2008) and fluxes of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-), where DON denotes dissolved organic nitrogen and N_2 denotes gaseous nitrogen.

4.5.2 Patterns and drivers of benthic nutrient fluxes in the Laurentian Channel MPA

Our study did not identify clear patterns of variation in benthic nutrient fluxes across the 6 stations sampled inside the Laurentian Channel MPA, nor between sea pen fields and other

sedimentary habitats (except for ammonium fluxes, as discussed in section *4.5.3*). Fluxes displayed very high intra-station variability (at the scale of meters) that exceeded inter-station variation (at the scale of kilometres), possibly obscuring any larger-scale pattern. These results can be linked to the relatively homogeneous conditions of the area, in terms of physico-chemical, sedimentary, and biological parameters, that might dampen any differences in benthic nutrient fluxes at larger scales (*e.g.*, between stations). In fact, previous studies detected clear differences in uni- or multi-variate benthic fluxes between sites characterized by large natural variation in environmental and/or biological factors, such as depth, temperature, oxygen concentrations (Belley et al., 2016), input of phytodetritus, and infaunal diversity (Link et al., 2013b). The high variability of fluxes among replicates that we measured was also possibly enhanced by the small diameter of the cores (sediment area 35 cm^2), noting that incubating larger sediment cores helps minimize the effect of sediment micro-heterogeneity, thereby producing less variable flux rates at a given site (Jahnke, 1985; Forja and Gómex-Parra, 1998). Other studies have found that organic matter remineralization exhibits substantial small-scale heterogeneity both vertically and horizontally within the sediment through effects of multiple factors such as organic matter particle distributions, the presence of suboxic niches, diffusion rates of elements, and faunal activities (Jahnke, 1985; Harper et al., 1999; Lewandowski and Hupfer, 2005). For instance, faunal bioirrigation activities strongly influence the intake and/or release of nutrients at the sediment-water interface, given that fluxes created by bioirrigation can exceed transport by molecular diffusion by as much as an order of magnitude (Kristensen and Holmer, 2000; Berelson et al., 2003; Hammond et al., 2004; Heilskov et al., 2006; Kristensen et al., 2012). The uneven distribution of organisms in sediments further enhances small-scale heterogeneity, and we observed fairly high small-scale variation of

macrofaunal density, diversity, and community composition between our replicate samples (Chapter 3).

Bottom water salinity and sedimentary concentration of total organic carbon were the best environmental predictors of variation of benthic nutrient fluxes. Salinity affects benthic nutrient fluxes through geochemical mechanisms, such as the availability of ions present in seawater as terminal electron acceptors used to convert nutrients (Seitzinger et al., 1991). Specifically, salinity affects the nitrogen cycle by determining the forms of nitrogen released from sediments and the relative importance of denitrification processes (Seitzinger et al., 1991; Hopkinson et al., 1999). We observed a general increase in ammonium release and a decrease in nitrate and nitrite release with increasing salinity, which aligns with other studies (*e.g.*, Hopkinson et al., 1999). However, the very limited salinity range in our study (from 34.99 to 35.01) leaves its actual importance in affecting benthic nutrient fluxes questionable. The influence of sedimentary organic matter quantity also aligns with other studies (Berelson et al., 1996; Jahnke, 1996), and we observed a general increase of effluxes of, nitrite, ammonium, and phosphate with increasing sedimentary concentrations of total organic carbon. The high percentage of variation not explained by the environmental variables considered in our study suggests the contribution of other factors. For example, sediment concentrations of manganese and iron have been linked to variation in benthic nutrient fluxes (Link et al., 2013b), potentially through the sequestration of phosphate by ferric iron (Paytan and McLaughlin, 2007). Additionally, density and diversity of sedimentary bacteria influence benthic nutrient fluxes (*e.g.*, Belley and Snelgrove, 2016), given their direct role in organic matter remineralization and nutrient regeneration (Jorgensen, 2006).

Biological variables related to macrofaunal diversity and relative taxa density explained variation in benthic nutrient fluxes better than environmental variables. The relative density of

scaphopods alone explained 15% of the total variation. These molluscs feed on foraminifera and other microorganisms using their distinctive tentacles (Ax, 2000; Reynolds, 2002) and in terms of bioturbation potential scaphopods are mainly considered upward and downward conveyors (Queiros et al., 2013), thus potentially relocating materials vertically between the sediment surface and deeper layers and *vice versa* (Kristensen et al., 2012) and affecting nutrient flux rates (Pierrejean et al., 2020). In our study, we observed a general increase in nitrate (**Figure 4.8, a**) and silicate (**Figure 4.8, c**) effluxes and a decrease in ammonium effluxes (**Figure 4.8, b**) with increasing densities of scaphopods. The total number of macrofaunal taxa explained 12% of total flux variation and we observed a general increase in nitrite (**Figure 4.8, d**), and phosphate (**Figure 4.8, e**) effluxes with increasing number of taxa. These findings support the hypothesis that higher taxonomical richness supports higher ecosystem functionality, likely by increasing bioturbation intensity, as reported by other studies (Emmerson et al., 2001; Marinelli and Williams, 2003; Hooper et al., 2005; Mermillod-Blondin et al., 2005; Ieno et al., 2006; Norling et al. 2007; Solan et al., 2008; Gamfeldt et al., 2015; Belley and Snelgrove, 2017). Notably, higher macrofaunal diversity and higher relative abundance of scaphopods characterized sea pen fields compared to barer sediments (Chapter 3), suggesting a potential effect of sea pen fields on benthic nutrient fluxes through their effect on macrofaunal communities. Such an effect can be seen in **Figure 4.6**, where sea pen fields separate in ordination space from other sedimentary habitats. Finally, the densities of onuphid polychaetes explained 7% of total flux variation. These omnivorous, semi-mobile polychaetes are considered surface modifiers (Queirós et al., 2013), even though some onuphid species form burrows made from sand grains embedded in a polysaccharide matrix (that we observed in our samples), which is permeable to diffusive exchange of solutes (Aller, 1983; Hannides et al., 2005; Waldbusser and Marinelli, 2009). Noting the lack of any specific

information, the characteristics of onuphid tubes and burrows suggest potential bioirrigation activities, known to markedly affect organic matter remineralization rates and nutrient exchange between pore water and the overlying water column (Aller, 1988; Kristensen and Andersen, 1987; Kristensen, 2000; Heilskov et al., 2006; Meysman et al., 2006). Interestingly, in our study, we observed a decline in all nutrient effluxes with increasing density of onuphids (*e.g.*, as shown for nitrate, ammonium, and silicate in **Figure 4.8**), suggesting that perhaps these polychaetes mostly flush their burrows toward the sediment deeper layers, therefore decreasing effluxes of nutrients toward the overlying water column. We recognize, however, the complexity of the mechanisms regulating bioirrigation mode of sedimentary organisms (Woodin et al., 2016; Renz et al., 2018) and the need for further studies assessing the specific bioturbation and bioirrigation potential of onuphid polychaetes and their role in affecting benthic nutrient fluxes.

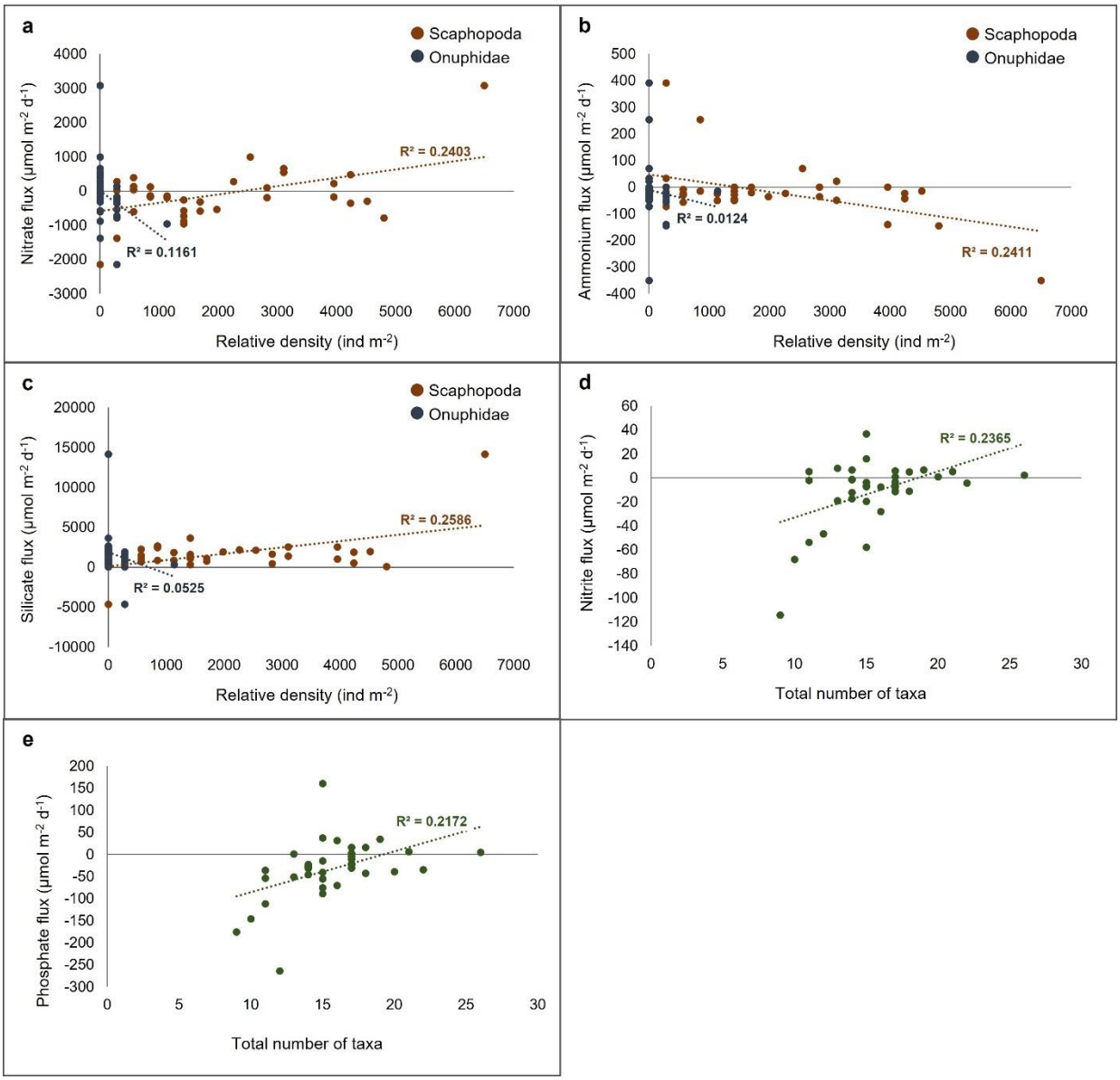

Figure 4.8: Scatterplots showing regressions between the relative densities of scaphopods and onuphid polychaetes and the measured fluxes of nitrate (**a**), ammonium (**b**), and silicate (**c**), or between the total number of taxa and the fluxes of nitrite (**d**) and phosphate (**e**). Each dot corresponds to the plotted values of one sediment core sample and lines show the best fit for the linear regression between the variables, with relative R^2 indicating the strength of the relationships.

Surprisingly, the cumulative community expression of biological traits that presumably relates directly to organic matter remineralization and nutrient fluxes (*e.g.*, bioturbation), explained less variation in benthic nutrient fluxes than taxonomic community composition. Link et al.

(2013b) reported similar findings, with taxonomic diversity indices explaining more variation in benthic oxygen and nutrient fluxes than functional indices. They attributed their findings to several mechanisms, such as the exclusion of important traits from the analysis because of lack of information (*e.g.*, bioirrigation), and the possible correlation between functional variables and some environmental and taxonomic diversity parameters that may result in the exclusion of functional indices from model selection (Link et al., 2013b). Similarly, we could not consider bioirrigation explicitly in our analysis because of the lack of data for the taxa considered. In addition, data on biological trait expression for the taxa considered were sometimes incomplete and often based on observations of species and taxa from a limited range of geographic locations and environmental conditions. Such data may therefore not always accurately reflect the actual behaviour of taxa inhabiting the sediment cores used for our analysis. Additionally, other studies have reported a low correlation between bioturbation intensity and nutrient generation in sediments (Solan et al., 2008), suggesting a more complex relationship between ecosystem functioning and bioturbation than anticipated, and the diverse interaction of different species with the benthic environment that cause variability in the effect of bioturbation on benthic fluxes (Ieno et al., 2006; Solan et al., 2008; Morata et al., 2020).

4.5.3 Sea pens and benthic nutrient fluxes

In our study, we detected a clear effect of sea pens in enhancing ammonium effluxes, at both small (effect of sea pen specimen collected in our sediment cores) and large scales (differences between sea pen fields and other sedimentary habitats). Similarly, Pierrejean et al. (2020), found the prevalence of ammonium release in sediments hosting biogenic structures (bamboo corals), compared to ammonium intake in bare sediments. Our finding suggests higher production of ammonium through ammonification, which could not be completely converted into

nitrate through nitrification, as well as higher rates of coupled nitrification and denitrification (Laverock et al., 2011), both resulting in higher release of ammonium into the water column. Such patterns point to the role of sea pens in stimulating ammonium release and nitrification rates in underlying sediments (de Froe et al., 2019), likely related to bioturbation activity. For example, the input of oxygenated water into deeper sediment layers and the extension of the oxic/anoxic interface caused by bioturbation activities of large organisms are known to stimulate microbial communities involved in the nitrogen cycle (Aller, 2001; Michaud et al., 2006; Laverock et al., 2011; Niemistö et al., 2018), together with the direct effect of bioturbation activities that increase the release of ammonium from sediments (Mermillod-Blondin et al., 2005). Higher release of ammonium near sea pens could also link to higher deposition of labile organic matter caused by the presence of sea pens that alter hydrodynamics at the sediment-water interface (Tissot et al., 2006; Cerrano et al., 2010; Kenchington et al., 2011), an interpretation supported by the higher quantity and quality of organic matter within sea pen fields found in this study. We indeed observed higher effluxes of ammonium from the sediments associated with higher sedimentary concentrations of total organic carbon. Finally, resuspension of sediment and organic matter particles at the sediment-water interface intensified by the presence of sea pens could also lead to more rapid effluxes of ammonium by enhancing degradation of organic materials, as reported by other studies (*e.g.*, Spagnoli and Bergamini, 1997; Niemistö et al., 2018; Niemistö and Lund-Hansen, 2019).

Sea pens, however, did not show clear effects on other nutrient flux rates, nor on the overall remineralization function of sediments. These findings were surprising given that we found different macrofaunal communities (Chapter 3) and sedimentary organic matter in sea pen fields compared to other sedimentary habitats within the Laurentian Channel MPA. Moreover, during

our sampling and experiments, we repeatedly observed burrowing behaviour in many sea pen species (*e.g.*, *Pennatula* spp., *Funiculina* spp.), especially when disturbed, suggesting that they could add significantly to bioturbation and thus increase flux rates at the sediment-water interface. Although we observed such an effect on ammonium fluxes in our incubation cores containing sea pens, other fluxes were not influenced by the presence of sea pens. Noteworthy, most of the sea pens collected in our cores reacted to the disturbance by partially retracting themselves into the sediment and subsequently did not move during the 48-hour incubation period. For individuals that did not retract, we did not observe any sediment burrowing. This behavioural change related to the disturbance of our sampling and incubation likely led to an underestimation of their effect on nutrient flux rates. Furthermore, the way many sea pens burrow into the sediment may potentially cause very little "disturbance" and bioturbation in the sediment. Sea pen species such as *Pennatula rubra* (Chimienti et al., 2018) contract their body considerably while burrowing, by closing their polyps and expelling most of the water contained within the colony (Hoare and Wilson, 1977). Contracted colonies can be more than three times smaller than the extended colonies, therefore only occupying the burrows that their burrowing peduncle would otherwise occupy (see explanatory Figure 1 in Chimienti et al., 2018). If this capacity exists for the sea pen species considered in our study, the colonies burrowing inside the sediment may have exerted little bioturbation effect, with minimal effect on associated sedimentary processes such as organic matter remineralization and nutrient fluxes. We note, however, a need for further studies to clarify the influence of sea pens on organic matter remineralization in deep-sea sediments that assess potential effects at different spatial and temporal scales (*e.g.*, possible seasonal variability).

4.6 Conclusion

Our results demonstrate the complexity of seafloor processes and the challenge of scaling relationships between ecosystem functioning and biota, particularly within a relatively homogeneous environment such as the Laurentian Channel MPA, where small-scale variation seems to underscore benthic processes. Successfully describing large-scale spatial variability in benthic nutrient fluxes requires a better understanding of the potentially interacting effects of biological and environmental processes, small-scale habitat heterogeneity, and temporal (*e.g.*, seasonal, interannual) variability, a gap identified by other studies (Hall et al., 1996). For instance, enabling the prediction of ecosystem processes from biological community data requires better knowledge of biological trait expression for the taxa considered. Macrofaunal behaviours such as bioirrigation, for instance, presumably strongly impact nutrient fluxes at the sediment-water interface, but lack of information on this biological trait for most macrofaunal organisms certainly limits our understanding of biodiversity and ecosystem functioning relationships and constrains our ability to predict functioning from functional diversity metrics.

From a conservation standpoint, our study points to the importance of including multiple habitats in MPA design and marine spatial planning in order to increase the likelihood of conserving multiple, essential processes and biological communities, especially for environments whose functioning remains unclear. In contrast to prioritizing rare, charismatic, and threatened species or habitats alone, researchers increasingly recognize the need for inclusion of a multitude of habitats and species as conservation targets (*e.g.*, through consideration of habitat diversity) as a key consideration in prioritizing locations for deep-sea conservation efforts (Danovaro et al., 2020).

4.7 Acknowledgements

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4.8 Appendices

Appendix 4A. Statistical results of ANOVA and independent t-tests for all the inorganic nutrient fluxes considered in our study among stations (factor "station"), between sea pen fields and other sedimentary habitats (factor "Biog. Hab."), and between cores containing sea pens and cores without (factor "Sea pen"). * indicates significant p-values (< 0.05). df: degrees of freedom; F: F-statistic; p: p-value; t: t-statistic.

