Towards The Priming Effect in Sediments of the St. Lawrence Estuarine System:

A Contribution using Specific Stable Carbon Isotope and Biomarker Analysis

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ABSTRACT

Towards the Priming Effect in Sediments of the St. Lawrence Estuarine System:

A Contribution using Specific Stable Carbon Isotope and Biomarker Analysis

Maria-Elena Radu

Aquatic systems, such as the St. Lawrence Estuary and Gulf (SLEG), are represented by a combination of recalcitrant Organic Matter (OM) derived from land-based carbon-based compounds, and marine labile OM, mostly composed from phytoplankton and zooplankton biomass. The consumption of marine labile OM components by aerobic heterotrophic bacteria results in sustained pressure of dissolved O_2 concentrations and accumulation of more recalcitrant OM fractions corresponding to slower degradation rates. The SLEG is a cold temperate estuarine system, characterized by large seasonal variations in riverine discharge rates resulting in sudden influxes of fresh and labile OM deposited into the sediment. The accumulation and remineralization of recalcitrant fractions (formed through *in situ* primary production) could be modulated by the priming effect (PE) where the change in the degradation rate of recalcitrant sediment is modulated by the addition of labile OM. The SLEG is an ideal site to study PE, where climate change has reduced dissolved O_2 levels, particularly in the depths of bottom waters and affected the marine life and may have contributed to the onset and worsening of hypoxia. To examine PE, through a batch incubation experiment we attempt to measure changes in the rate of degradation in recalcitrant sediment from SLEG by adding fresh labile OM, specifically ^{13}C-depleted phytoplankton. A time course study was developed to examine the pathway of fresh OM,

particularly carbon, remains in the artificial system. Phytoplankton fixes inorganic carbon, specifically CO_2 (aq), and transforms it into organic matter via photosynthesis. Since this process preferentially selects carbon-12, we expect to observe an increase of carbon-12 in the system (a lowering of the 13C/12C ratio) over time. Most of the organic matter in sediment originates from primary production in the water column, therefore the sediment's initial delta13C value can be altered by the amount of CO_2 (aq), plankton, nutrients, and light. We performed Dissolved Organic Carbon (DOC) and stable isotope analysis on the seawater samples as well as stable isotope analysis of the samples that underwent incubation. Quenching of the incubations followed by the extraction, quantification and isotopic characterization of the bacterial fatty acids and hydrocarbons allows us to examine whether there is any effect of labile OM on the mineralization of recalcitrant sedimentary OM. Finally, the experimental evaluation of the reactivity of recalcitrant OM indicated that the sedimental bulk was not reactive on the time scale of 20 and 32 days respectively, such that only additions of labile OM were quickly consumed.

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I dedicate this work to my parents, who keep being my biggest supporters. You taught me to persevere and even in the darkness of times, to never forget who I am and where I come from. As I look back, only now I realize how much you have sacrificed for me. I only wish to make my you proud of my small achievements. When I started this program, I had no idea of how many psychological challenges I would have to face, but I learned to keep my head high and keep going. Va iubesc !