Nitrate flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Station	ANOVA type III (Tukey)	4.927e +6	5	985338.645	1.676	0.171
Res		1.764e +7	30	587853.576		
Source	**Test**		**t**	**df**	**p**	
Biog. Hab.	t-test (Student)		0.345	34	0.732	
Sea pen	t-test (Student)		-0.430	34	0.670	

Appendix 4A (continued)

Nitrite flux

Source	Main test	Sum of Squares	df	Mean Square	F	p
Station	ANOVA type III (Tukey), Brown-Forsythe corr.	3496.534	5	699.307	0.943	0.486
Res		22246.525	12.835	1733.22		

Source	Test	t	df	p
Biog. Hab.	t-test (Student)	-0.714	34	0.48
Sea pen	t-test (Student)	-0.402	34	0.690

Ammonium flux

Source	Main test	Sum of Squares	df	Mean Square	F	p
Station	ANOVA type III (Tukey), Brown-Forsythe corr.	134099.535	5	26819.907	2.951	0.065
Res		272689.323	10.609	25703.509		

Source	Test	t	df	p
Biog. Hab.	t-test (Student)	-2.194	34	0.035*
Sea pen	t-test (Welch)	-2.411	7.870	0.043*

Phosphate flux

Source	Main test	Sum of Squares	df	Mean Square	F	p
Station	ANOVA type III (Tukey)	29511.622	5	5902.324	1.317	0.283
Res		134405.061	30	4480.169		

Source	Test	t	df	p
Biog. Hab.	t-test (Student)	-0.805	34	0.426
Sea pen	t-test (Student)	-0.694	34	0.492

Silicate flux

Source	Main test	Sum of Squares	df	Mean Square	F	p
Station	ANOVA type III (Tukey), Brown-Forsythe corr.	3.256e +7	5	6.513e +6	1.038	0.453
Res		1.882e +8	8.742	2.153e +7		

Source	Test	t	df	p
Biog. Hab.	t-test (Student)	0.464	34	0.645
Sea pen	t-test (Student)	0.240	34	0.812

Appendix 4B. Statistical results of PERMANOVA main test of multivariate benthic nutrient fluxes among stations (factor "station"), between sea pen fields and other sedimentary habitats (factor "Biog. Hab."), and between cores containing sea pens and cores without (factor "Sea pen"). df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Benthic nutrient fluxes						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Station	5	27.199	5.4397	1.1041	0.3272	9894
Res	30	147.8	4.9267			
Biog. Hab.	1	6.4985	6.4985	1.3113	0.2448	9951
Res	34	168.5	4.9559			
Sea pen	1	10.473	10.473	2.1643	0.1049	9944
Res	34	164.53	4.839			

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CHAPTER 5 – SEDIMENTARY ORGANIC MATTER SHAPES MACROFAUNAL COMMUNITIES BUT NOT BENTHIC NUTRIENT FLUXES IN CONTRASTING HABITATS ALONG THE NORTHWEST ATLANTIC CONTINENTAL MARGIN*

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5.1 Abstract

The heterogeneous topography of continental margins can influence patterns of resource availability and biodiversity in deep-sea sediments, potentially altering ecosystem functioning (*e.g.*, organic matter remineralization). Noting a lack of studies that address the latter, we contrasted spatial patterns and drivers of benthic nutrient fluxes and multiple characteristics of macrofaunal communities in shelf, slope, canyon and inter-canyon sedimentary habitats along the Northwest Atlantic continental margin. Replicate sediment push cores were collected from 10 stations (229-996 m depth), incubated for ~48 hours to estimate fluxes of nitrate, nitrite, ammonium, phosphate, and silicate (as a measure of organic matter remineralization) and subsequently analyzed to characterize macrofaunal communities. We also considered various environmental factors, including sedimentary organic matter quantity and quality, and assessed their influence on fluxes and macrofauna. Comparatively high macrofaunal density and distinct community composition and trait expression characterized Georges Canyon, where elevated sedimentary organic matter suggested important lateral transport mechanisms along this canyon axis, with deposition of organic matter strongly affecting biological communities but not benthic nutrient fluxes. Lower penetration of macrofauna into the sediments, distinct community

composition, biological traits, and higher nutrient flux rates characterized inter-canyon habitats compared to slope habitats at similar depths. Within inter-canyons, intermediate to low organic matter suggested hydrodynamic forces inhibiting organic matter deposition, affecting biological and functional processes. The input of fresh phytodetritus to the seafloor was the best predictor of macrofaunal density and diversity and contributed to variation in macrofaunal community composition and biological trait expression, together with latitude, depth, and other measures of organic matter quantity and quality. Benthic nutrient fluxes revealed complex variation, with disproportionate effects of few key macrofaunal taxa, together with bottom water oxygen concentration, and sediment granulometry. Our results suggest a relationship between resource availability and macrofaunal density, diversity, and taxonomic and trait composition, whereas organic matter remineralization exhibited a more complex response, which we suggest reflected variation in hydrodynamics and/or physical disturbance in heterogeneous continental margin habitats.

5.2 Introduction

Many abiotic and biotic factors affect ecosystem processes and biodiversity in deep-sea sediments (Snelgrove and Smith, 2002). For example, because most deep-sea organisms rely on the availability of surface-derived or advected organic material, their density usually declines with increasing depth and distance from shore (Rowe et al., 1982; Rex et al., 2006; Smith et al., 2008). Other factors complicate these general patterns at different scales, including habitat heterogeneity, sediment grain size, oxygen availability, and biological interactions (Levin et al., 2001, 2010). Similar factors can also affect benthic organic matter remineralization (Link et al., 2013a,b; Stief, 2013; Alonso-Pérez and Castro, 2014; Belley and Snelgrove, 2016; Belley et al., 2016), the

important ecosystem process of breaking down complex organic particles into their simplest inorganic forms that primary producers can reuse (Jahnke, 1996; Nixon, 1981).

Continental margins, the transitional zones between the thick continental crust and the thin ocean crust, cover approximately 11% of the global ocean seafloor (Jahnke, 2010), and are characterized by high heterogeneity of geomorphological, geochemical, and hydrographic features. This mosaic of different habitats and ecosystems supports high biodiversity and ecosystem functioning (Levin and Sibuet, 2012). Submarine canyons provide a major source of habitat heterogeneity along continental margins and can act as major conduits for transporting organic matter from shallow to deeper areas. These conduits increase food availability in the food-limited deep sea and possibly alter sediment characteristics (Levin et al., 2010; Harris and Whiteway, 2011; Puig et al., 2014; Amaro et al., 2015; De Leo et al., 2014; De Leo and Puig, 2018; Robertson et al., 2020). For these reasons, numerous studies describe submarine canyons as biodiversity hotspots in the deep sea (Levin and Sibuet, 2012; Robertson et al., 2020), with enhanced levels of abundance, diversity, and biomass of organisms compared to adjacent areas, and often hosting distinct communities in term of species composition (De Leo et al., 2010, 2014; Robertson et al., 2020). However, strong heterogeneity in the processes regulating organic matter deposition and ecological processes among different canyons (Pusceddu et al., 2010), and even between axes of the same canyon (Bianchelli et al., 2008), constrains any simple generalization. For instance, canyons that incise the continental shelf likely experience lateral transport of materials (*e.g.*, turbidity currents; Puig et al., 2014) more often than canyons that terminate on the continental shelf (known as blind canyons; Harris and Whiteway, 2011). Other topographic features and hydrodynamic forces can affect organic matter deposition and associated biological communities (Vetter et al., 2010; Harris and Whiteway, 2011; Campanyà-Llovet et al., 2018). By

potentially altering the fluxes of particulate organic matter to the deep sea and changing hydrodynamics, submarine canyons could affect benthic-pelagic coupling and sedimentary processes, as well as act as a source of carbon storage, possibly playing a major role in regulating global climate (Fernandez-Arcaya et al., 2017).

Only a few studies have examined in detail the biodiversity patterns of macro-infaunal organisms in canyon habitats (*e.g.*, McClain and Barry, 2010; De Leo et al., 2014; Leduc et al., 2015; Campanyà-Llovet et al., 2018; Bernardino et al., 2019; Robertson et al., 2020; Shantharam et al., 2021). Even though some of these studies report higher macrofaunal diversity within canyons compared to adjacent slope habitats, they also highlight high intra-canyon heterogeneity, often observed at small scales (De Leo et al., 2014; Campanyà-Llovet et al., 2018). Some studies report contrasting findings, such as a reduction of biodiversity nearby canyon walls, likely caused by increased sedimentation and/or bioturbation disturbance (McClain and Barry, 2010). Even fewer studies have explored the slope areas between canyons, termed inter-canyons (Quattrini et al., 2015). Finally, whereas previous studies reported higher oxygen consumption in canyons compared to the adjacent continental slope, suggesting higher organic matter remineralization (Duineveld et al., 2001), we are unaware of studies comparing benthic inorganic nutrient fluxes in canyon and inter-canyon environments. Despite relatively routine use of oxygen consumption to estimate organic matter remineralization in marine sediments, understanding biogeochemical cycles requires measurements of inorganic nutrient fluxes at the sediment-water interface (Giller et al., 2004; Bourgeois et al., 2017), which oxygen consumption may not always accurately represent (Berelson et al., 2003; Link et al., 2013a,b). The lack of information on variation in sedimentary habitat processes along continental margins points to a need for more studies. For instance, understanding the environmental and biological drivers of ecosystem processes such as

organic matter remineralization in marine sediments can improve management and conservation efforts (Hooper et al., 2005; Loreau, 2010; Snelgrove et al., 2014). This aspect is particularly relevant considering the increased attention continental margins have received in recent years as focal areas for conservation efforts (Levin and Sibuet, 2012; Davies et al., 2014; Fernandez Arcaya et al., 2017; Metaxas et al., 2019).

In this study, we contrast spatial patterns of a wide range of environmental, biological, and functional variables in deep-sea sediments across shelf, slope, canyon, and inter-canyon habitats along the highly heterogeneous Northwest Atlantic continental margin (Canada and USA). We use fluxes of nitrate, nitrite, ammonium, phosphate, and silicate at the sediment-water interface to compare benthic organic matter remineralization in sediments, which can release and take up dissolved inorganic nutrients. We then assess macrofaunal communities in terms of density, taxonomic diversity, vertical distribution, community composition, and biological trait expression. We investigate the environmental drivers (including several measures of sedimentary organic matter quantity and quality) of variation in benthic nutrient fluxes and macrofaunal density, diversity, community composition, and biological trait expression, as well as the role of macrofauna in regulating benthic nutrient fluxes. To our knowledge, our study represents the first attempt to quantify organic matter remineralization through the measurement of benthic nutrient fluxes in canyon and inter-canyon habitats. We aim for this work to provide a starting point to fill in some of the knowledge gaps regarding biodiversity and ecosystem functioning in these underexplored habitats. Noting evidence of enhanced biomass and elevated diversity in some canyon studies and a lack of data on inter-canyon habitats, we predict that our western Atlantic canyons will exhibit elevated densities and diversity of macrofauna, along with distinct taxa and biological trait composition, with the possibility of some spillover to inter-canyon habitats relative

to broad continental slope. We also expect elevated benthic nutrient fluxes will characterize canyons in response to higher input of organic materials and greater macrofaunal activities.

5.3 Materials and methods

5.3.1 Field sampling and stations description

Samples were collected along the Atlantic continental margins of Canada and the United States during a research cruise on board the NOAA research vessel *Henry B. Bigelow* (June 2017). We collected sediment push cores (i.d. = 6.7 cm, L = 35 cm) using the Remotely Operated Vehicle (ROV) ROPOS (www.ropos.com). We sampled 10 different stations (**Figure 5.1**; **Table 5.1**) spanning a depth range of 230-996 m and encompassing continental shelf (Western Jordan Basin), continental slope (Fiddler's Cove and outside Georges Canyon), submarine canyon (Corsair Canyon and Georges Canyon), and inter-canyon (Munson-Nygren Inter-canyon and Nygren-Heezen Inter-canyon) sedimentary environments. We named each station using the location's followed by the habitat's abbreviation (Sh for continental shelf; Sl for continental slope; C for canyons; I for inter-canyons), as shown in **Table 5.1**. At each station, we collected 4-7 push cores at randomly selected locations 10s meters away from each other. We dedicated 1-2 cores at every station to the analysis of sediment properties and the remaining cores (3-5) to the evaluation of benthic nutrient fluxes and macrofaunal diversity. At every station, we also collected bottom water samples using the (2-4) Niskin bottles mounted on the ROV for nutrient analysis and for water exchange during incubations. The ROPOS CTD Seabird 19plus mounted on the ROV recorded depth, bottom water temperature, salinity, and dissolved oxygen, and we also estimated the shortest distance from the shore for each station.

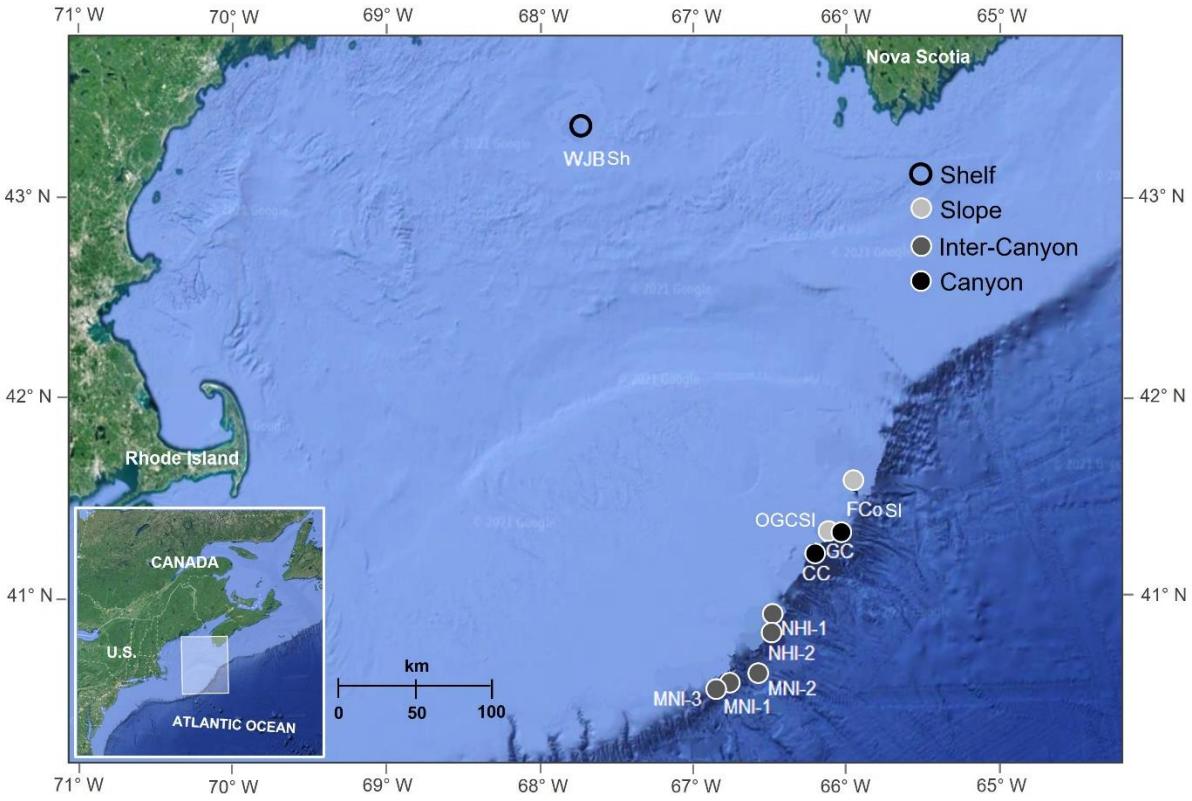

Figure 5.1: Map showing sampling area location and the 10 stations sampled in June 2017 for the evaluation of inorganic nutrient fluxes at the sediment-water interface, macrofaunal diversity, and environmental characteristics.

Table 5.1: Description of stations and main environmental variables. Stations are ordered from shallowest to deepest. n: number of replicate sediment cores used for characterization of benthic nutrient fluxes and macrofaunal communities; B T: bottom water temperature; B S: bottom water salinity; B [O_2]: bottom water oxygen concentration.

Station	Location	n	Latitude (N)	Longitude (W)	Habitat	Depth (m)	B T (°C)	B S (PSU)	B [O_2] (ml · L^{-1})
WJBSh	Western Jordan Basin (Canada)	4	43° 20.67'	67° 50.92'	Shelf	229	8.98	34.22	3.21
OGCSl	Outside Georges Canyon (Canada)	3	41° 16.43'	66° 11.27'	Slope	605	4.84	34.40	1.70
GC	Georges Canyon (Canada)	4	41° 15.98'	66° 14.51'	Canyon	636	4.90	34.98	5.00
MNI-1	Munson-Nygren Inter-canyon (USA)	3	40° 37.39'	66° 50.44'	Inter-canyon	793	4.70	34.98	5.34
FCoSl	Fiddler's Cove (Canada)	3	41° 35.84'	65° 51.98'	Slope	795	4.37	34.89	5.35
NHI-1	Nygren-Heezen Inter-canyon (USA)	3	40° 52.06'	66° 32.94'	Inter-canyon	837	4.20	34.95	5.63
NHI-2	Nygren-Heezen Inter-canyon (USA)	3	40° 51.94'	66° 32.89'	Inter-canyon	870	4.20	34.95	5.48
MNI-2	Munson-Nygren Inter-canyon (USA)	3	40° 37.17'	66° 32.94'	Inter-canyon	874	4.45	34.97	5.50
CC	Corsair Canyon (Canada)	3	41° 19.19'	66° 05.87'	Canyon	980	4.37	34.96	5.50
MNI-3	Munson-Nygren Inter-canyon (USA)	3	40° 37.02'	66° 49.87'	Inter-canyon	996	4.37	34.96	5.50

5.3.2 Sedimentary organic matter and granulometric properties

We collected sediment for analysis of organic matter and grain size from the 0-2 cm top layer of the 1-2 dedicated cores at each station, homogenizing the sediment and then placing it in Whirl-Pak bags prior to storage in the dark at -20 °C until analyzed. In this study, we use Total Organic Matter (TOM) and Total Organic Carbon (TOC) as a measure of food quantity. We use total nitrogen (TN) and total organic carbon to total nitrogen ratio (C: N) as a measure of organic matter quality over long time scales, with higher TN and lower C: N indicating fresher and higher quality organic matter (Godbold and Solan, 2009; Le Guitton et al., 2015; Campanyà-Llovet et al., 2017). We use concentrations of chlorophyll *a* (Chl *a*), phaeopigments (Phaeo), total pigments (Tot Pigm), as well as chlorophyll *a* to phaeopigments ratio (Chl *a*: Phaeo), and chlorophyll *a* to total organic carbon ratio (Chl *a*: TOC) as measures of phytodetritus input to the seafloor and short-term organic matter quality and freshness. In this sense higher concentrations of pigments and higher Chl *a*: Phaeo and Chl *a*: TOC indicate higher inputs of fresh phytodetritus to the seafloor (Pusceddu et al., 2009; Le Guitton et al., 2015;). We use % sand, % silt, and % clay, as well as mean grain size of the sortable silt fraction (MGS), as a measure of sediment particle size and distribution.

Sediment total organic matter (TOM) was calculated as the difference between dry (desiccated at 60 ºC for 24 hours) and calcinated (muffle furnace at 450 ºC for 4 hours) weight, and expressed as mg · g DW^{-1} (Danovaro, 2010).

Total organic carbon (TOC) and total nitrogen (TN) were determined by drying a sediment subsample of 1-5 g (wet weight) at 60 °C for 24 h, grinding it to a fine powder, and then weighing and acidifying (with pure HCl fumes) for 24 h to eliminate inorganic carbon. Samples were dried again at 60 °C for 24 h before starting the analysis. We then weighed an aliquot of dried

decarbonated sediments (15 mg) and folded it tightly into a tin capsule. A Carlo Erba NA1500 Series II elemental analyser (EA) determined the sediment concentration of TOC and TN, expressed as mg · g DW^{-1}.

Sedimentary concentrations of chloroplastic pigments (chlorophyll *a* and phaeopigments) were determined using a spectrophotometer following Danovaro (2010). Pigments were extracted with 90% acetone (24 h in the dark at 4 °C). After centrifugation (800 x g), the supernatant was used to determine the functional chlorophyll-a and acidified with 0.1 N HCl to estimate the amount of phaeopigments. We then dried the sediment at 60 ºC for 24 h prior to weighing. Sediment concentrations of pigments were expressed as μg · g DW^{-1}. Total phytopigment concentrations were defined as the sum of chlorophyll *a* and phaeopigment concentrations (Pusceddu et al., 2009).

We digested a subsample of sediment with hydrogen peroxide to eliminate any organic material present and then freeze-dried sediments before analysis with the Beckman Coulter LD13-320 laser diffraction analyzer to determine granulometric properties. Sieving was performed prior to analysis to ensure the elimination of large particles (gravel fraction). For each sample, we determined % of gravel, sand, silt and clay. We also determined mean grain size of the sortable silt fraction (MGS, μm). Sediments were classified following the sediment classification scheme based on the percentages of sand, silt, and clay (Shepard, 1954).

5.3.3 Benthic inorganic nutrient fluxes

To evaluate fluxes of nitrate, nitrite, phosphate, ammonium and silicate, we incubated sediment cores (sediment volume: 527.6 ± 98.4 cm^3; water volume: 705.8 ± 98.4 cm^3) and overlying water for approximately 48 hours and removed water samples for dissolved inorganic nutrients analysis at regular intervals during the incubation. A total of 32 incubations were run on board during the 2-week cruise.

After collection, sediment cores were acclimated for about 12 hours, allowing sediment particles in suspension to settle back to the sediment surface. Several hours before the beginning of the experiment, the overlying water was carefully (without resuspending the sediment) exchanged with fresh, oxygenated bottom seawater collected *in situ*, allowing it to overflow in the surrounding water bath. This addition prevented hypoxia in the cores and removed toxic metabolites produced by community metabolism. To start the incubation, all visible bubbles were removed from the surface, chambers were sealed with acrylic caps equipped with magnetic stirrers, and the sediment cores were incubated in a refrigerator kept at in situ temperature (~4.5 °C) and in the dark for about 48 hours. Stirrers were working for the duration of the experiment at approximately 3 revolutions per minute to prevent anoxia in the sediment, without resuspending the sediment. Each 48-h experiment comprised three ~ 12-hour sequential incubation segments. At the beginning of every incubation, we extracted ~30 ml of water using a 60-ml acid-rinsed plastic syringe. We used ~ 5 ml of water to rinse the syringe and sample bottle and then removed ~25 ml of water to store in 30-ml acid-rinsed HDPE plastic bottles at -80 °C in upright position for subsequent analysis of dissolved inorganic nutrients. At the end of every 12-h incubation segment, we removed the lids and resampled water for nutrient analysis. During the following 1-6 hours, overlying water in the chamber was carefully exchanged with fresh, oxygenated bottom seawater collected at each station to prevent hypoxia in the chambers and to remove toxic metabolites produced by community metabolism.

The concentrations of dissolved inorganic nutrients (nitrate, nitrite, ammonium, phosphate, and silicate) in the water sampled from the incubations as well from the bottom water samples were determined using a continuous segmented flow analyzer (Seal AutoAnalyzer 3) at the Bedford Institute of Oceanography (Darmouth, NS). Analyses were performed following the

Industrial Method 186-72W for silicates, the Industrial Method 158-71W (adapted from Armstrong et al., 1967; Grasshoff, 1969) for nitrate and nitrite, the Industrial Method 155-71W (adapted from Murphy and Riley, 1962; Aoyama et al., 2012) for orthophosphate and the fluorometric method developed by Aminot and Kérouel (1997) for ammonium. Nutrient fluxes, expressed as $\mu mol \cdot m^{-2} \cdot d^{-1}$, were determined from the measured concentration changes in the overlying water as a function of time, water volume, and sediment area, summed over the three ~12-hour incubation segments.

5.3.4 Macrofaunal identification and taxonomic diversity

At the end of each ~48-h incubation, we removed all cores from the water bath and immediately processed them for subsequent analysis. Following extrusion of the sediment from the cores and sectioning into 0-5 and 5-10 cm sediment layers using inert plastic spatulas, we fixed the unsieved sediment in 4% buffered formaldehyde seawater in 500-ml plastic jars for later faunal identification. In the laboratory, we processed samples over a 300-μm sieve prior to subsequent transfer to 70% ethanol until we could complete microscopic analysis. Before identification, samples were stained with a few drops of Rose Bengal ($0.5 g \cdot L^{-1}$) to facilitate sorting of the samples and identification of organisms.

For each sample, we sorted macrofaunal organisms and assessed abundances of the major taxa (Classes: Polychaeta, Oligochaeta, Amphipoda, Isopoda, Copepoda, Ostracoda, Bivalvia, Gastropoda, Scapopoda, Sipunculidea, Ophiuroidea, Asteroidea, Holothuroidea, Echinoidea, and Subclasses: Hexacorallia, Octocorallia). We further identified polychaetes to the family level because they represented the most abundant taxon in the majority of our samples. In addition, the high taxonomic and functional diversity of polychaetes make them good indicators of environmental quality, as well as effective surrogates for total biodiversity in ecological studies

(Pocklington and Wells, 1992; Dauvin et al., 2003; Olsgard et al., 2003). The large number of samples and the time-consuming nature of taxonomic analysis precluded species identification, however, we justify our decision to assess polychaete diversity at the family level based on previous studies that demonstrated the efficacy of this approach as a valid alternative to species level analysis when investigating patterns of community structure, functional diversity, species distribution and effects of environmental variables on biological communities (Fauchald and Jumars, 1979; Jumars et al., 2015; Checon and Amaral, 2017). We calculated total macrofaunal densities (ind · m^{-2}), total number of taxa (including classes, subclasses and polychaete families), and Pielou's evenness (J') for each sample combining the data from the entire 10-cm cores. We also assessed the vertical distribution of macrofauna by calculating the proportion of organisms in the top 5-cm layer. Diversity indices were calculated in PRIMER 6+ using the DIVERSE routine.

5.3.5 Macrofaunal biological trait expression

In order to evaluate biological trait expression, we used Biological Trait Analysis (Bremner et al., 2003), which uses multivariate ordination to describe patterns of biological trait composition over the entire macrofaunal assemblage and quantifies the types of trait present in assemblages and the relative frequency with which they occur, thereby providing a means to explore patterns in assemblage functional structure and functioning (Bremner et al., 2006). We selected 6 biological traits related to different aspects of life histories and functioning, and subdivided them into 26 categories that characterized behaviour/strategies in more detail (**Table 5.2**). We used a fuzzy coding approach to assign trait categories to the taxa (classes, subclasses, or families) that allowed each taxon to represent more than one trait category, therefore capturing inter- and intraspecific variation in trait expression. We adopted a scoring range of 0 to 5, with 0 reflecting no affinity for the given trait category, 1, 2, 3, or 4 reflecting partial, increasing affinity, and 5 denoting exclusive

affinity. We derived information on trait expression for all the taxa from several published sources (Fauchald and Jumars, 1979; Highsmith and Coyle, 1991; Hyne, 2011; Queirós et al., 2013; Jumars et al., 2015; Polytraits, 2020) as well as from direct observations on our specimens. When information was unavailable for a given taxon, we obtained information from one taxonomic rank higher (*e.g.*, from orders of polychaetes). In a few cases where no information at all was available for a given taxon, we distributed the 5 scores equally among all the plausible trait categories. To obtain the community trait expression in each sample, we multiplied trait categories for each taxon present in a sample by its density (ind · m^{-2}) in that sample, and then summed over all taxa present in each core to obtain a single value for each trait category in each sample (Bremner et al., 2006). We then explored the resulting matrix using multivariate analysis.

Table 5.2: Biological traits and categories used in trait analysis.

Trait	Modalities
Motility	Motile
	Discretely motile
	Sessile/sedentary
Feeding mode	Suspension/filter feeder
	Surface deposit feeder
	Subsurface deposit feeder
	Omnivore
	Predator
	Scavenger
Bioturbation	None
	Surface modifier
	Biodiffuser
	Upward conveyor
	Downward conveyor
	<1 year

Table 5.2 (continued)

Lifespan	1-3 years
	3-5 years
	>5 years
Larval development	Direct
	Indirect
	Benthic
	Pelagic
Fecundity	Low
	Medium
	High
	Very high

5.3.6 Statistical analysis

The opportunistic nature of our sampling resulted in an unbalanced design with different habitats represented differently at different depths (*e.g.*, most shelf and slope stations were at shallower depths and most inter-canyon stations were at deeper depths). This confounding complicated the comparison between habitats because of well-established effects of depth (and its correlates) on biological communities and functional processes (Rex et al., 2006). To address this concern, we ran a preliminary analysis to understand the effect of depth on macrofaunal community structure (chosen because community structure showed the most obvious differences among stations) among all the habitats. To do so, we assigned depth classes to each station as follows: WJBSh 200 m; OGCSl and GC 600 m; FCoSl, MNI-1, and NHI-1 800 m; MNI-2 and NHI-2 900 m; CC and MNI-3 1000 m. We then assessed variation in macrofaunal community composition among depth classes using multivariate analysis of variance (PERMANOVA) on Bray-Curtis similarity matrices of square-root transformed density data and we ran the pair-wise comparisons as post hoc analyses to identify which depth classes differed in terms of macrofaunal composition. Based on these results, we defined four *a posteriori* depth classes (**Table 5.3**) that

we used to investigate variation in benthic nutrient fluxes and macrofauna across habitats. We also note the unbalanced distribution of our stations, with clear geographic separation of WJBSh from all other stations, and the clustering together of all inter-canyon stations apart from other stations (see **Figure 5.1**). We acknowledge that this distribution represents a limitation of this study and might have affected our findings.

Table 5.3: *A posteriori* depth class classification of stations used to determine differences among habitats in subsequent analysis. Classification derived from significant differences (PERMANOVA, $p < 0.05$) in terms of macrofaunal community composition. * was removed from the analysis comparing habitats as the only station in the 200 m depth class as well as in shelf habitat.

Station	*A posteriori* depth class	Habitat
WJBSh*	200 m	Shelf
OGCSl	600 m	Slope
GC		Canyon
MNI-1	800-1000 m	Inter-Canyon
FCoSl		Slope
NHI-1		Inter-Canyon
NHI-2		Inter-Canyon
MNI-2		Inter-Canyon
CC		Canyon
MNI-3		Inter-Canyon

To investigate variation in each nutrient flux (nitrate, nitrite, phosphate, silicate, and ammonium), taxonomic diversity index (total macrofaunal density, total number of taxa, and Pielou's evenness), and vertical distribution of macrofauna (% organisms in the 0-5 cm layer) among habitats we used two-way type III univariate analysis of variance (ANOVA). We ensured that our data met homogeneity of variance (with Levene's tests) and normality (with Q-Q plots of residuals) assumptions prior to analysis. We then performed post hoc pair-wise comparisons of

significant effects ($p < 0.05$) using standard Tukey's tests.We also investigated variation in multivariate benthic nutrient fluxes, macrofaunal community composition and biological trait expression (separately) among habitats using multivariate analysis of variance (PERMANOVA) performed with 9999 random permutations of appropriate units. We ran the pair-wise comparisons as post hoc analysis whenever we found significant differences ($p < 0.05$). We verified homogeneity of multivariate dispersions using the PERMDISP routine. For both ANOVA and PERMANOVA analyses we used a nested design with the fixed factors "depth" (3 levels), and "habitat (depth)" (2 levels within the 600 m depth class: slope, canyon; 3 levels within the 800-1000 m depth class: slope, inter-canyon, canyon).

We visualized separation of multivariate benthic nutrient fluxes in ordination space using Principal Component Analysis (PCA), and separation of macrofaunal community composition and biological trait expression (separately) in ordination space using non-metric multidimensional scaling (nMDS) ordinations of similarity matrices. We also identified the taxa and biological trait categories that distinguished assemblages among habitats using a percent similarity procedure (SIMPER) analysis (Clarke and Gorley, 2006).

We next ran a set of analyses to identify the environmental drivers of benthic nutrient fluxes and macrofauna. We first explored correlations between total macrofaunal density, total number of taxa, and Pielou's evenness (separately) and all available environmental variables using Draftman's plots and correlation analysis. We next used a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001) to identify which set of environmental variables predicted variation of multivariate benthic nutrient fluxes, macrofaunal community composition, and biological trait expression (in 3 separate analyses). We used resemblance matrixes of multivariate benthic nutrient flux data (based on Euclidean distances), and macrofaunal

community composition and biological trait expression (based on Bray-Curtis similarity, calculated from square root transformed data) as a measure of between-samples similarities. The predictive environmental variables allowed to enter the models were: latitude, distance from shore, depth, bottom water temperature, salinity, oxygen concentration, bottom water concentrations of nitrate, silicate, phosphate ad ammonium, sedimentary concentrations of TOM, TOC, TN, Chl *a*, Phaeo, and Tot Pigm, C: N, Chl *a*: Phaeo, Chl *a*: TOC ratios, % gravel, % sand, % silt, % clay, and MGS. Variables were standardised to mean 0 and standard deviation 1 prior to analysis. We assessed normality and collinearity of predictor variables using Draftsman's plots, ensuring that highly correlated variables did not appear simultaneously in the final models.

Finally, we identified the biological drivers of benthic nutrient fluxes in order to assess underlying biodiversity and ecosystem functioning (BEF) relationships. To do so, we used a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001), with macrofaunal taxonomic diversity indices, as well as community composition and biological trait expression matrices as predictive variables. The use of matrices of community composition and biological trait expression in this analysis allowed testing of the relevance of each taxon density and trait category expression, respectively, on multivariate flux variation. We used the resemblance matrix of multivariate benthic nutrient flux data based on Euclidean distances as a measure of between-samples similarities. Noting that the number of predictor variables in this case greatly exceeded the number of samples, we did preliminary testing of the influence of each group of biological variables (taxonomic diversity indices, macrofaunal community composition, and macrofaunal biological trait expression) on benthic nutrient fluxes separately. Taxa that appeared in fewer than three samples (echinoids, amphinomids, heterospionids, phyllodocids, serpulids) were removed from the community composition matrix to further reduce the number of predictor

variables and the number of zeros. Taxonomic diversity indices were standardised to mean 0 and standard deviation 1, whereas community composition and biological trait expression data were square-root transformed prior to analysis. For each group, the variable(s) that correlated best with benthic nutrient fluxes were selected and combined in a final analysis to determine the best biological model explaining variation in benthic nutrient fluxes.

For all DistLM analyses, we ran stepwise routines with 9999 permutations and used AICc (Akaike's information criterion corrected) selection criterion, which is recommended for analyses with a small number of samples relative to the number of predictor variables (Anderson et al., 2008). We examined R^2 to identify the best model and determine the proportion of the variation explained by that model, visualizing results with distance-based redundancy analysis (dbRDA; Anderson et al., 2008). All multivariate analyses, including PERMANOVA, PERMDISP, SIMPER, PCA, nMDS, DistLM, and dbRDA analyses were performed in PRIMER v6 (Anderson et al., 2008).

5.4. Results

5.4.1 Sedimentary organic matter and granulometric properties

In terms of sedimentary organic matter quantity (**Table 5.4**) GC, CC, and WJBSh were characterized by the highest concentrations of TOM and TOC. In terms of sedimentary organic matter quality (**Table 5.4**), WJBSh was characterized by the highest long-term quality (*e.g.*, high TN and low C: N), whereas GC had the highest input of phytodetritus (high Chl *a*, Chl *a*: TOC) of all stations. Other stations presented intermediate levels of organic matter quantity and quality, with the MNI stations showing the lowest input of phytodetritus.

In terms of granulometric properties (**Table 5.5**), most sediments were classified as silty sand according to the Shepard (1954) classification scheme, except for sandy sediments at GC and MNI-2, sandy silt at MNI-1 and clay-silt at WJBSh. MGS ranged from 20 µm to 39 µm at WJBSh and NHI-1, respectively.

Table 5.4: Summary of main organic matter quantity and quality parameters measured in the stations (average ± standard deviation derived from 2 or 3 replicate samples per station) *indicates values derived from only one replicate sample per station. TOM: total organic matter; TOC: total organic carbon; TN: total nitrogen; C: N: carbon to nitrogen ratio; Chl *a*: chlorophyll *a*; Tot Pig: total phytopigmemts; Chl *a*: Phaeo: chlorophyll *a* to phaeopigments ratio; Chl *a*: TOC: chlorophyll *a* to total organic carbon ratio.

Station	**TOM** (mg · g DW^{-1})	**TOC** (mg · g DW^{-1})	**TN** (mg · g DW^{-1})	**C: N**	**Chl *a*** (µg · g DW^{-1})	**Tot Pig** (µg · g DW^{-1})	**Chl *a*: Phaeo**	**Chl *a*: TOC**
WJBSh	27.96 ± 5.60	13.63 ± 1.30	1.69 ± 0.15	7.88 ± 0.89	2.08 ± 0.60	50.11 ± 9.39	0.05 ± 0.02	0.16 ± 0.05
OGCSl	16.89*	6.79*	0.62*	10.87*	3.67*	61.79*	0.06*	0.54*
GC	29.67*	12.96*	1.11*	11.71*	6.85 ± 3.23	89.79 ±	0.08 ± 0.02	0.53 ± 0.25
MNI-1	12.78*	9.04*	0.71*	12.71*	0.64*	6.43*	0.11*	0.07*
FCoSl	14.54 ± 7.19	13.84 ± 5.25	0.87 ± 0.61	18.29 ± 6.74	2.07 ± 0.97	61.29 ± 3.70	0.04 ± 0.02	0.15 ± 0.01
NHI-1	13.30 ± 3.22	6.79 ± 2.22	0.42 ± 0.24	17.72 ± 4.84	2.07 ± 0.48	79.45 ±	0.03 ± 0.00	0.31 ± 0.03
NHI-2	17.40 ± 0.22	8.08 ± 0.05	0.45*	17.94 ± 0.12	1.74 ± 0.56	72.28 ±	0.03 ± 0.01	0.22 ± 0.07
MNI-2	23.12 ± 2.77	12.11 ± 5.34	0.87 ± 0.09	13.63 ± 4.66	1.21 ± 0.01	60.85 ±	0.02 ± 0.00	0.11 ± 0.05
CC	29.49*	12.93*	1.02*	12.69*	2.37*	73.50*	0.03*	0.18*
MNI-3	12.15 ± 0.91	8.52 ± 1.48	0.44 ± 0.18	20.19 ± 4.79	0.99 ± 0.13	75.70 ±	0.01 ± 0.00	0.12 ± 0.04

Table 5.5: Summary of granulometric properties of sediments (average ± standard deviation derived from 2 or 3 replicate samples per station). *indicates values derived from only one replicate sample per station. MGS: mean grain size of the sortable silt fraction. Sediments were classified following the Shepard (1954) sediment classification scheme based on relative percentages of sand, silt, and clay.

Station	% gravel	% sand	% silt	% clay	MGS (μm)	Sediment class.
WJBSh	5.24 ±	22.06 ±	43.65	29.06 ±	20.56 ± 0.61	Clavey silt
OGCSl	0.00 ±	72.21 ±	20.08	7.70 ±	31.42 ± 0.28	Silty sand
GC	0.00*	75.63*	18.10*	6.28*	32.09*	Sand
MNI-1	0.00*	24.08*	51.51*	24.41*	24.62*	Sandy silt
FCoSl	0.00 ±	44.64 ±	40.60	14.76 ±	30.62 ± 1.20	Silty sand
NHI-1	1.29 ±	73.81 ±	18.15	6.75 ±	38.70 ± 0.43	Silty sand
NHI-2	0.00*	72.30*	20.45*	7.25*	38.37*	Silty sand
MNI-2	0.00 ±	77.13 ±	15.34	7.53 ±	28.82 ± 0.40	Sand
CC	0.00 ±	50.43 ±	36.09	13.49 ±	32.75 ± 1.26	Silty sand
MNI-3	0.00*	53.26*	30.43*	16.3*	30.92*	Silty sand

5.4.2 Benthic nutrient fluxes

Nitrate flux (**Figure 5.2, a**) ranged from -725.4 μmol · m^{-2} · d^{-1} to 1288.4 μmol · m^{-2} · d^{-1} across our replicate cores and the average values were directed toward the water column (release of nitrate) at all stations. Nitrite flux (**Figure 5.2, b**) ranged from -245.7 μmol · m^{-2} · d^{-1} to 62.0 μmol · m^{-2} · d^{-1} across our replicate cores and the average values were directed toward the sediment (uptake of nitrite) at all stations except MNI-2 and CC. Ammonium flux (**Figure 5.2, c**) ranged from -2705.9 μmol · m^{-2} · d^{-1} to 404.0 μmol · m^{-2} · d^{-1} across our replicate cores and the average values were directed toward the sediment (or uptake of ammonium) at all stations except GC and FCoSl. Phosphate flux (**Figure 5.2, d**) ranged from -108.9 μmol · m^{-2} · d^{-1} to 778.3 μmol · m^{-2} · d^{-1} across our replicate cores and the average values were directed toward the water column (release of phosphate) at all stations except FCoSl. Silicate flux (**Figure 5.2, e**) ranged from -7225.4 μmol

$\cdot m^{-2} \cdot d^{-1}$ to 6473.5 μmol $\cdot m^{-2} \cdot d^{-1}$ across our replicate cores and the average values were directed toward the water column (release of silicate) at all stations except FCoSl. We detected significant differences in silicate fluxes between habitats nested in depth classes (ANOVA, $p < 0.05$, **Appendix 5A**) and post hoc pair-wise analysis revealed significantly higher silicate effluxes in inter-canyon compared to slope habitats within the 800-1000 m depth class (**Figure 5.2, e**). Other fluxes did not differ significantly among habitats nested in depth classes (ANOVA, $p > 0.05$, **Appendix 5A**).