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List of Abbreviations

OC = Organic Carbon

OM = Organic Matter

DCM = Dichloromethane

DOC = Dissolved Organic Carbon

DOM = Dissolved Organic Matter

DP = Double Pulse

EA-IRMS = Elemental Analyzer- Isotope Ratio Mass Spectrometer

GC-FID = Gas Chromatography-Flame Ionization Detector

GC-IRMS = Gas Chromatography- Isotope Ratio Mass Spectrometer

GC-MS = Gas Chromatography-Mass Spectrometry

PE = Priming Effect

POM= Particulate Organic Matter

SLEG = Saint Lawrence Estuary and Gulf

SOM = Soil Organic Matter

SP = Single Pulse

LOCP= Labile Organic Carbon Pool

LOM = Labile Organic Matter

ROCP= Recalcitrant Organic Carbon Pool

Chapter I: Introduction

1.1. Carbon Cycle

The significance of carbon and its association to the Organic Carbon (OC) cycle plays a pivotal role in both the foundation of life and the dynamics of the global climate.[1] Within biogeochemistry, the study of carbon is essential as it underpins the foundational framework for ecosystem functionality, influences climate dynamics, and eventually facilitates a comprehensive understanding of the interplay between living and non-living components within the Earth's system. Acquiring additional knowledge will allow us to address environmental challenges by developing sustainable practices *e.g.,* climate regulations. Carbon, an abundant element, plays an indispensable role for all life forms on Earth, as it is essential for life.[1] Despite the ongoing consumption of carbon, its abundance ensures a perpetual supply as it circulates continually through all ecosystems.[1,2] Carbon constitutes more than 50% of the elemental composition of organic matter (OM), a paramount factor when investigating the chemical dynamics of carbon exchange between different reservoirs.[2] Carbon exists in both organic and inorganic forms. In aquatic systems, the latter includes mostly $CO_2(g)$, as well as forms of weak carbonic acid ($H_2CO_3^-$), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}).[3,4] On the other hand, carbon within living and formerly living organisms is labeled as organic carbon.[5,6]

The carbon cycle in ocean significantly differs from that on land.[5,8] In marine ecosystems, organic carbon is derived from both autochthonous (native to the system *e.g.,* microalgae, benthic fluxes, macrophytes) and allochthonous (*e.g.,* terrestrial inputs from groundwater and the atmosphere) organic matter.[7] In addition, both non-humic heterogeneous organic substances and humic (organic compounds found in soil) substances contribute to the aquatic organic carbon pump.[8] When comparing terrestrial organic matter to marine organic matter, the latter originates in the surface waters of estuaries or gulfs or is produced by autotrophic phytoplankton whereas a significant amount of marine organic carbon is produced by phytoplankton. Phytoplankton are primary producers; they create organic compounds, such as cellulose for survival, from CO_2 (*aq*) in the surface waters. Overall, aquatic organic matter ultimately consists of a heterogeneous mixture of living and non-living sources, such as plankton, bacterial cells, or detritus.[9,10]

The organic carbon cycle encompasses the flow of carbon among Earth's reservoirs; the atmosphere, oceans, soil and living organisms (see Figure 1.1).[11] The carbon cycle maintains the necessary balance for sustaining life and is critical to address a wide range of environmental and societal challenges. However, human activities have significantly altered this cycle through anthropogenic influences. Deforestation and the burning of fossil fuels have released vast amounts of carbon dioxide into the atmosphere. The organic carbon cycle sustains various transformations, including the conversion of CO_2 and its deposition in coastal sediment, as well as its transfer to

the deep open ocean.[13] Therefore, the considerable rise in greenhouse gases and the accumulation of carbon has affected all Earth's reservoirs.[12]