Multivariate analysis combining all the fluxes provided comparison of the overall remineralisation function of the sediments, for which we detected significant differences (PERMANOVA, $p < 0.05$, **Appendix 5B**) among habitats nested in depth classes. Post-hoc tests revealed significantly different benthic nutrient fluxes between inter-canyon and slope habitats within the 800-1000 m depth class. In the PCA plot (**Figure 5.3**), the first two PC axes explained 68% of the variation of benthic nutrient fluxes and analysis of the eigenvectors showed that no single flux dominated the multivariate similarity pattern among samples, with nitrite flux correlating most strongly with the first PCA axis (positively), and silicate flux correlating most strongly with the second PCA axis (negatively).

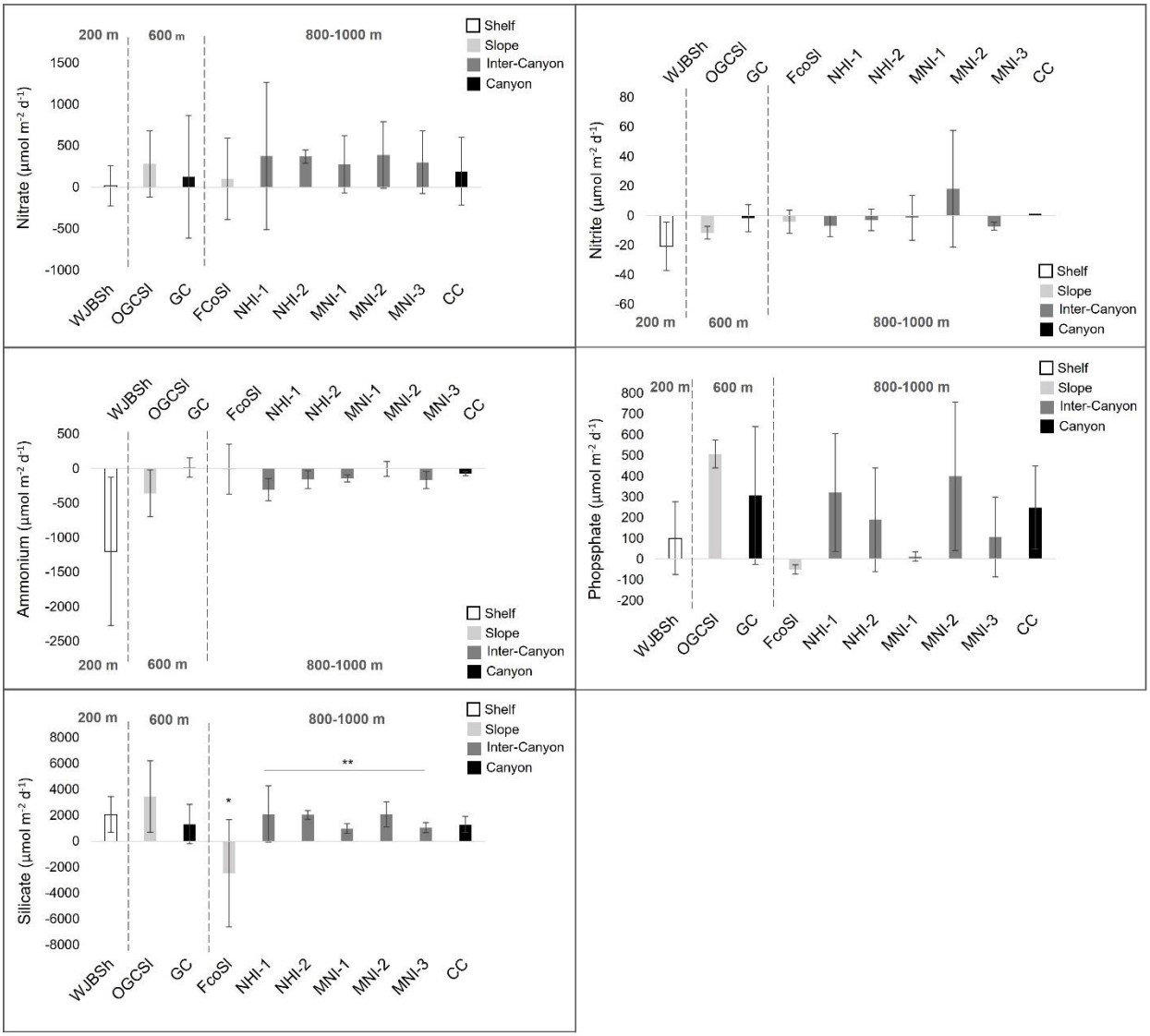

Figure 5.2: Inorganic nutrient fluxes at the sediment-water interface in the 10 stations (average and standard deviation), expressed as µmol · m^{-2} · d^{-1}. **a**) Nitrate flux, **b**) Nitrite flux, **c**) Ammonium flux, **d**) Phosphate flux, **e**) Silicate flux. Positive values indicate release of nutrients from the sediment into the water column (efflux), whereas negative values indicate uptake by the sediment (influx). Vertical dash lines indicate the 3 three depth classes used in the analysis (200 m, 600 m, 800-1000 m). Symbols (*, **), highlight significant differences among habitats within each depth class (ANOVA and t-tests, $p < 0.05$)

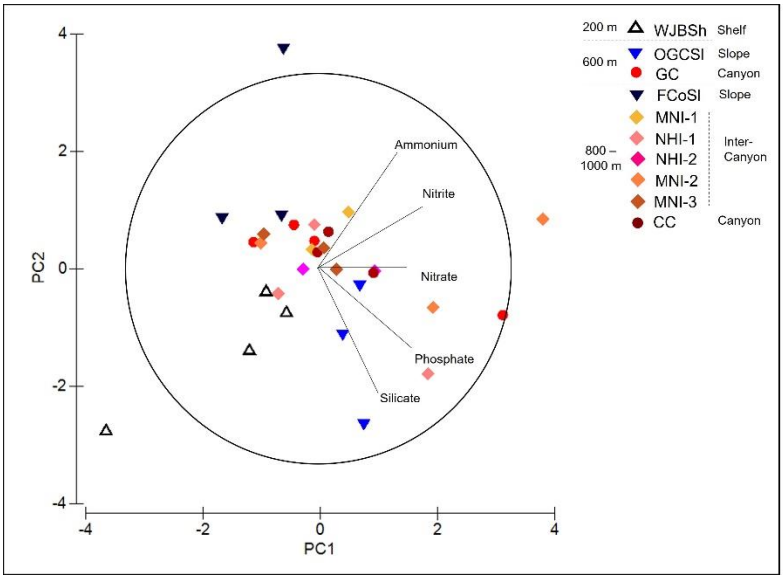

Figure 5.3: Principal Component Analysis (PCA) of all the inorganic nutrient fluxes at the sediment-water interface. Vectors show the strength and direction of nutrient fluxes contributing to the variation of benthic nutrient fluxes.

5.4.3 Macrofaunal density, taxonomic diversity, vertical distribution and community composition

We sorted 1,257 macrofaunal organisms in total, representing 40 different taxa. Densities varied from 2,554 to 27,526 ind · m^{-2} at MNI-2 and WJBSh, respectively. Polychaetes were the dominant taxon in most samples (average 49% of total macrofauna), followed by amphipods (average 15% of total macrofauna). Among the polychaetes, the families Cirratulidae, Paraonidae and Polynoidae dominated across stations, representing average 22%, 12%, and 9% of total polychaetes, respectively.

Total macrofaunal density (**Figure 5.4, a**) varied significantly between habitats nested in depth classes (ANOVA, $p < 0.05$, **Appendix 5C**) and post-hoc tests revealed higher density in canyon compared to slope habitat within the 600 m depth class. Total number of taxa (**Figure 5.4, b**) and Pielou's evenness (**Figure 5.4, c**) did not vary significantly among habitats (ANOVA, $p > 0.05$, **Appendix 5C**). The proportion of macrofaunal organisms in the upper 5-cm layer ranged from 0.83 to 1 and varied significantly (ANOVA, $p < 0.05$, **Appendix 5C**) between habitats nested

in depth classes. Post hoc tests revealed significantly lower proportion of organisms in the upper sediment layer in slope compared to inter-canyon habitats (**Figure 5.4, d**).

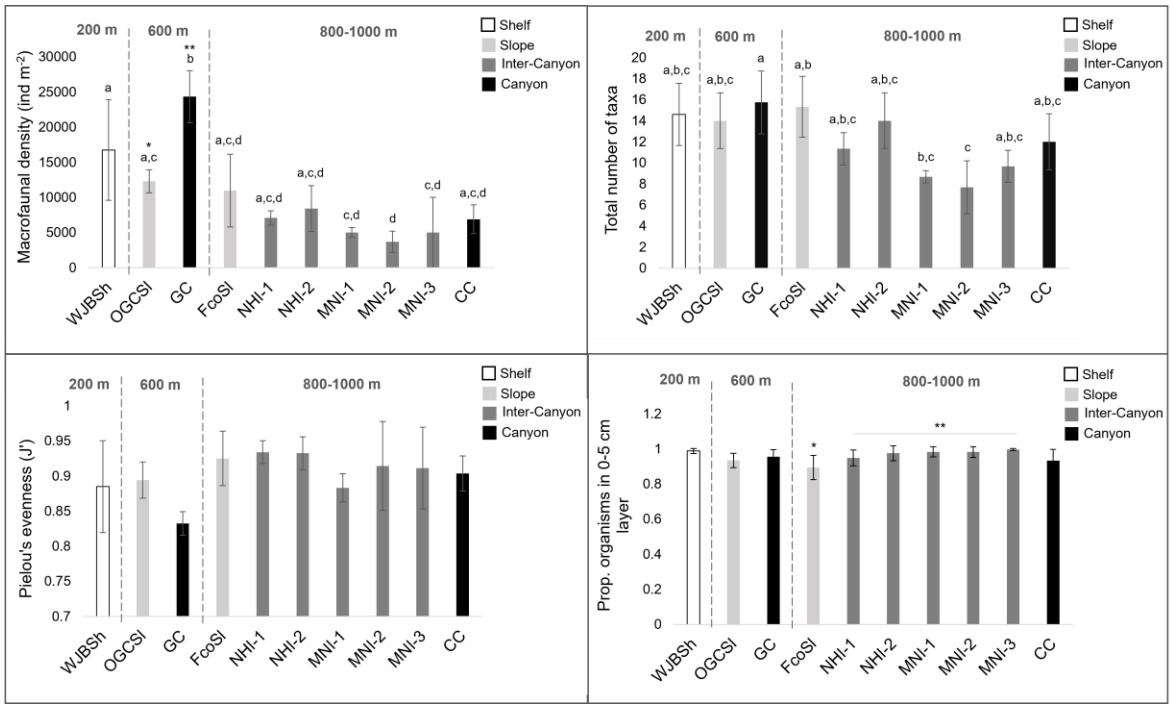

Figure 5.4: Macrofaunal density, taxonomic diversity and vertical distribution in the 10 stations (average ± standard deviation). **a**) Total macrofaunal density, **b**) Total number of taxa, **c**) Pielou's evenness (J'), **d**) Proportion of organisms in the 0-5 cm layer. Vertical dashed lines indicate the 3 three depth classes used in the analysis (200 m, 600 m, 800-1000 m. Symbols (*), if any, highlight significant differences among habitats within each depth class (ANOVA, $p < 0.05$).

Macrofaunal community composition differed significantly (PERMANOVA, $p < 0.05$, **Appendix 5D**) among habitats nested in depth classes and post-hoc tests revealed different macrofaunal composition between slope and canyon habitats within the 600 m depth class, and between inter-canyon and other habitats within the 800-1000 m depth class. Stations and habitats also separated in ordination space based on our nMDS analysis (**Figure 5.5**).

According to our SIMPER analysis, ophiuroids, amphipods, cossurids, polynoids, ostracods, cirratulids, and bivalves were the taxa that mostly contributed to differences between

slope and canyon habitats within the 600 m depth class, and all were more abundant in the canyon, whereas opheliids were more abundant on the slope. The average dissimilarity between Georges Canyon and adjacent slope was 43 % and these taxa contributed to 52 % of the variation. Bivalves, cirratulids, eunicids, maldanids, ostracods, lumbrinerids, amphipods, and hesionids contributed most to differences between inter-canyons and other habitats within the 800-1000 m depth class were more abundant in slope and canyon habitats, whereas ophiuroids, paraonids, and nereids were more abundant in inter-canyons. The average dissimilarity between inter-canyon and other habitats was 62 % and these taxa contributed 52 % of the variation.

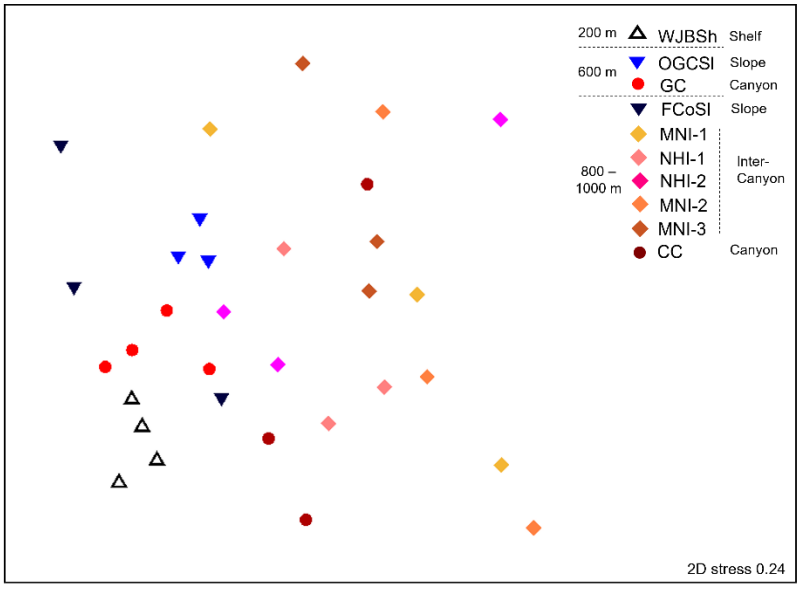

Figure 5.5: Nonmetric multidimensional scaling (nMDS) of the macrofaunal communities in the 10 stations, based on Bray-Cutis similarity of square root transformed density data.

5.4.4 Macrofaunal biological trait expression

In terms of biological traits, motile, deposit-feeders, surface modifier organisms with indirect, pelagic larval development, and medium lifespan dominated sediments overall. Macrofaunal community biological trait expression differed significantly (PERMANOVA, $p < 0.05$, **Appendix 5E**) among habitats nested in depth classes and post hoc tests revealed different

trait expression between slope and canyon habitats within the 600 m depth class, and between inter-canyon and slope habitats within the 800-1000 m depth class. Stations and habitats also separated in ordination space based on our nMDS analysis (**Figure 5.6**).

According to our SIMPER analysis, greater expression of motile, pelagic, indirect and direct larval development, high and very high fecundity, surface modifiers and lifespan 3-5 years in canyon habitats contributed most to trait modality differences between Georges Canyon and the adjacent slope. The average dissimilarity between slope and canyon was 18% and these trait modalities contributed 50% of the variation. Greater expression of indirect, pelagic larval development, sessile, discretely motile and motile, suspension and filter feeder, upward and downward conveyors, lifespan 3-5 years, and medium fecundity in slope habitats contributed most to trait modality differences between inter-canyon and slope habitats within the 800-1000 m depth class. The average dissimilarity between inter-canyon and slope was 20% and these trait modalities contributed 51% of the variation.

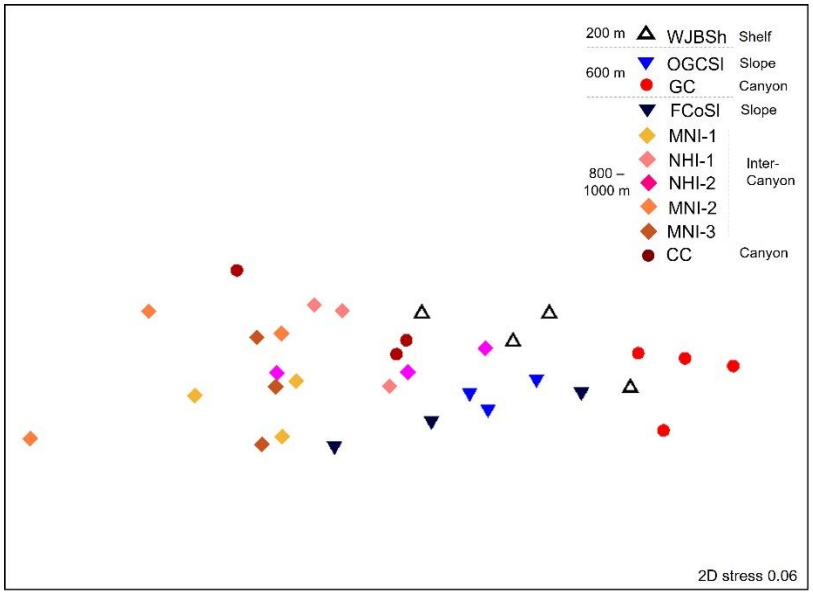

Figure 5.6: Nonmetric multidimensional scaling (nMDS) of the macrofaunal biological trait expression in the 10 stations, based on Bray-Curtis similarity of square root transformed trait expression data.

5.4.5 Environmental and biological drivers of variation of multivariate benthic nutrient fluxes

The best environmental distance-based linear model (DistLM), explaining 39% of the overall variation in benthic nutrient fluxes, included the variables bottom water oxygen concentration, distance from shore, and percentage of gravel (**Table 5.6**, **Figure 5.7, a**). The best biological distance-based linear model (DistLM) based on all biological variables, explained 49% of the overall variation in benthic nutrient fluxes, and included the relative densities of orbinid, onuphid, and maldanid polychaetes and sipunculids (**Table 5.6**, **Figure 5.7, b**).

Table 5.6: Statistic results of DistLM analysis for fitting environmental and biological factors to benthic nutrient fluxes. Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AICc (Akaike Information Criteria corrected), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model.