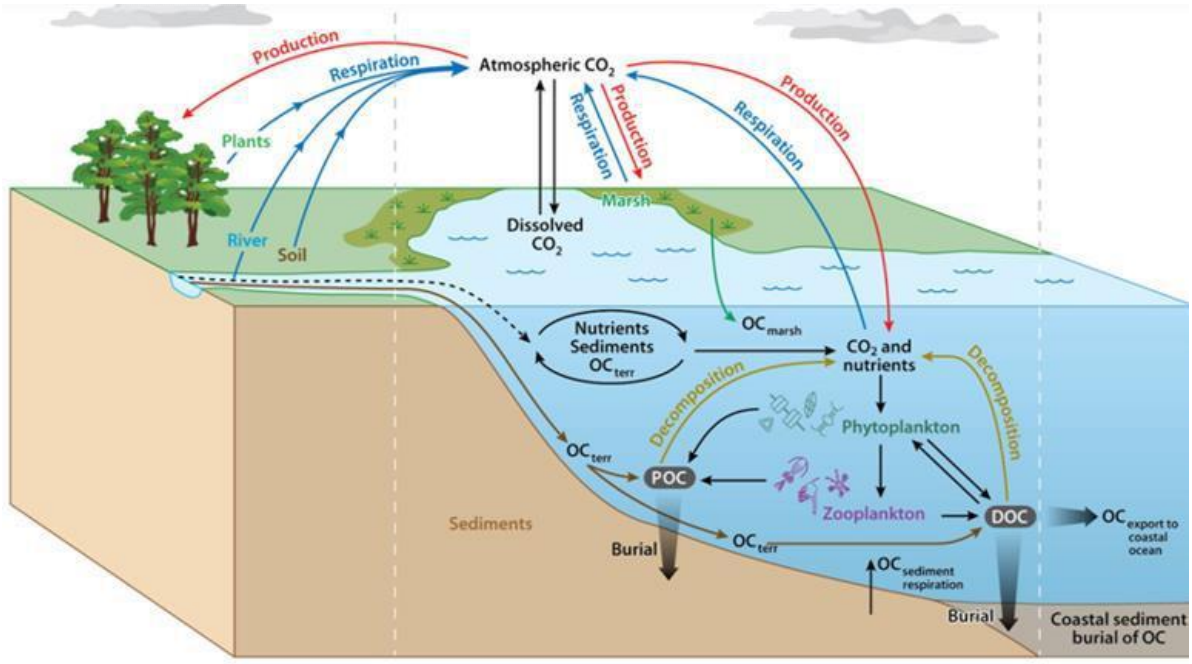

Figure 1.1 The carbon cycle depicted in the river-estuary-coastal ocean.[13]

Within aquatic systems, phytoplankton are not only responsible for the uptake of dissolved carbon dioxide, but also for assimilating other carbon source, such as bicarbonate and carbonate ions. This acquisition occurs through the solubility equilibrium that exists between the atmosphere and the ocean, where carbon dioxide (CO_2) undergoes transformation into dissolved CO_2 and subsequently reacting with H_2O to form carbonic acid (H_2CO_3) (Equation 1).[4]

$$CO_2 + H_2O \rightleftharpoons \underset{\text{carbonic acid}}{H_2CO_3} \rightleftharpoons H^+ + \underset{\text{bicarbonate}}{HCO_3^-} \rightleftharpoons 2H^+ + \underset{\text{carbonate}}{CO_3^{2-}}$$

Equation 1.

In addition to phytoplankton activities, osmotrophic microorganisms in the water column consume OC for nutrition and decompose animal and plant residuals, dead and living microorganisms, root exudates or soil biota. This activity releases additional OC in the form of nutrients and energy into the ocean's food web.[14,15] Soluble organic substances, such as dissolved organic carbon (DOC) are liberated, act as a significant energy reservoir, and connects the carbon flow from primary producers to other ecosystem components.[16]

Once carbon is fixed into organic compounds via primary production, it primarily is utilized for growth and development, while a small fraction is sequestered.[17] This fixed organic carbon escapes immediate decomposition and undergoes burial or enters long-term storage as fossil fuels (*e.g.,* coal, oil, and natural gas). Over geological time scales these carbon-rich deposits are gradually transformed into underground reservoirs, sequestering carbon from the atmosphere for millions of years.

Organic carbon is also transferred from terrestrial ecosystems to aquatic environments via various processes, such as weathering, erosion, and river transport. Once in the water, organic carbon is further processed by aquatic organisms or settles as recalcitrant sediment.[18] Recalcitrant sediment is the organic matter that resists decomposition and accumulates on the seabed.[19,20] This carbon reservoir acts as a carbon sink, sequestering organic carbon away from the carbon cycle for potentially thousands of years. The recalcitrant carbon reservoir significantly influences the net carbon exchange between the atmosphere and aquatic systems, making it a critical focus for

studying the overall carbon budget. However, there is limited research on the microbial utilization of this bulk material.[18,14]

Around 90% of organic matter in the water column undergoes decomposition before it reaches the seabed.[21] After depositing as sediment, this organic matter experiences further degradation, as a result a mere 0.5% of OM is preserved within sediment.[21] To comprehend how the remineralization of this preserved sediment occurs, it is important to acknowledge the presence of diverse carbon pools.

A thorough comprehension of global carbon pools is essential for investigating the Priming Effect in aquatic environments. The global cycle comprises four major reservoirs that facilitate the exchange of carbon among Earth's various components. These reservoirs encompass the ocean (the largest reservoir of them all), the atmosphere, the terrestrial ecosystem, and the earth's crust.[22,23]

Each of these components plays a unique and interrelated role in sustaining the carbon equilibrium through carbon fluxes. The overall knowledge of carbon sources and sinks offers insight into the interactions among organisms and processes affected by shifts in carbon availability and helps to assess the role of the St. Lawrence Estuary and Gulf in nutrient cycling. While most of the carbon residing at the ocean's surface undergoes cycling through biological processes like photosynthesis, respiration, and the decomposition of aquatic vegetation, this thesis focuses on the fraction of organic carbon sequestered in the sediment.

1.2. The Research Site

The St. Lawrence Estuary and Gulf (SLEG) in eastern Canada is the largest semi-enclosed system in the world, spanning ~240,000 km^2 in area with depths of 350 m at the center of the Laurentian Channel.[24,25] As one of the most significant commercial water routes, it marks the southern boundary of the Subarctic region and connects the SLEG to the Atlantic Ocean via the Strait of Belle Isle (depth ~50-80m) and the Cabot Strait (depth ~480m).[6,25, 26,27,28]

The SLEG represents an ecosystem rich in natural resources and biodiversity. In the Gulf of St. Lawrence portion of the SLEG, freshwater from the Great Lakes and the St. Lawrence River meet the salty Atlantic Ocean.[10] The estuaries portion of the SLEG, often divided into the Upper St. Lawrence Estuary (USLE) and the Lower St. Lawrence Estuary (LSLE), is characterized as the most nutrient-rich and biologically productive area.

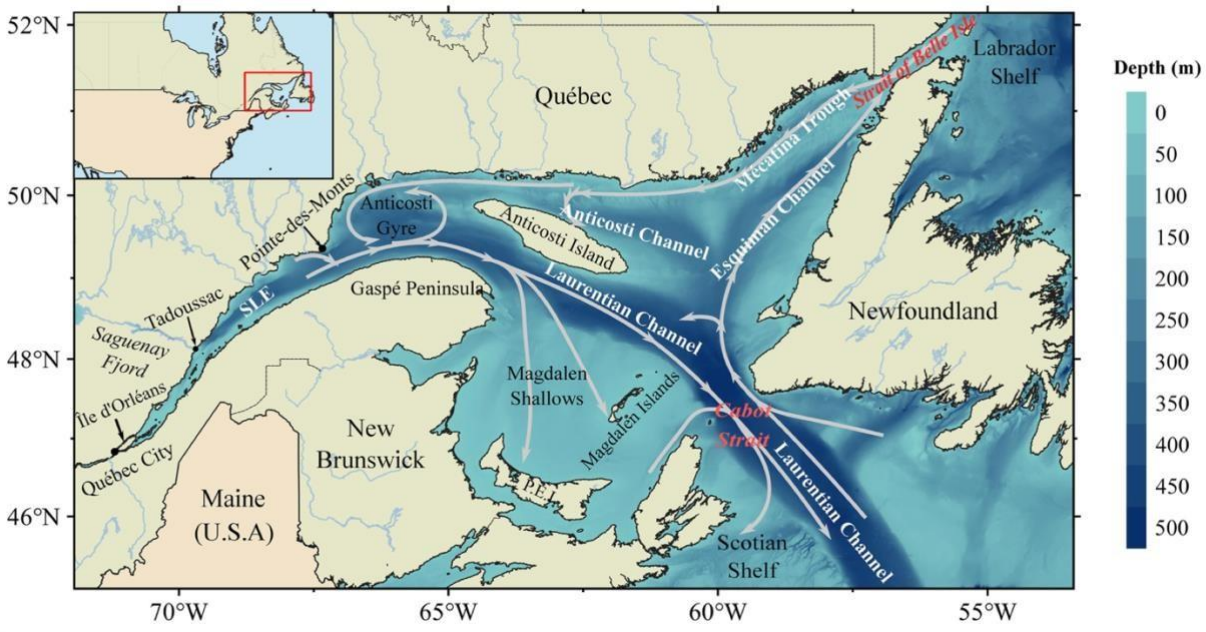

Figure 1.2. Geographical location of St. Lawrence Estuary and Gulf, the study area.[28]