Benthic nutrient fluxes - Environmental drivers							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AICc**	**R^2**	**RSS**
B [Oxygen]	17296000	3.6321	0.0714	0.1113	472.66	0.386	9.5371e +7
Distance from shore	16005000	3.6706	0.0648	0.103			
% gravel	26724000	7.5656	0.025	0.17197			
Benthic nutrient fluxes - Biological drivers							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AICc**	**R^2**	**RSS**
Orbinidae density	30295000	7.0229	0.0229	0.19496	469.95	0.487	7.9688e +7
Onuphidae density	16392000	4.2222	0.0652	0.10549			
Maldanidae density	12096000	3.3804	0.0721	0.077839			
Sipunculidae density	16924000	5.5217	0.0167	0.10891			

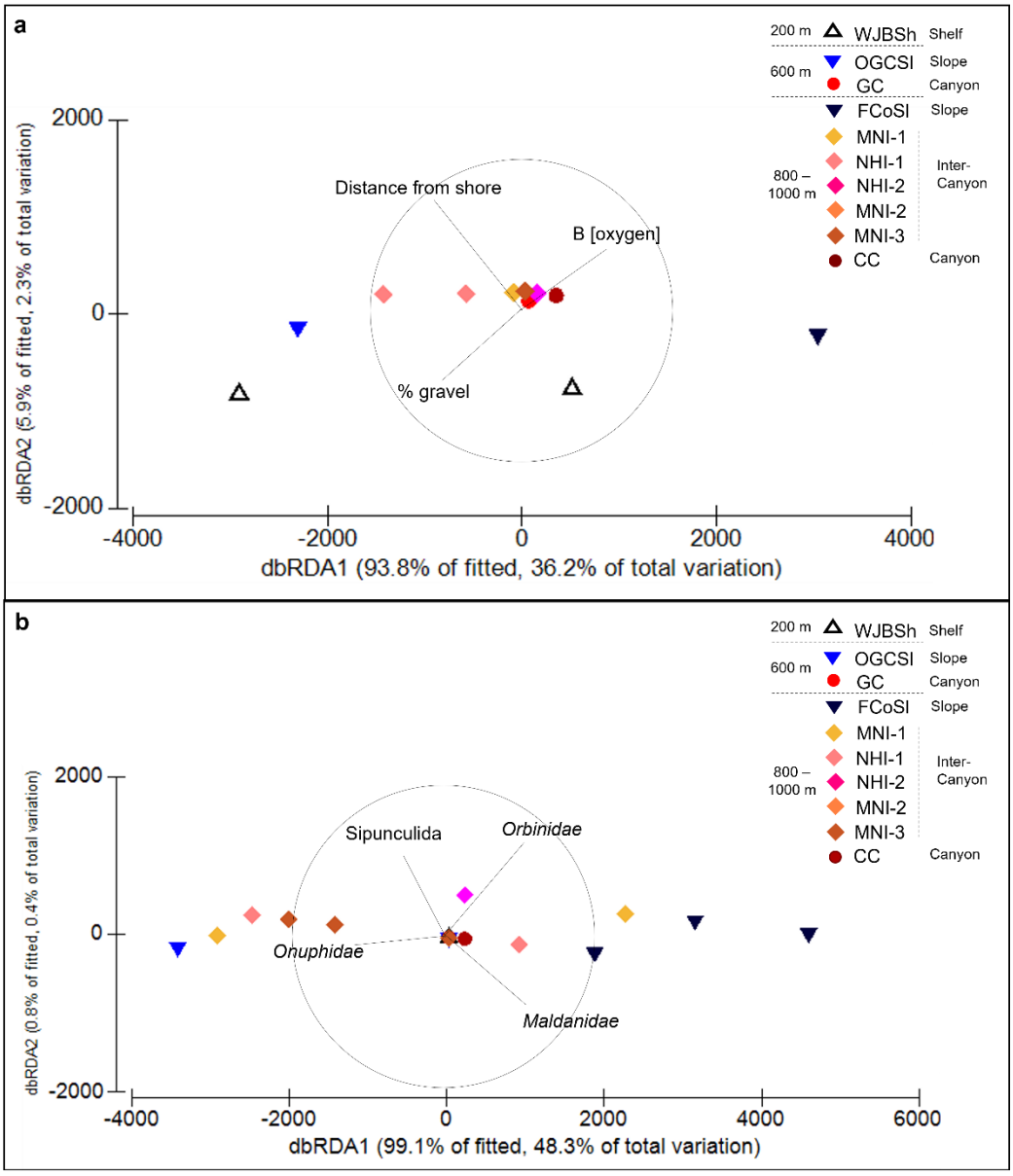

Figure 5.7: **a**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes and all environmental variables. Vectors show direction and strength of environmental variables contributing to variation in benthic nutrient fluxes. B [oxygen] refers to the bottom water concentration of oxygen. **b**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes and all selected biological variables. Vectors show direction and strength of taxa and polychaete families (*italic*) contributing to variation in benthic nutrient fluxes.

5.4.6 Environmental drivers of macrofaunal diversity, and variation of community composition and biological trait expression

Draftsman's plots and correlation analysis identified sedimentary concentration of chlorophyll *a* as the single variable that best correlated with total macrofaunal density (R = 0.85, **Figure 5.8**), total number of taxa (R = 0.47), and Pielou's evenness (R = -0.56).

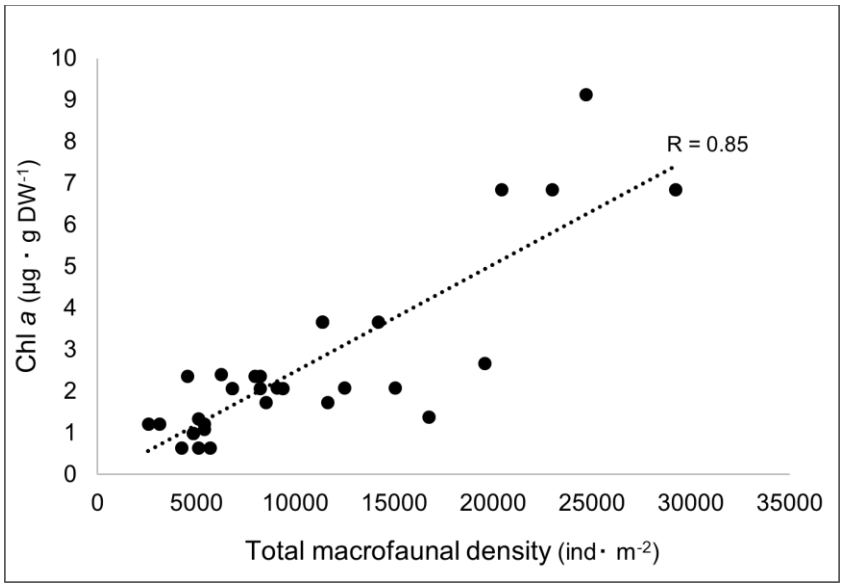

Figure 5.8: Linear correlation between total macrofaunal density and sedimentary concentrations of chlorophyll *a* in the stations sampled. R indicates Draftsman's correlation.

The best environmental distance-based linear model (DistLM), which explained 35% of the overall variation in macrofaunal community composition, included the variables latitude, Chl *a*: TOC ratio, sedimentary concentration of TOM, and depth (**Table 5.7**,). The best environmental distance-based linear model (DistLM) explained 72% of the overall variation in macrofaunal biological trait expression and included the variables sedimentary concentration of Chl *a* and TN, latitude, and sedimentary concentration of Phaeo (**Table 5.7**, **Figure 5.9, b**).

Table 5.7: Statistic results of DistLM analysis (final model) for fitting environmental variables to benthic macrofaunal community composition and biological trait expression. Table includes sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AICc (Akaike Information Criteria corrected), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model.

Macrofaunal community composition							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AICc**	**R^2**	**RSS**
Latitude	7116.1	4.3947	0.0001	0.12777	237.05	0.355	35912
Chl *a*: TOC	5541.3	3.734	0.0001	0.0949			
TOM	3693.73430.7	2.6288	0.0025	0.066322			
Depth	3544.3	2.5793	0.0049	0.061599			
Macrofaunal biological trait expression							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AICc**	**R^2**	**RSS**
Chl *a*	3894.3	25.92	0.0001	0.46352	149.83	0.72	2352.8
TN	496.49	4.9519	0.0055	0.059094			
Latitude	363.85	4.0205	0.0111	0.043307			
Phaeo	265.72	3.0493	0.0239	0.031627			

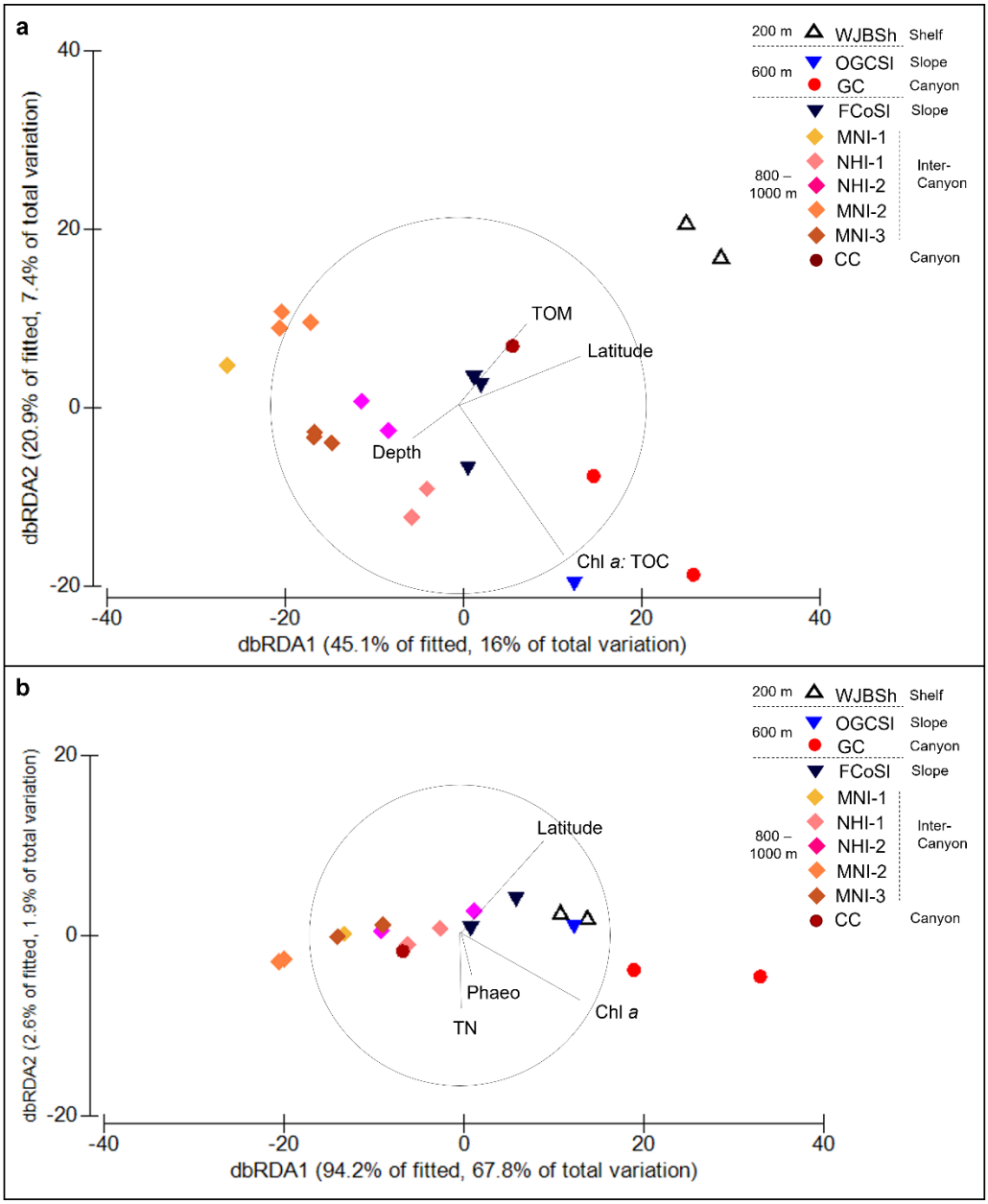

Figure 5.9: **a**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of macrofaunal community composition and all environmental variables. Vectors show the direction and strength of environmental variables contributing to variation in macrofaunal community composition. TOM: total organic matter; Chl a: TOC: chlorophyll *a* to total organic carbon ratio. **b**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of macrofaunal biological trait expression and all environmental variables. Vectors show the direction and strength of environmental variables contributing to variation in macrofaunal biological trait expression. TN: total nitrogen; Phaeo: phaeopigments; Chl *a*: chlorophyll *a*.

5.5 Discussion

Our study simultaneously evaluated benthic inorganic nutrient fluxes (as a measure of organic matter remineralization), and macrofaunal taxonomic and functional diversity parameters in different habitats along the Northwest Atlantic continental margin, contrasting pattern and process drivers among canyons, inter-canyons, and slope environments. Our results confirm our hypothesis of enhanced densities of macrofauna, distinct taxa and biological trait composition at Georges Canyon, but not at Corsair Canyon; inter-canyons did not exhibit increased density or diversity of macrofauna compared to broad continental slope, in contrast to our expectations. Benthic nutrient fluxes were not enhanced by increased organic matter input and elevated macrofaunal densities found at Georges Canyon, showing unclear patterns among the continental margin habitats sampled.

5.5.1 Benthic nutrient fluxes

Benthic nutrient fluxes did not show clear variation patterns among the different habitats sampled. Fiddler's Cove, on the continental slope, was the station that differentiated most from the others, mostly because of the higher intake and/or lower release of phosphate and silicate. The source of such differences remains open to investigation as this station did not differentiate from others in terms of physico-chemical variables, granulometric properties, sedimentary organic matter, or macrofaunal diversity. At the same time, sediments in Georges Canyon, which were characterized by distinct sedimentary organic matter composition and macrofaunal communities, did not differ from others in terms of benthic nutrient fluxes, pointing to different mechanisms shaping biological communities and benthic remineralization processes. The complexity of biogeochemical processes occurring in marine sediments also complicates their understanding and our ability to identify their patterns clearly (Hall et al., 1996). For example, up to 75% of the

ammonium formed during mineralization of sedimentary organic matter is nitrified and subsequently denitrified in the sediment, consistent with the absence of significant ammonium in- or effluxes across the sediment-water interface during intense organic matter remineralization (Enoksson, 1993; Devol and Christensen, 1993). A similar decoupling of remineralization and nutrient fluxes has been reported for other nutrients (Hall et al., 1996).

Percentage of gravel, bottom water oxygen concentration, and distance from shore were the best environmental predictors of multivariate benthic nutrient fluxes. The effect of the presence of gravel in the sediments (detected in some of the samples from WJBSh and NHI-1) on flux rates might be related to the higher permeability of gravel that affects solute transport (*e.g.*, dominance of porewater advection in gravel and sand and dominance of molecular diffusion in muddy sediments; Janssen et al., 2005). Bottom water oxygen concentration was found to correlate positively with fluxes of nitrate, nitrite, and ammonium, and negatively with fluxes of silicate and phosphate. The higher release of phosphate into the water column in lower oxygen environments (*e.g.*, OGCSl) can be explained by sedimentary redox conditions and depletion of ferric iron that diminish the capacity of sediments to retain phosphate (Ingall and Jahnke, 1994; Paytan and McLaughlin, 2007). Finally, we observed higher nutrient fluxes offshore, which contrasts with other studies that detected higher fluxes nearshore on the continental shelf (*e.g.*, Friedrich et al., 2002) and could relate to lower bottom water oxygen concentrations at our nearshore stations.

Surprisingly, variables related to sedimentary organic matter quantity and quality did not contribute substantially to variation of benthic nutrient fluxes, in contrast to other studies (*e.g.*, Link et al., 2013a,b; Belley et al., 2016; Miatta and Snelgrove, 2021), indicating the overriding importance of other environmental factors in determining benthic flux variation among our stations. Importantly, topographic features, such as canyons and inter-canyons, influence

sedimentary organic matter deposition, hydrodynamic forces, and disturbance events (*e.g.*, sediment deposition and resuspension) and might affect benthic nutrient fluxes, decoupling organic matter input and its remineralization. For instance, previous studies reported that sediment resuspension influence oxygen and nutrient effluxes in marine sediments (Tengberg et al., 2003; Niemistö et al., 2018), and intense sedimentation rates in slope sediments were also found to alter benthic nutrient fluxes (Hensen et al., 2000). The absence of other studies measuring benthic nutrient fluxes in canyon and inter-canyon habitats limits our capacity to contrast our findings with other regions, and points to the need for more studies investigating benthic processes in these understudied environments. Other environmental factors not considered in our study might also contribute to the variation of benthic fluxes. These include, for instance, sediment concentrations of manganese and iron, linked to variation in benthic nutrient fluxes (Link et al., 2013b) through the sequestration of phosphate by ferric iron (Paytan and McLaughlin, 2007), as well as density and diversity of sedimentary bacteria (Belley and Snelgrove, 2016), as they are directly responsible for organic matter remineralization and nutrient regeneration in marine sediments (Jorgensen, 2006).

Overall, biological variables explained more variation in benthic nutrient fluxes than environmental variables, with relative densities of orbiniid, onuphid, and maldanid polychaetes and sipunculids among the best biological predictors. These taxa likely affect fluxes through their activities, including bioturbation. Orbiniid polychaetes and sipunculids contribute to bioturbation mostly by biodiffusion and up- and downward conveyor (Queirós et al., 2013), similarly to maldanid polychaetes, which are also considered funnel feeders and were reported as important contributors to benthic fluxes in a previous study (Belley and Snelgrove, 2016). Onuphids are tube-forming polychaetes that build thin, flimsy burrows made from sand grains embedded in a

polysaccharide matrix (Waldbusser and Marinelli, 2009) that are permeable to diffusive exchange of solutes (Aller, 1983; Hannides et al., 2005). Despite a lack of available specific information, the characteristics of onuphid tubes and burrows suggest potential bioirrigation activities, which markedly increase nutrient exchange between pore water and the overlying water column (Aller, 1988; Kristensen and Andersen, 1987; Heilskov et al., 2006; Meysman et al., 2006). We observed heterogeneity in the effect of different organisms on each nutrient flux, which underscores the complexity of biodiversity and ecosystem functioning relationships, which is further complicated by interactions between organisms and environmental characteristics. For example, the effect of bioturbation activities on benthic nutrient fluxes is usually more pronounced in muddy than sandy sediments, where hydrological processes tend to determine porewater advection (Mermillod-Blondin, 2011). However, Waldbusser and Marinelli (2009) reported that, whereas environmental variables such as sediment granulometry and physical forces may drive large-scale variability in porewater advection in permeable sediments, type and abundance of bioturbating infauna significantly affect smaller-scale variation. The effect of bioturbation on benthic fluxes can also be solute-specific, further complicating its understanding. For instance, whereas bioirrigation generally increases nutrient fluxes, it can negatively affect silicate and phosphate effluxes (Waldbusser and Marinelli, 2009), potentially by increasing oxygenation of the sediment that increases the capacity of sediments to absorb and retain inorganic phosphorus (Ingall and Jahnke, 1994; Paytan and McLaughlin, 2007).

Macrofaunal density and taxonomic diversity indices correlated poorly with multivariate benthic nutrient fluxes, adding evidence for the greater importance of taxonomic and functional identity over communities' diversity in regulating ecosystem processes (Hooper et al., 2005). In our study, the lower importance of biological trait expression in determining variation of benthic

nutrient fluxes compared to the relative densities of key taxa is likely a consequence of our analytical method. For instance, the low taxonomic resolution used in our study, the lack of data on trait expression for certain taxa, the exclusion of potentially important traits (*e.g.*, bioirrigation, biodeposition) due to lack of information, the use of literature trait data rather than direct observation of individuals traits, and the use of relative densities rather than biomass to weight trait expression might reduce the accuracy of our trait analysis. Interestingly, other studies reported a low correlation between bioturbation intensity and nutrient generation in sediments (Solan et al., 2008), suggesting that the relationships between ecosystem functioning and bioturbation might not be so straightforward and that different species might interact with the benthic environment differently, causing variability in how bioturbation influences benthic fluxes (Ieno et al., 2006; Solan et al., 2008).

5.5.2 Macrofauna

In contrast to benthic nutrient fluxes, macrofauna displayed clear variation patterns among habitats, with community composition proving more sensitive than diversity indices and biological trait expression in characterizing patterns. Differences in macrofauna appeared to be driven mostly by the quantity and quality of sedimentary organic matter. In particular, the sedimentary concentration of chlorophyll *a*, indicative of input of fresh marine-derived, highly labile organic matter to the seafloor, was the best predictor of macrofaunal density and taxonomic diversity. These findings support the role of phytodetritus as important source of nutrition for benthic organisms and the importance of food quality over quantity in sustaining benthic communities (Campanyà-Llovet et al., 2017; Leduc et al., 2020). Input of labile organic matter has been previously reported to sustain high macrofaunal biomass (Pilditch et al., 2015; Leduc et al., 2020), and higher biodiversity through the creation of new niches that enhance the coexistence of different

taxa (Levin et al., 2001). Lower Pielou's evenness associated with higher Chl *a* suggests a particular advantage for a few opportunistic taxa that respond to the pulses of highly labile food (Levin et al., 2001). Depth was overall less important than food availability in determining macrofaunal diversity, and we attribute the disruption of the typical depth-diversity relationship to the presence of lateral or down-slope transport of organic matter at some of our stations (Flach and Heip, 1996; Levin et al., 2001; Wei et al., 2010).