Since the beginning of the industrial revolution in the St. Lawrence Valley, the SLEG has been affected by anthropogenic activities. As a result, industrial and agricultural pollution has led to eutrophication (*i.e.,* nutrient enrichment) and landscape deterioration, which culminated in the depletion of dissolved oxygen in its bottom waters.[29] In addition, changes in oceanic circulation have increased the discharge of terrestrial OM leading to biodiversity losses in the St. Lawrence River and associated tributaries. Other factors that contribute to hypoxia and the global carbon cycle are the elevated primary production owing to nutrients discharged by rivers or upwelled from bottom waters.[29]

The presence of a versatile habitat between the St. Lawrence River and the northwest Atlantic Ocean is the result of the freshwater to saltwater gradient and strong stratification of water layers

at different water depths, (See Figure 1.2). The primary production of organic matter prevails in diatoms and dinoflagellates, while the fauna consisting of a wide range of species, *e.g.,* pelagic, or bottom-feeding fish (*e.g.,* capelin, mackerel, Atlantic cod), crustaceans (*e.g.,* lobster), plankton or marine mammals (*e.g.,* beluga and North Atlantic right whales) depending on the geographical location.[26] The principal nutrient for this fauna is phytoplankton and the sedimentary organic matter (OM) from different input series, which makes the SLEG an attractive site for the study of estuarine biogeochemical processes.[30]

Since OM ranges from both freshwater sources (*e.g.,* the Great Lakes) and saltwater sources (*e.g.,* the Gulf that originates from the Atlantic Ocean), its molecular composition can host microbial communities at densities up to 1000 times higher than those observed in other water columns.[31] The deposition of organic matter at different depths of the SLEG is a complex amalgamation of organic matter, derived from sources such as overlying vegetation, root systems, *in situ* microbial and fungal activities, as well as carbon transported *via* above-ground pathways. With increasing depths, the quantity and composition of sedimentary organic matter undergo substantial modifications, making it further recalcitrant due to the influence of microbial processes and transitions between the solid-water phase, mediated by sorption and desorption.[32]

1.3. Labile and Recalcitrant Organic Matter

In this research we have examined both recalcitrant and labile organic matter. We have acquired organic matter originating allochthonously (originating outside its current location or environment) from the terrestrial biomes, as well as autochthonously (originating from its current location or environment), from primary and secondary production. Sediment from 10 stations was collected.

The organic carbon pool is defined on its utilization by heterotrophic microorganisms as substrates and carbon sources. Two fractions associated with the organic carbon pool are known as: (1) the labile organic carbon pool (LOCP) and (2) the recalcitrant organic carbon pool (ROCP).[14,33,34] Overall, the labile organic carbon pool consists of a mixture of proteins (approximately 45%), along with supplementary proportions of lipids (17%), carbohydrates (approximately 25%), nucleic acids (around 12%), and pigments (approximately 1%).[12,35] In contrast, ROCP is a component of sediment organic matter, heterogeneous in carbon with diverse chemical structures and functions that are resistant to degradation.[36,37] LOCP can be rapidly consumed within minutes to hours, allowing the incorporation of more readily available organic compounds into the water column in a short timeframe. [39] Despite the differing rates of utilization, both pools serve as distinct carbon and energy source for microbes.[40] Recalcitrant material ends up encompassing a wide spectrum of aliphatic hydrocarbons with complex polymeric structures, due

to diverse inputs. The complexity of hydrocarbons varies depending on the specific SLEG station and the degree of biologically productivity as well as the source of organic material involved.[24,41]