Input of phytodetritus together with latitude, organic matter quantity and quality, and depth were the best environmental predictors of macrofaunal community composition and biological trait expression. These findings align with other studies (*e.g.*, Levin and Gage, 1998; Wei et al., 2010; Robertson et al., 2020). For example, Wei et al. (2010) found that patterns of macrofaunal composition correlated strongly with depth and flux of particulate organic carbon from surface production, whereas Käß et al. (2021) found great effect of labile, phytodetrital organic matter in determining macrofaunal biological traits. Similarly, sedimentary concentration of Chl *a* alone explained nearly half of the total variation of biological trait expression among our stations. The low proportion of community composition variability explained by our model points to the important role of other factors in determining community assemblages. For instance, our study did not explicitly consider hydrodynamic patterns and physical disturbance, which are important drivers of benthic community composition along continental margins (Levin et al., 2001; McClain and Barry, 2010; Cunha et al., 2011; Robertson et al., 2020). The presence of sedimentary mega-epifauna (*e.g.*, sea pens) can also influence macroinfaunal communities (Chapter 3) and we observed differences in mega-epifaunal density and diversity among stations during our ROV dives.

The shallowest sediments on the continental shelf at Western Jordan Basin (**Figure 5.10, continental shelf**), where sedimentary organic matter properties point to the accumulation of organic material (Campanyà-Llovet et al., 2018) that indeed typically characterizes sediments on the continental shelf, displayed relatively high macrofaunal density and diversity, with distinct taxa and trait composition (statistical results not reported here). Here, greater presence of predators and a reduced role for deposit feeders probably relate to higher environmental stability and long-term availability of organic matter that favours the occupation of multiple ecological niches and the presence of taxa with contrasting feeding strategies and higher trophic levels (Simboura et al., 2000). Higher presence of biodiffusers living closer to the sediment-water interface, as well as sessile mega-epifauna (author's personal observation**; Figure 5.11, a**) likely benefitted from greater availability of particulate organic matter, coupled with lower hydrodynamic forces (Harris, 2014; Pierdomenico et al., 2019), as suggested by lower mean grain size at this station (Van Rijn, 1993).

The organic enrichment measured in the sediments from Georges Canyon (**Figure 5.10, shelf-incising canyon**), together with signs of deposition (*e.g.*, presence of large detritus in some areas of the canyon, **Figure 5.11, c**) and strong currents (*e.g.*, presence of ripples, **Figure 5.11, c**; higher mean grain size) point to potential occurrence of turbidity and tidal currents, sediment gravity flows and other lateral transport mechanisms (de Stigter et al., 2007; Puig et al., 2014). Reports from other canyons indicate similar findings (*e.g.*, Pusceddu et al., 2010; Amaro et al., 2015; Campanyà-Llovet et al., 2018; Pierdomenico et al., 2019; Leduc et al., 2020). The high concentrations of phytopigments and high Chl *a*: Phaeo ratio in the sediments from GC point to rapid downward transport of marine-derived organic matter along the canyon axis, contributing to supplying labile organic matter at depths far below the sinking of particulates from productive

surface waters (Campanya-Llovet et al., 2018). The presence of sea pigs (class Holothuroidea, order Elasipodida) on the seafloor in some parts of Georges Canyon (author's personal observation) also confirms recent input of fresh organic matter, because sea pigs are mobile deposit feeders that form transient, dense aggregations on the seafloor in response to high influx of fresh organic matter that they consume in a timespan of hours to days (Miller et al., 2000). The increased organic matter quantity and quality in Georges Canyon likely contributed to high macrofaunal density, low evenness and dominance of deposit feeders that characterized sediments in this canyon compared to the adjacent slope. These findings align with other studies (*e.g.*, De Leo et al., 2010; Cunha et al., 2011; De Leo et al., 2014). Organic enrichment usually contributes to high biomass and depressed biodiversity and evenness because it favours high densities of a small number of opportunistic species (Levin et al., 1991; Rosenzweig and Abramsky, 1993; Levin and Gage, 1998; Levin et al., 2001). Moreover, the occurrence of turbidity flows can affect biological communities through periodic physical disturbance (de Stigter et al., 2007; De Leo et al., 2014; Puig et al., 2014; Liao et al., 2017). For instance, we observed scarce presence of sedimentary mega-epifauna (**Figure 5.11, c**), but occurrences of corals and sponges on the canyon's walls and boulders, where they can take advantage of abundant flux of particulate organic matter with minimal disturbance through sediment deposition and resuspension (Harris, 2014; Pierdomenico et al., 2019). Moreover, macrofaunal traits such as dominance of mobile deposit feeders with high fecundity and shorter life span and presence of opportunistic taxa in the sediments from Georges Canyon, supports the effect of disturbance events. Periodic disturbance caused by turbidity flows creates repeated opportunities for recolonization and maintains the benthic fauna in an early successional state dominated by opportunists, with high abundance and biomass of organisms that can tolerate disturbance and exploit the increased food availability (Levin et al., 2001; McClain

and Barry, 2010; Vetter et al., 2010). Disturbance can also help maintain high biodiversity by supporting small-scale heterogeneity that maintains habitat niches (Snelgrove, 1999; Levin et al., 2001), which likely contributed to the relatively high number of taxa in Georges Canyon. Our findings support the argument that gradients of productivity, sedimentary processes, and physical disturbance play an important role in shaping biological communities in canyon habitats (Levin et al., 2001; McClain and Barry, 2010; Robertson et al., 2020).

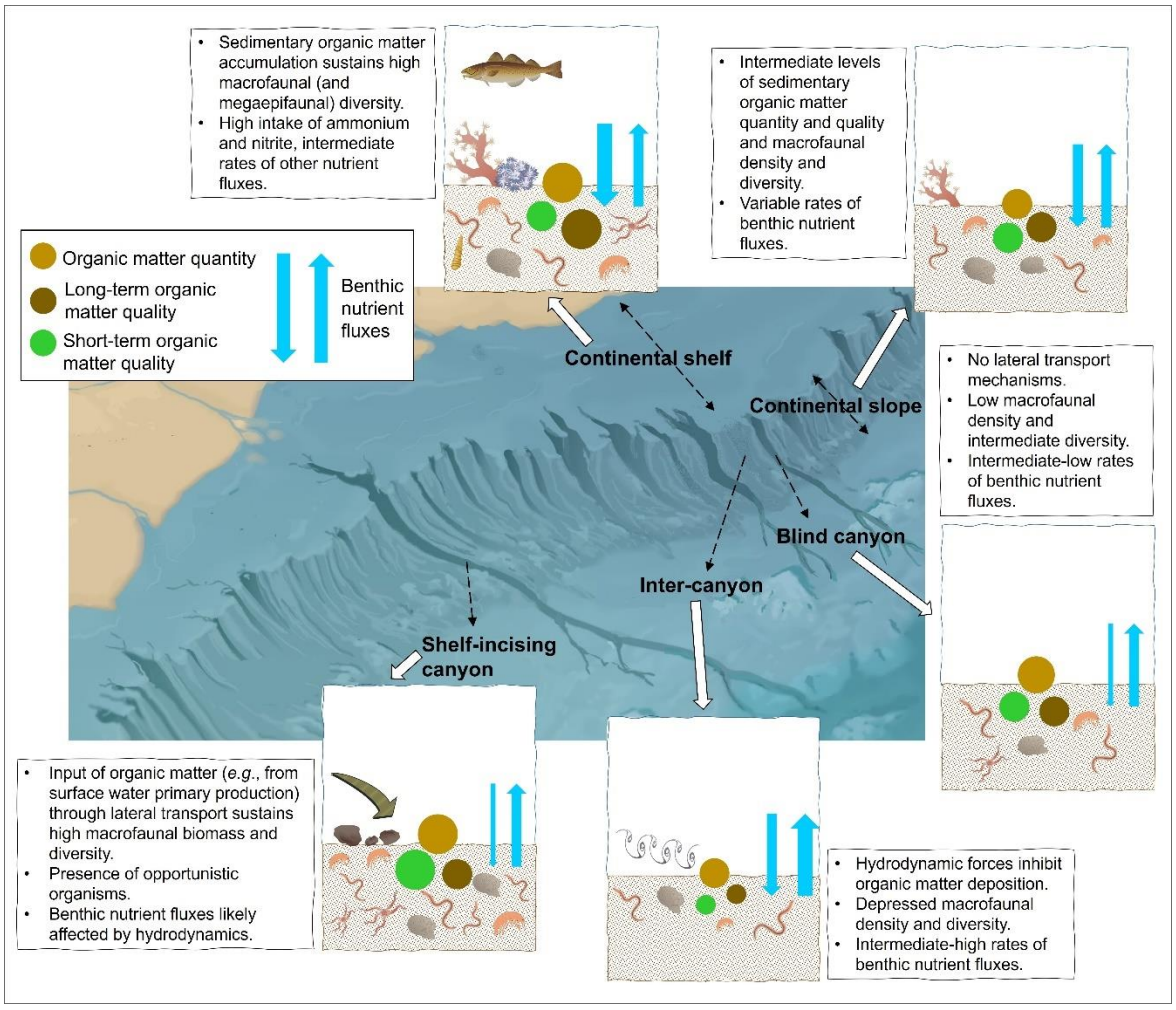

Figure 5.10: Schematic summary of the main characteristics of different habitats along continental margins, based on our findings relative to the stations sampled in this study. Dimensions of dots and arrows are proportional to the amount of sedimentary organic matter (quantity, short-term and long-term quality) and benthic nutrient flux rates (influxes and effluxes), respectively. Diagram of the continental margin was modified from *Encyclopædia Britannica Inc.*; organisms' icons were provided by Integration and Application Network (ian.umces.edu/media-library).

In contrast, macrofaunal communities from Corsair Canyon (**Figure 5.10, blind canyon**) did not differentiate from other stations at comparable depth, suggesting that this canyon is unaffected by lateral transport mechanisms such as turbidity currents that influence biological communities through organic enrichment and physical disturbance. Previous studies report no evidence for turbidity currents or down-axis mass movement of sediment in Corsair Canyon (Dillon and Zimmerman, 1970). Granulometric properties with higher presence of mud in our sites in Corsair Canyon contrasting with sandy sediments in Georges Canyon, also suggests that Corsair Canyon is a slope canyon that does not indent the continental shelf edge (Jobe et al., 2011), therefore limiting the lateral transport of organic materials along this canyon (Harris and Whiteway, 2011). However, we cannot rule out that differences in depth, sediment type, or other variables could also contribute to differences between Georges and Corsair canyons (as reported in other systems by Williams et al. 2009; Robertson et al., 2020). We also acknowledge evidence of substantive small-scale heterogeneity in resource availability and biodiversity in canyons not only between canyon axes but also within the same canyon axis at scales < 100 m (McClain and Barry, 2010; Cunha et al., 2011; Campanyà-Llovet et al., 2018). The limited sampling opportunities in our study within each canyon limits our ability to draw clear conclusions on the physical and ecological processes in Georges and Corsair Canyons at larger scales.

Finally, higher percentage of organisms in the upper sediment layers characterized inter-canyons (**Figure 5.10, inter-canyon**), along with differences in community composition and biological trait expression compared to slope sediments at comparable depth. Moreover, even though differences were not significant, density and diversity tended to be lower in inter-canyons (especially at Munson-Nygren Inter-canyon) than in slope sediments. These findings suggest the presence of different mechanisms influencing macrofaunal communities in inter-canyons,

although the absence of comparative studies limits our capacity to draw strong inferences. Perhaps, a combination of limited resource availability and intense bottom currents determined the observed patterns. Previous studies have reported negative effects of intense hydrodynamics on benthic communities (*e.g.*, Levin et al., 2001) and in our study strong currents are suggested by the coarse mean grain size in inter-canyons (especially NHI), as well as the lower quantity and quality of sedimentary organic matter, that can result from decreased rates of organic matter deposition in relation to strong hydrodynamics (Vetter et al., 2010). We also observed very high densities and diversity of mega-epifauna (*e.g.*, corals, sponges, bivalves) on the inter-canyon walls and boulders, whereas sediments were generally bare (**Figure 5.11, e**), as also reported by Quattrini et al. (2015), another indication of strong hydrodynamics that can disturb sedimentary mega-epifauna through sediment resuspension (Pierdomenico et al., 2019). Other findings, such as high C: N ratio values at some inter-canyon sites, point to presence of non-canyon related lateral transport mechanism that conveys organic matter of terrestrial origin (Flach and Heip, 1996) from the continental shelf along inter-canyons. A previous study also documented the presence of unconsolidated sediments, and deposition of larger material, suggesting a highly geologically dynamic nature for inter-canyon areas (Quattrini et al., 2015). In our study, the high contribution of terrestrial material to the organic carbon pool in inter-canyons may also contribute to the depress density and diversity of macrofauna (Cuhna et al., 2011; Leduc et al., 2020), as suggested by Leduc et al. (2020) who recently identified marine organic matter as the main limiting factor shaping macrofaunal communities in New Zealand submarine canyons.

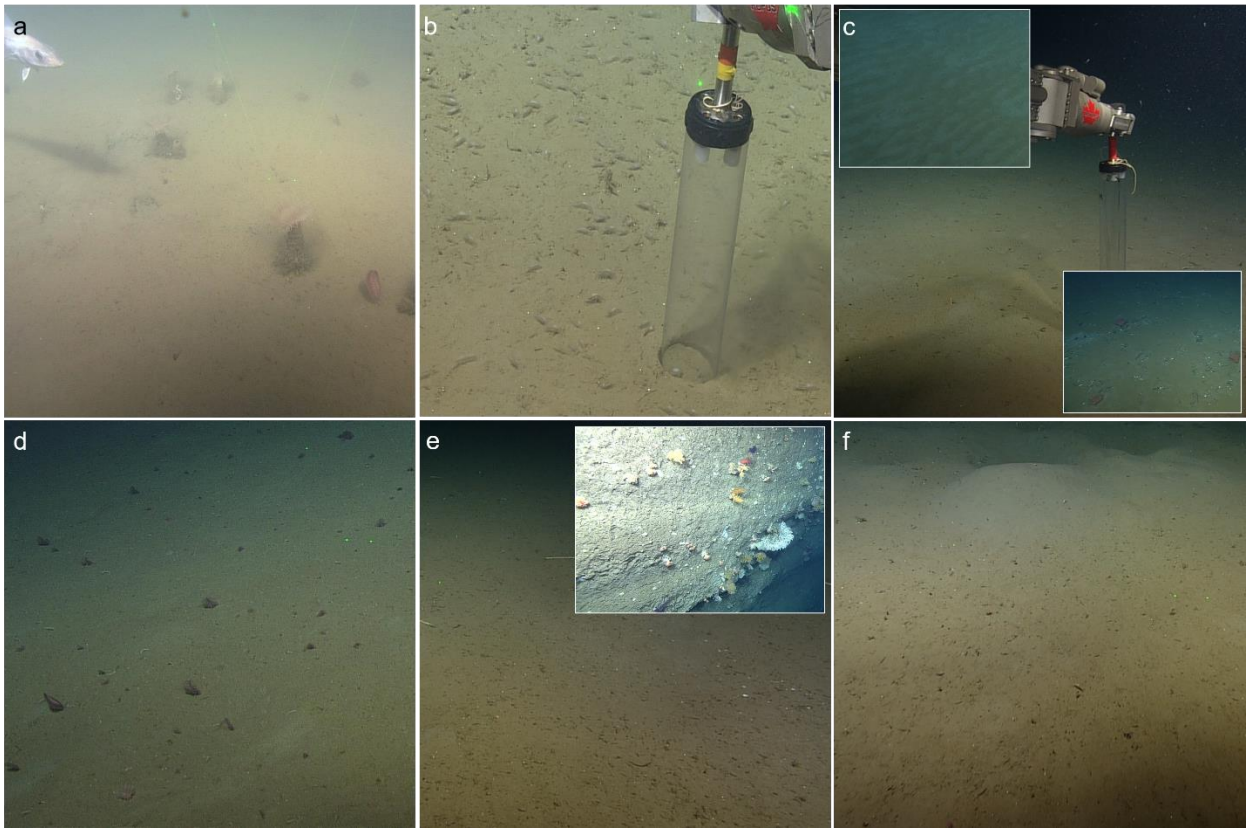

Figure 5.11: Submarine images of some of the stations sampled in our study (photo credits CSSF ROPOS). **a**) Megafauna-rich seafloor at the sediment sampling site in Western Jordan Basin (continental shelf); **b**) ROPOS arm collecting a push core at the continental slope outside Georges Canyon where some sea pigs are visible on the seafloor; **c**) ROPOS arm collecting a sediment push core, ripples (top left) and signs of material deposition in Georges Canyon (bottom right) **d**) *Pennatula* sea pen field at sediment sampling site in Fiddler's Cove (continental slope) **e**) Mostly bare sediments at the sediment sampling site in Nygren-Heezen Inter-canyon and inter-canyon wall colonized by mega-epifauna in Munson-Nygren Inter-canyon (top right) **f**) Mostly bare sediments in Corsair Canyon at the sediment sampling site with signs of bioturbation.

5.6 Conclusion

We documented clear patterns of macrofaunal communities related to variation in sedimentary organic matter quality and quantity along the highly heterogeneous Northwest Atlantic continental margin. We infer occurrence of lateral transport of materials in Georges Canyon that increased resource availability and helped sustain denser macrofaunal communities with distinct taxa and biological trait composition. In contrast, inter-canyons displayed low organic

matter quantity and quality, with some observable effects on macrofauna, likely related to strong hydrodynamics that inhibit deposition of particulates and lead to disturbed communities. We found a strong relationship between the input of fresh phytodetritus to the seafloor and macrofaunal density, taxonomic diversity, and biological trait expression. Benthic nutrient fluxes proved more variable and confirmed the complexity of biogeochemical processes in marine sediments and the challenge of generalizing patterns. Benthic fluxes were uncoupled from sedimentary organic matter and macrofaunal diversity, with disproportionate effects of a few macrofaunal taxa and influences of environmental variables mostly related to oxygen availability and sediment granulometry. We recognize the need for further studies assessing patterns and drivers of benthic nutrient fluxes along heterogeneous continental margins.

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5.8 Appendices

Appendix 5A. Statistical results of ANOVA main tests for all benthic nutrient fluxes considered in our study. * indicates significant p-values (< 0.05). df: degrees of freedom; F: F-statistic; p: p-value.

Nitrate flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	42212	1	42212	0.177	0.678
Habitat (depth)		210797	3	70266	0.295	0.828
Res		5234503	22	237932		
Nitrite flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	161	1	160.62	0.648	0.429
Habitat (depth)		218	3	72.68	0.293	0.83
Res		5454	22	247.9		
Ammonium flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	2808	1	2808	0.076	0.7859
Habitat (depth)		301903	3	100634	2.71	0.0697
Res		816839	22	37129		
Phosphate flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	247012	1	247012	4.275	0.0506
Habitat (depth)		251192	3	83731	1.449	0.2557
Res		1271225	22	57783		
Silicate flux						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	8313555	1	8313555	2.506	0.12768
Habitat (depth)		49912070	3	16637357	5.015	0.00845*
Res		72981769	22	3317353		

Appendix 5B. Statistical results of PERMANOVA main tests of benthic nutrient fluxes. * indicates significant p-values (< 0.05). df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Benthic nutrient fluxes						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Depth	2	30470000	15235000	0.97979	0.5157	180
Habitat (depth)	3	50676000	16892000	4.7058	0.0055*	9947
Res	25	89741000	3589600			

Appendix 5C. Statistical results of ANOVA main tests for all macrofaunal diversity indices considered in our study. * indicates significant p-values (< 0.05). df: degrees of freedom; F: F-statistic; p: p-value.

Total macrofaunal density						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	813140744	1	813140744	103.94	<0.001*
Habitat (depth)		314160502	3	104720167	13.39	<0.001*
Res		179924702	23	7822813		
Total number of taxa						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	74.3	1	74.3	9.371	0.0055*
Habitat (depth)		71.46	3	23.82	3.004	0.0512
Res		182.35	23	7.93		
Pielou's evenness (J')						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	0.01648	1	0.016475	13.14	0.0014*
Habitat (depth)		0.00726	3	0.00242	1.93	0.15296
Res		0.02884	23	0.001254		
Vertical distribution						
Source	**Main test**	**Sum of Squares**	**df**	**Mean Square**	**F**	**p**
Depth	ANOVA type III (Tukey)	0.00083	1	0.00083	0.466	0.5015
Habitat (depth)		0.02031	3	0.006769	3.802	0.0238*
Res		0.04095	23	0.001781		

Appendix 5D. Statistical results of PERMANOVA main tests of macrofaunal community composition. * indicates significant p-values (< 0.05). df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Macrofaunal community composition						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Depth	2	8127	4063.5	1.4749	0.1047	180
Habitat (depth)	3	8766.7	2922.2	2.1136	0.001*	9874
Res	26	35946	1382.6			

Appendix 5E. Statistical results of PERMANOVA main tests of macrofaunal biological trait expression. * indicates significant p-values (< 0.05). df: degrees of freedom; SS: sum of squares; MS: mean sum of squares; Pseudo-F: F value by permutation; p(perm): p-value based on 9999 random permutations; Unique perms: number of unique permutations.

Macrofaunal biological trait expression						
Source	**df**	**SS**	**MS**	**Pseudo-F**	**p (perm)**	**Unique perms.**
Depth	2	2200	1100	2.8484	0.1322	180
Habitat (depth)	3	1256.7	418.88	3.5575	0.0095*	9950
Res	26	3061.4	117.75			

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CHAPTER 6 – CONCLUSIONS AND FUTURE DIRECTIONS

6.1 Exploring regional-scale drivers of biodiversity and ecosystem functioning

After characterizing the small- and medium-scale patterns of macrofauna and benthic nutrient fluxes and identifying the main drivers of such variability (Chapters 3-5), I considered the drivers of larger-scale (*e.g.*, regional) variability. I, therefore, combined all the data collected for this thesis during three distinct sampling occasions to identify the main drivers of variability of macrofauna and benthic nutrient fluxes. Larger-scale studies can help in understanding patterns of biodiversity and ecosystem functioning (Snelgrove et al., 2014), and in developing models that can predict effects of environmental change and human impacts, therefore maximizing conservation efforts. Because my analyses upscaled processes from studies conduced at smaller scales, however, I acknowledge the limitations of my conclusions. For example, the sampling design used in my study may not accurately capture larger-scale patterns, and drivers of spatial variability at different scales often prove challenging to identify (Silberberger et al., 2019).

6.1.1 Methods

In these analyses, I utilized data on macrofauna from 98 core replicates collected in the two study areas (**Figure 6.1**) on three occasions: Laurentian Channel 2017 (LC 2017), Laurentian Channel 2018 (LC 2018), and Gulf of Maine 2017 (GoM 2017); I also used data on benthic nutrient fluxes from 67 core replicates collected on two occasions; Laurentian Channel 2017 (LC 2017) and Gulf of Maine 2017 (GoM 2017). The environmental factors used as predictive variables included: latitude, depth, bottom water temperature, salinity, oxygen concentration, sedimentary concentrations of TOM, TOC, TN, Chl *a*, Phaeo, and Tot Pigm, C: N, Chl *a*: Phaeo, Chl *a*: TOC ratios, % gravel, % sand, % silt, % clay, and MGS. Chapters 3-5 present the materials and methods

used to collect and process samples and data, and here I describe only the statistical methodology used for these analyses.

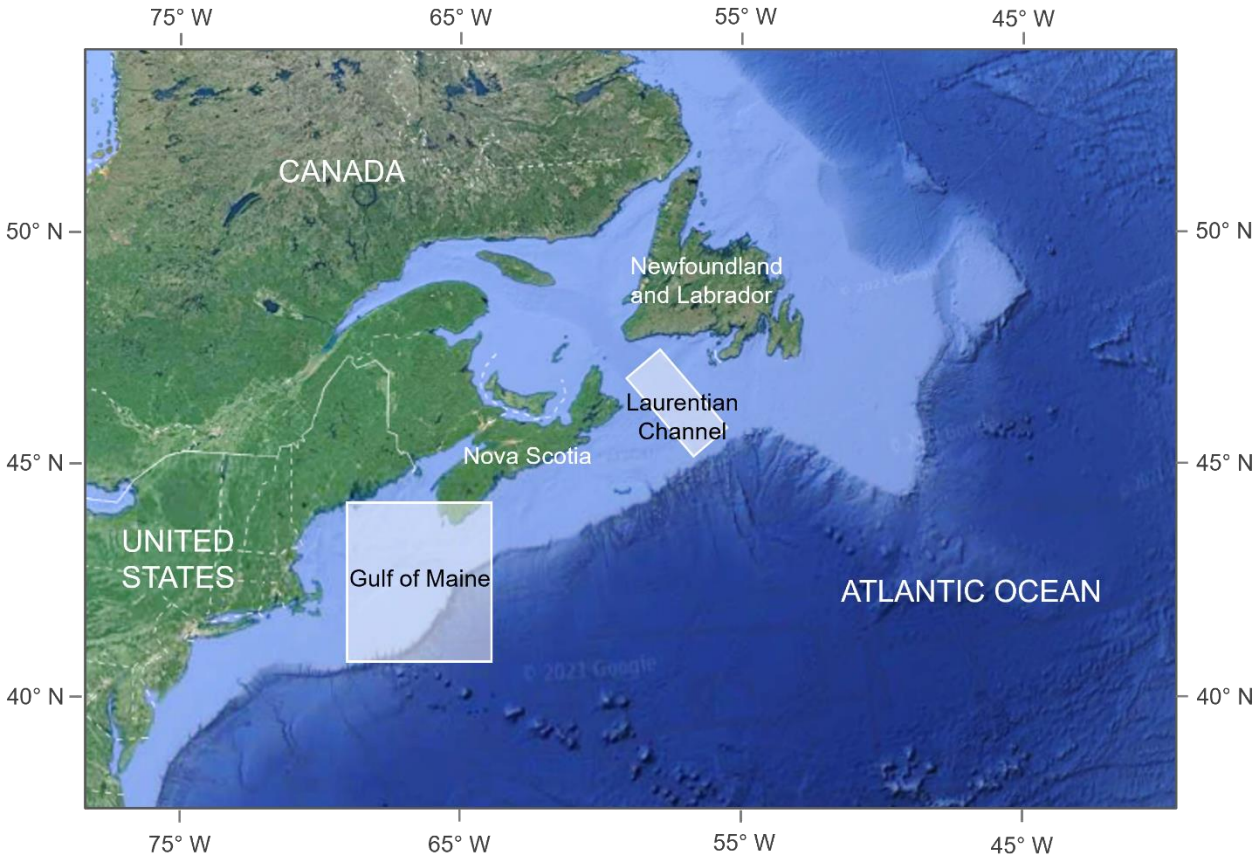


Figure 6.1: Map showing location of the two study areas (Laurentian Channel and Gulf of Maine) sampled in this research to characterize macrofaunal communities and benthic nutrient fluxes.

To identify the environmental drivers of macrofaunal density, total number of taxa, and Pielou's evenness, I used Draftman's plots and correlation analysis, identifying the environmental variables with the highest correlation (R) values. To identify which set of environmental variables predicted multivariate benthic nutrient fluxes, macrofaunal community composition, and biological trait expression (in 3 separate analyses), I used a stepwise distance-based linear model permutation test (DistLM; McArdle and Anderson, 2001). Resemblance matrices of multivariate benthic nutrient flux data (based on Euclidean distances), and macrofaunal community

composition and biological trait expression (based on Bray-Curtis similarity, calculated from square root transformed data) provided a measure of between-sample similarities. Predictor variables were standardised to mean 0 and standard deviation 1 prior to analysis, and I assessed their normality and collinearity using Draftsman's plots to ensure that highly correlated variables did not appear simultaneously in the final models.

To identify the biological drivers of benthic nutrient fluxes, I used stepwise distance-based linear model permutation tests (DistLM; McArdle and Anderson, 2001), with resemblance matrix of multivariate benthic nutrient flux data based on Euclidean distances as a measure of between-samples similarities. Macrofaunal taxonomic diversity indices (standardised to mean 0 and standard deviation 1), as well as community composition and biological trait expression matrices (square-root transformed) were used as predictive variables. Noting that the number of predictor variables in this case greatly exceeded the number of samples, I did preliminary testing of the influence of each group of biological variables (taxonomic diversity indices, macrofaunal community composition, and macrofaunal biological trait expression) on benthic nutrient fluxes separately. Next, the variable(s) from each group that correlated best with benthic nutrient fluxes were selected and combined in a final analysis to determine the best biological model to explain variation in benthic nutrient fluxes. All DistLM analyses were run with 9999 permutations and AIC (Akaike's information criterion) selection criterion; R^2 was examined to identify the best model and determine the proportion of the variation explained by that model, and results were visualized with distance-based redundancy analysis (dbRDA; Anderson et al., 2008). All analyses were performed in PRIMER v6 (Anderson et al., 2008).

6.1.2 Results and discussion

Draftsman's correlation analysis identified sedimentary organic matter quantity (TOM and TOC) as the best predictor of total macrofaunal density (R = 0.82; **Figure 6.2, a**), total number of taxa (R = 0.6; **Figure 6.2, b**), and Pielou's evenness (R= -0.72; **Figure 6.2, c**). These results confirm that macrofaunal density and taxa richness respond positively to increased food availability in deep-sea sediments, whereas evenness tends to decrease with increased organic matter quantity in response to increases in opportunistic taxa that can rapidly consume organic materials (Levin et al., 2001; Johnson et al., 2007; Pilditch et al., 2015; Leduc et al., 2020).

According to DistLM analysis, the best environmental model explained 39% of the variation in macrofaunal community composition and included the variables latitude, TOM, TOC, and Chl *a*: TOC (**Appendix 6A**; **Figure 6.2, d**). Interestingly, the same combination of variables also explained 68% of the variation in macrofaunal biological trait expression (**Appendix 6A**). Latitude was the strongest contributor to differences in community composition, likely by reflecting environmental differences between the two sampling regions, and gradients in other ecologically relevant variables, such as food supply. Total organic matter explained over half of the variation in biological trait expression, aligning with the findings of other studies (*e.g.*, Henkel and Nelson, 2018; Käß et al., 2021). In contrast with other studies (*e.g.*, Wei et al., 2010; Brandt et al., 2019), depth was not a good predictor of macrofaunal diversity or community structure, and we attribute this result to latitudinal gradients and to the presence of geological features and habitats that might have disrupted the usual exponential decline of flux of particulate organic carbon to the seafloor with depth (Suess, 1980; Pace et al., 1987; Rex et al., 2006).

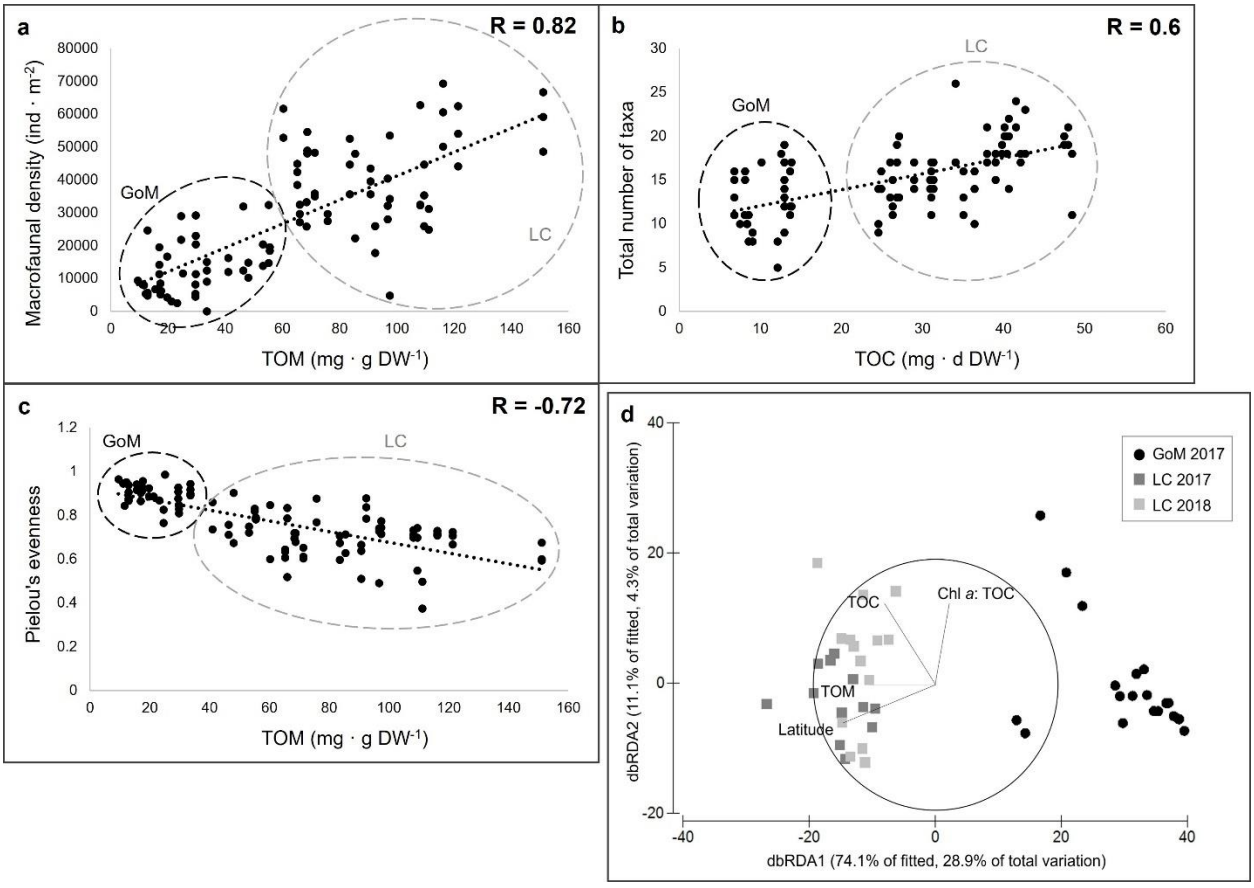


Figure 6.2: Scatterplots showing the correlation between sedimentary concentration of total organic matter (TOM) and macrofaunal density (**a**), sedimentary concentration of total organic carbon (TOC) and total number of taxa (**b**), and sedimentary concentration of total organic matter (TOM) and Pielou's evenness (**c**), where R indicates Draftsman correlation and contours highlight data from the two separate study areas. **d**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of macrofaunal community composition (based on Bray-Curtis similarity of square-root transformed data) and environmental variables (based on standardized data). Vectors show direction and strength of the environmental variables explaining variation in macrofaunal community composition. GoM 2017: Gulf of Maine 2017 sampling; LC 2017: Laurentian Channel 2017 sampling; LC 2018: Laurentian Channel 2018 sampling; TOC: sedimentary concentration of total organic carbon; TOM: sedimentary concentration of total organic matter; Chl *a*: TOC: chlorophyll *a* to total organic carbon ratio.

DistLM analysis based on environmental predictors showed that sediment granulometry and physico-chemical bottom water properties such as salinity and oxygen concentration, best explained variation in multivariate benthic nutrient fluxes, explaining a combined 25% of the total variation (**Appendix 6B**; **Figure 6.3, a**). The best biological model based on macrofaunal

diversity, community composition and biological trait expression explained 32% of the total variation in benthic nutrient fluxes and included 5 variables (**Appendix 6B**; **Figure 6.3, b**). Drivers of variation of benthic nutrient fluxes at regional scales did not generally differ from those identified at smaller scales (Chapters 4 and 5), and the relative density of a few macrofaunal taxa had the largest overall influence on fluxes, once again confirming the importance of macrofauna for benthic sedimentary processes. Environmental and biogenic variables influenced each flux differently, confirming the complexity of sedimentary processes involved in organic matter remineralization and the release or uptake of inorganic nutrients (Hall et al., 1996).

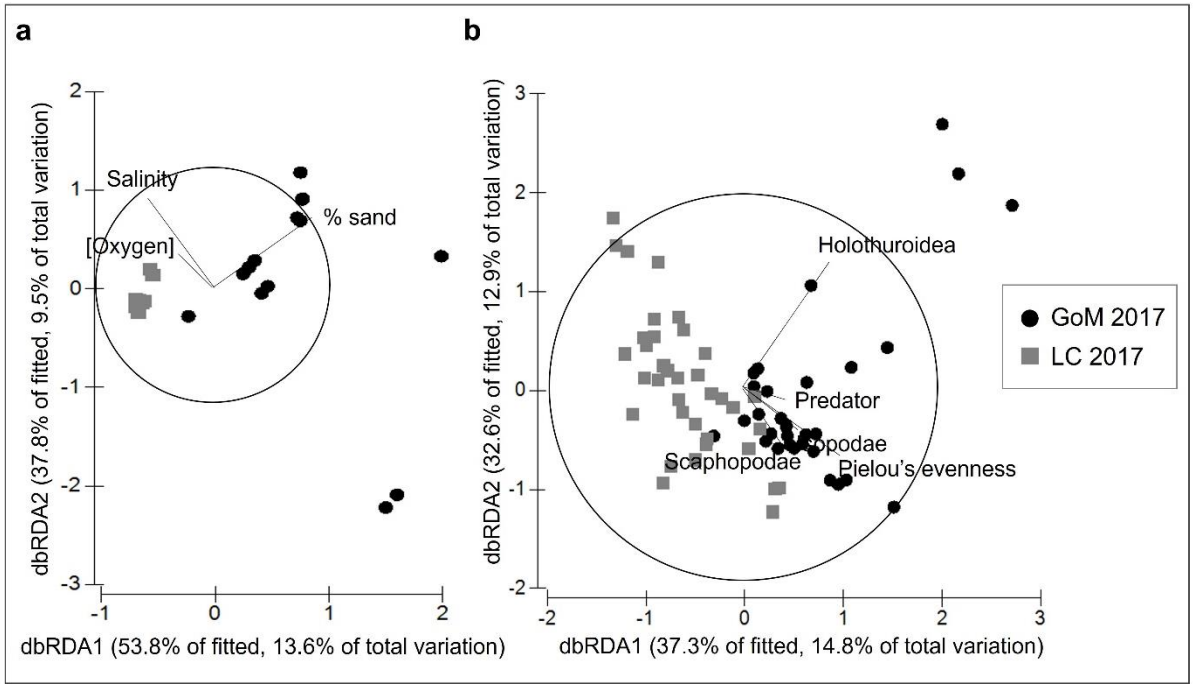


Figure 6.3: a) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes and all environmental variables. Vectors show direction and strength of environmental variables contributing to variation in benthic nutrient fluxes. [oxygen] refers to the bottom water concentration of oxygen. **b**) Redundancy analysis (dbRDA) from the best distance-based linear model (DistLM) of benthic nutrient fluxes and all selected biological variables related to macrofaunal diversity and composition. Vectors show direction and strength of taxon density, expression of biological trait categories, and taxonomic indices contributing to variation in benthic nutrient fluxes. GoM 2017: Gulf of Maine 2017 sampling; LC 2017: Laurentian Channel 2017 sampling.

6.2 General conclusions and significance of this study

My doctoral thesis explored patterns of biodiversity and ecosystem processes in contrasting marine habitats along the Northwest Atlantic continental shelf and margin and identified the main drivers of their variation at different scales. Overall, my findings demonstrate the importance of biogenic and geophysical habitats in affecting local biodiversity of macrofauna in deep-sea sediments, with less clear effects on biogeochemical sedimentary processes. Within the Laurentian Channel MPA, I identified the potential role of sea pen field fields as biogenic habitat for macrofauna (Chapter 3), even though I did not detect a clear effect of sea pens on organic matter remineralization rates (Chapter 4). Along the canyon-incised continental margins explored in Chapter 5, I confirmed the important role of geophysical habitats such as submarine canyons for macrofaunal communities, likely through increased organic matter input, but I did not find the same effect on benthic nutrient fluxes, which I suggest derives from a decoupling of organic matter input and its remineralization. At larger scales (*section 6.1*), my analyses reiterated the role of food availability (*e.g.*, sedimentary organic matter quantity) in determining the density and diversity of macrofauna and its taxa and trait composition. I also found that overall macrofaunal community composition had the greatest influence on variability in benthic flux rates, supporting the important ecological role of macrobenthos. Throughout the thesis (Chapters 3-5), I demonstrate how macrofaunal biological trait expression analysis can complement more traditional methods and provide information on the environmental drivers that determine community composition and dynamics, response to stressors, as well as functional roles of organisms. Based on these advantages, I argue for the integration of trait-based approaches into conservation strategies in order to improve their outcomes (Chapter 2).

6.2.1 Macrofauna in deep-sea sedimentary habitats

Deep-sea biota could potentially represent the most diverse assemblage on Earth (Snelgrove, 1998), and it clearly plays a role in key ecosystem processes (Braeckman et al., 2010; Mermillod-Blondin, 2011; Kristensen et al., 2012); macrofauna (see **Appendix 6C** for some examples) therefore merits consideration in ecological studies and in conservation planning. Yet our understanding of local and regional patterns of macrofaunal diversity and community composition in deep-sea sediments and what drives such variability remains limited. Furthermore, relatively few studies and many assumptions form the basis of this knowledge (*e.g.*, Gray, 2001: Levin et al., 2001; Gage, 2004; Ingole et al., 2010). For example, many studies identify depth and sediment grain size as the most important drivers of macrofaunal patterns (Snelgrove and Butman, 1994; Bergen et al., 2001; Cummings et al., 2010; Buhl-Mortensen et al., 2012), and these variables form at times the basis of habitat classifications used as surrogates to describe biodiversity (*e.g.*, Greene et al., 1999; Valentine et al., 2005) and inform conservation strategies. However, in most cases, such classifications tend to over-simplify patterns and do not capture biological habitat complexity, or finer scales mechanisms (Guarinello et al., 2010). Even more importantly, the functional structure and diversity of deep-sea macrofaunal communities and the macrofaunal biodiversity and ecosystem function (BEF) relationships have been overlooked for a long time, leaving a substantial gap in our understanding of the role of macrofauna for ecosystem processes (Baldrighi et al., 2017; Sivadas et al., 2020).

In my study, I found that local factors such as sea pen density, depth, and granulometric properties explained most of the observed variability of macrofaunal communities and their biological traits. These factors likely reflect differences in food availability, hydrodynamics, and habitat heterogeneity among habitats. Indeed, measures of food availability (organic matter

quantity and quality) also explained part of the variation in macrofauna. The presence of distinct macrofaunal communities in both biogenic (*e.g.*, sea pen fields; Chapter 3) and geophysical (*e.g.*, submarine canyons; Chapter 5) habitats, highlights the importance of habitat heterogeneity in sustaining regional biodiversity and ecosystem functioning (Thrush et al., 2006). At larger scales, measures of food availability explained most of the variability in macrofaunal variables (*section 6.1*). In particular, organic matter quantity and quality strongly influenced density, taxonomic diversity, and biological trait expression of macrofaunal communities, in accordance with other studies (Levin et al., 2001; Johnson et al., 2007; Wei et al., 2010; Käß et al., 2021). The importance of food quantity and quality in shaping the biological traits of organisms is not surprising, given that food availability and source largely determine the trophic structure of communities (Grall et al., 2006; Campanyà-Llovet and Snelgrove, 2018), which directly relates to the expression of some biological traits (*e.g.*, feeding mode, motility, bioturbation). Such strict relationships between macrofauna and food availability have ramifications for predicting climate change effects on benthic biodiversity and functioning. For example, ocean warming can alter surface water productivity, leading to reduced input of phytodetritus to the seafloor (Bopp et al., 2005; Buesseler et al., 2008; Smith et al., 2008; Morán et al., 2010, 2015), with potential alterations to benthic communities and their functional role (Ruhl and Smith, 2004). Alterations in organic matter input may be even more pronounced in submarine canyons, where warming may reduce density-driven cascading events (Canals et al., 2006), a fundamental source of delivery of organic materials in these habitats (Puig et al., 2014).

6.2.2 Benthic nutrient fluxes in deep-sea sedimentary habitats

Organic matter remineralization in marine sediments is one of the key processes driving benthic-pelagic coupling and represents an important function of benthic environments (Snelgrove

et al., 2014; Griffiths et al., 2017), whose rates can be estimated through measurement of fluxes of inorganic nutrients at the sediment-water interface (Rowe and Phoel, 1992; Giller et al., 2004; Bourgeois et al., 2017). Only a few studies have measured benthic fluxes in deep-sea sediments (*e.g.*, Hensen et al., 2000; Link et al., 2013a,b; Belley and Snelgrove, 2016; Belley et al., 2016). These studies report the influence of several environmental factors on fluxes, including organic matter quantity and quality, oxygen concentration, temperature, and granulometric properties (Link et al., 2013a; Belley et al., 2016), as well as the importance of macrofaunal taxonomic and functional richness, and the density of key taxa or functional groups in determining variation of flux rates (Link et al., 2013b; Belley and Snelgrove, 2016). In my research, I identified similar environmental drivers of flux variation at different spatial scales (Chapters 4 and 5 and *section 6.1*). Overall, environmental variables explained less variation in flux rates than biological variables related to macrofaunal diversity and community composition, suggesting the overriding influence of biological communities on ecosystem processes, as suggested by studies in other environments (Solan et al., 2004; Hooper et al., 2005). To explore biodiversity and ecosystem functioning (BEF) relationships, I used an innovative analytical method to understand the role of taxa and biological traits in determining variation of multivariate nutrient fluxes (representing the overall remineralization function performed by sediments), finding disproportionate effects of a few macrofaunal taxa and polychaete families, in addition to measures of taxonomic diversity. Onuphid polychaetes were among the best taxa predicting flux variation in both our analyses (Chapters 4 and 5) and alone explained up to 11% of total flux variability, suggesting their importance for sedimentary processes, potentially through bioturbation activities. In Chapter 4, I also documented effect of sea pen octocorals on ammonium flux rates, at both small- and large-

scales, suggesting that these mega-epifaunal organisms influence organic matter deposition and sedimentary biogeochemical processes.

In my study, benthic nutrient fluxes displayed high small-scale variability (*e.g.*, among replicate cores from the same station), especially in Laurentian Channel sediments (Chapter 4). Spatial patterns of benthic fluxes appeared more complex than those I documented for macrofaunal communities, and they were not always clearly related to obvious differences in other a/biotic characteristics, nor clearly different among contrasting biogenic or geophysical habitats (*e.g.*, sea pen fields, canyons, inter-canyons). My findings confirm the challenge of describing large-scale spatial variability in benthic nutrient fluxes and scaling up relationships between ecosystem functioning and its drivers, which requires a better understanding of the potentially interacting effects of biological and environmental processes, small-scale habitat heterogeneity, and temporal (*e.g.*, seasonal, interannual) variability (Hall et al., 1996; Aller, 2014). Because of these constraints, the explicit inclusion of benthic nutrient fluxes as a key ecosystem function in marine spatial planning and conservation strategies remains difficult. Nevertheless, understanding the main a/biotic factors that drive organic matter remineralization in marine sediments remains a key objective necessary in order to predict, and possibly prevent, changes in functional processes related to biodiversity loss, climate change, and other alterations of the environment caused by anthropogenic impacts.

6.2.3 Biological traits of marine organisms

Biological traits refer to measurable characteristics of organisms that link them to their environment and to the role they perform in the ecosystems (Díaz and Cabido, 2001; Violle et al., 2007; Mlambo, 2014; Pawar et al., 2015; Wong and Dowd, 2015). Evaluation of organism traits can therefore elucidate drivers of taxa and community occurrence, as well as the effect of taxa and

communities on ecosystem functioning; for these reasons they can be helpful in improving the design of targeted and effective MPAs (Bremner, 2008; Frid et al., 2008; Wong and Dowd, 2015; Beauchard et al., 2017), as I thoughtfully explore in Chapter 2. Among trait-based approaches that quantify measures of functional diversity and structure, my thesis focused on Biological Trait Analysis, a methodology developed by Bremner et al. (2003) to quantify the relative expression of selected biological trait categories over entire biological communities. Although this approach cannot quantify ecosystem functioning *per se*, it defines the functional structure of communities and can help in understanding drivers of community composition and the potential role communities play in ecosystem processes and functioning (Bremner, 2008). In my research, biological trait analysis added interesting insights on macrofaunal communities from contrasting habitats that I could not extrapolate from other biodiversity metrics alone, as reported by other studies (*e.g.*, Wong and Dowd, 2015). For instance, I found that macrofaunal communities inhabiting sea pen fields in the Laurentian Channel MPA display traits that characterize relatively stable environments and make them more susceptible to change and impacts, an important factor to consider when designing conservation strategies (Chapter 3). Additionally, in Chapters 3 and 5, I identified measures on organic matter quantity and quality as the main drivers of variation in macrofaunal biological trait expression, confirming findings from shallow-water environments (Grebmeier et al., 2006; Käß et al., 2021; Sivadas et al., 2021). This result portends the potential effect of climate change and other impacts that, by altering food sources and availability, could affect the functional structure of deep-sea macrofaunal communities, altering ecosystem functioning. In my analyses, however, the expression of macrofaunal traits commonly considered important for benthic ecosystem functioning linked poorly to variation in organic matter remineralization (Chapters 4 and 5). I mostly attribute this result to the lack of data on biological

trait expression for some deep-sea benthic organisms and traits (*e.g.*, bioirrigation) and other analytical limitations (as previously discussed in Chapters 4 and 5). I also suggest that the complexity of organic matter remineralization processes could complicate efforts to identify a clear link between organism traits and remineralization rates. In fact, many concurrent and interacting abiotic and biotic factors regulate biogeochemical cycles and act over different spatial and temporal scales, resulting in benthic flux rates that defy prediction with reductionist approaches. Pakeman (2011) also highlighted the difficulty in identifying the traits that directly and/or indirectly affect ecosystem functions. Potentially, other important functions performed by biological communities (*e.g.*, productivity, habitat provisioning, communities' resilience) might link more easily to their traits (for instance, see Bolam and Eggleton, 2014) and allow easier incorporation into conservation strategies.

6.2.4 Lessons on conservation from the Laurentian Channel MPA

Bringing together several researchers across Eastern Canada, the CHONe Laurentian Channel project advanced scientific knowledge of the benthic ecosystems in the Laurentian Channel MPA. For example, the project shed light on the factors that determine the distribution of sea pens and other mega-epifauna (S.N. de Mendonça and A. Metaxas, unpublished data), and on the ecological role of sea pens and other mega-epifauna in providing suitable habitat for fishes (M. Boulard, E. Edinger, and P. Lawton, unpublished data) and macrofauna (Chapter 3) and in affecting sedimentary processes and ecosystem functioning (Chapter 4). Advancing conservation outcomes fundamentally requires such scientific knowledge assuming the desire to base conservation choices on scientific evidence of ecological systems (Walsh et al., 2014; Lemieux et al., 2018), a rule not always followed by decision-makers (Sutherland and Wordley, 2017; Gardner et al., 2018). Importantly, adaptive management should periodically update MPA regulations

based on the newest available evidence, a practice still rarely used for Canadian MPAs (Mills et al., 2015). In the absence of such an adaptive mechanism, monitoring efforts may become pointless. Applying the newest scientific knowledge to spatial management might prove particularly important in the Laurentian Channel MPA, where some available scientific knowledge was disregarded during the design phases. Moreover, the desire to minimize conflicts with stakeholders may have compromised the effectiveness of this MPA in protecting some species of conservation priority, as some argue (Muntoni et al., 2019).

In order to evaluate the efficacy of the Laurentian Channel MPA in protecting sea pens and associated biodiversity and functioning, further research should determine population connectivity and larval dispersal of sea pens within the MPA boundaries and in adjacent areas. Such information could address the adequacy of the size and boundaries of the MPA in protecting sea pen populations (Kenchington et al., 2019). Imagery tools, including ROVs and drop cameras, can provide an effective method to assess mega-epifaunal abundance and diversity with minimal disturbance to the seafloor (de Mendonça and Metaxas, 2021); monitoring protocols can use such tools to assess how populations respond to management actions.

Quantitative sampling of sediments collected through ROV push cores or multicorers can enable monitoring of the associated infauna. Numerous monitoring programs use macrofauna as effective indicators of ecosystem status because quantitative sampling is relatively easy with a more readily available scientific literature than for other types of organisms (Patricio et al., 2012). Importantly, the relatively sedentary nature of most macrofaunal organisms make them reliable indicators of ecosystem status, noting that they also integrate the effects of environmental conditions over relatively long periods of time (Patricio et al., 2012). Consideration of biological traits of macrofauna also provides insights into the mechanisms driving potential shifts in

communities, and possible repercussions for ecosystem functioning. From my experience, a few replicate cores from each location may be sufficient to understand basic patterns, and monitoring macrofaunal communities in Laurentian Channel may require a less intensive sampling effort than that used for my study (Chapter 3), reducing cost and time for sample analyses. Polychaete analysis at a taxonomic resolution of family level might provide sufficient information on the taxonomic and functional diversity and structure of communities and provide a good surrogate for full taxonomic analysis, further reducing the time and effort necessary to analyse samples.

6.3 Future directions

In order to improve scientific knowledge of benthic marine systems and ensure the effectiveness of conservation strategies, future research should focus on:

- Increasing knowledge regarding biological traits of marine organisms. Despite recent efforts to increase the knowledge and availability of trait databases (*e.g.*, *Polytraits*), we still lack information for many marine taxa (Faulwetter et al., 2014). Moreover, in contrast with some fairly well-studied traits (*e.g.*, feeding mode), others remain largely unexplored (*e.g.*, bioirrigation). Such information gaps constrain our ability to use traits as ecological indicators and to include trait-based methodologies in conservation strategies. In terms of methodology, weighting trait expression by organism biomass instead of density might be a better way to describe macrobenthic community functioning through biological trait (Bolam and Eggleton, 2014; Darr et al., 2014), though other authors offer contrasting opinions (Gusmao et al., 2016; Kun et al., 2019).
- Clarifying the ecological role of sea pens in deep-sea sedimentary habitats requires further studies on whether sea pens effect on macrofaunal communities and sedimentary processes (as I suggest in Chapters 3 and 4) reflects a biogenic process or environmental filtering. Future

studies should focus on testing the role of different sea pen species and contrasting environments (*e.g.*, depth) and geographic locations on associated biodiversity and functioning, examining more specifically the role of sea pens at small scales (*e.g.*, on organic matter, oxygen penetration, infauna), and assessing potential seasonal variability.

- Identifying relationships between organisms, their biological traits, and benthic flux rates will enable better prediction of spatial variability in benthic fluxes, and the effect of biodiversity shifts on ecosystem functioning. Researchers could assess these relationships both through field observations and through manipulative experiments, which could also support better characterization of biological trait expression, including poorly understood traits such as bioirrigation.

6.4 Appendices

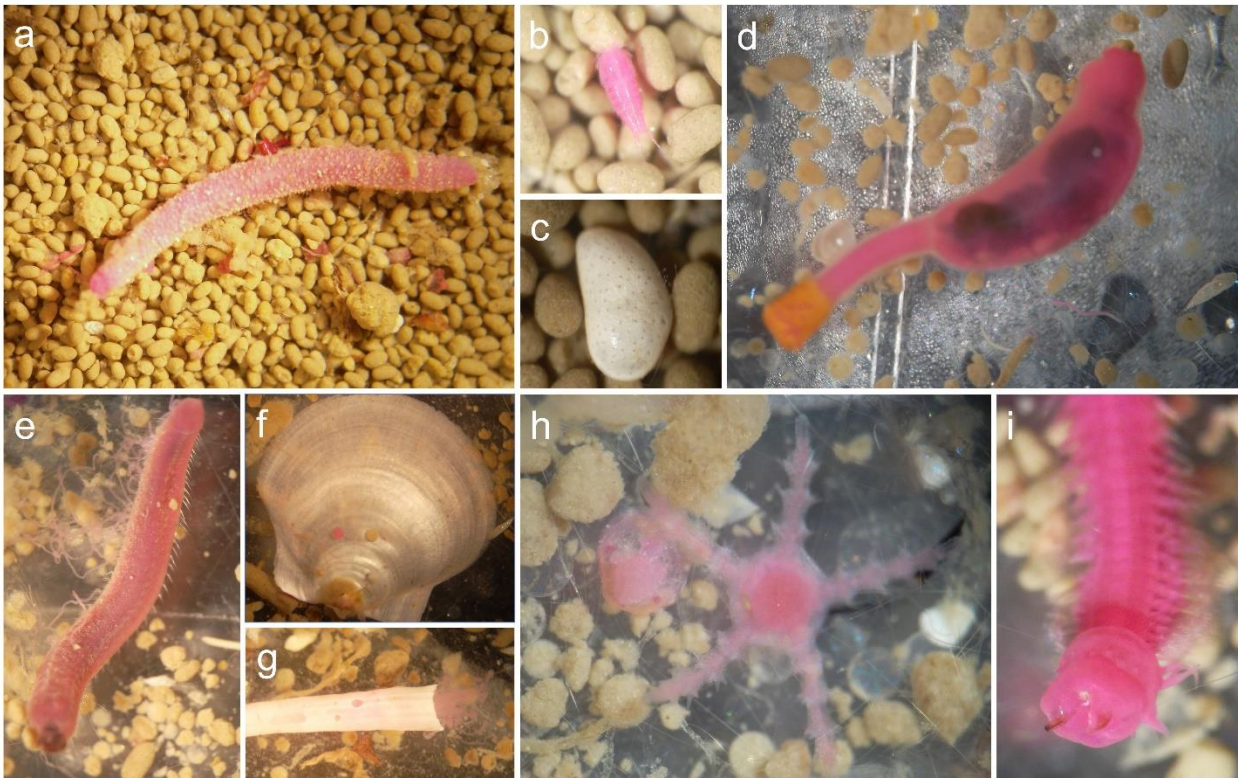
Appendix 6A. Statistical results of DistLM analysis (final model) for fitting environmental variables to macrofaunal community composition and biological trait expression. Tables include sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AIC (Akaike Information Criteria), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model. TOM: total organic matter; TOC: total organic carbon; Chl *a*: TOC: chlorophyll *a* to total organic carbon ratio.

Macrofaunal community composition							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
Latitude	39878	35.483	0.0001	0.27	678.78	0.389	90156
TOM	32480	27.046	0.0001	0.22			
TOC	32476	27.042	0.0001	0.22			
Chl *a*: TOC	13427	9.6025	0.0001	0.1			
Biological trait expression							
Variable	**SS (trace)**	**Pseudo-F**	**p**	**Prop.**	**AIC**	**R^2**	**RSS**
TOM	20210	109.29	0.0001	0.53	476.78	0.698	11476
Latitude	28202	88.429	0.0001	0.48			
TOC	17225	79.746	0.0001	0.45			

Appendix 6B. Statistical results of DistLM analysis (final model) for fitting environmental and biological variables to multivariate benthic nutrient fluxes. Tables include sequential tests results for each variable: SS(trace) (portion of sum of squares relative to the analysed predictor variable), Pseudo-F values, p-values, and Prop (proportion of variation explained by each variable), as well as AIC (Akaike Information Criteria), R^2 (proportion of variation explained by the model) and RSS (Residual Sum of Squares) of the best model. B salinity: bottom water salinity; B [Oxygen]: bottom water oxygen concentration.

Benthic nutrient fluxes - Environmental drivers							
Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**	**AIC**	**R^2**	**RSS**
% sand	38.903	8.6869	0.0001	0.12	95.34	0.252	246.72
B Salinity	37.463	8.324	0.0001	0.11			
B [Oxygen]	12.043	2.4619	0.0491	0.04			
Benthic nutrient fluxes - Biological drivers							
Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**	**AIC**	**R^2**	**RSS**
Holothuroidea density	38.061	8.4742	0.0008	0.11	92.6	0.324	223.11
Pielou's evenness	33.951	7.4542	0.0002	0.10			
Isopoda density	20.797	4.3718	0.0194	0.06			
Scaphopoda density	17.348	3.6065	0.0137	0.05			
Predator expression	3.9505	0.7876	0.5179	0.01			

Appendix 6C. Representative images of some of the macrofaunal specimens sampled and analysed in this study. **a**) Flabelligerid polychaete; **b**) Amphipod crustacean; **c**) Ostracod crustacean; **d**) Sipunculid worm; **e**) Cirratulid polychaete; **f**) Bivalve mollusc; **g**) Scaphopod mollusc; **h**) Ophiuroid; **i**) Nereid polychaete. Photo credit Marta Miatta.



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