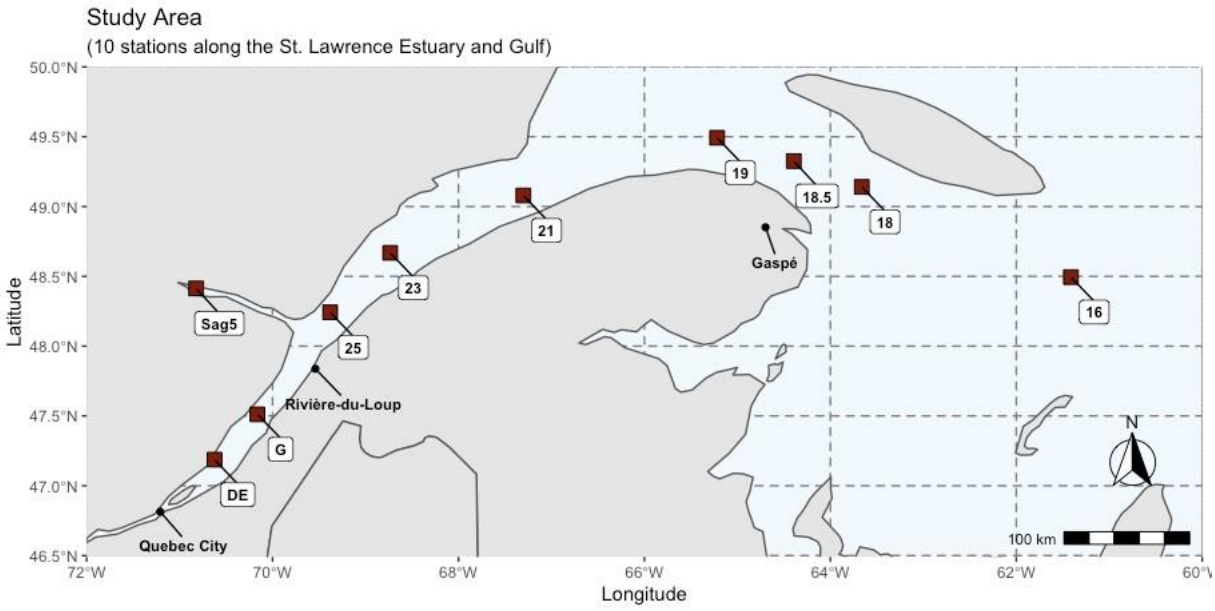

Figure 1.3. Study map area of the SLEG.[21] Potential sampling stations indicated.

The formation of sediment is directly linked with the accumulation of recalcitrant organic matter, which is contingent on the geographical location. For example, OM in stations close to the coast, such as station DE and G exhibit lower levels of recalcitrance compared to stations in open water, such as station 16. This is attributed to higher turbidity levels in the waters in proximity to land relative to the Gulf. Changes in river runoff or wind patterns can impact the nutrient outflow, the levels of primary production, and the upcycling of organic matter. These effects tend to heighten nutrient productivity in areas where freshwater from land interferes with seawater. Whereas sediment from stations situated at greater distances from land contains recalcitrant organic matter

resistant to degradation, owing to low hydrodynamic processes, tides, salinity, currents, and seasons (Figure 1.3).[42]

As humic substances, such as cellulose, find their way into the marine environment, a portion of them gradually sink to the bottom of the SLEG and ultimately lead to the gradual formation of sediment in the sediment layers. This deposit facilitates the preservation of organic matter within sedimentary layers and enhances the resistance of this organic matter to microbial decomposition, making the sediment recalcitrant.[43] Excluding the presence of aliphatic hydrocarbons, other certain recalcitrant organic compounds, such as microbial peptides, carbohydrates, chitin, fatty acids, and lipids, enhance the resistance of organic matter to microbial degradation. This allows organic carbon to be stored, to persist, and to accumulate within sedimentary layers.[44]

Measuring changes in the organic matter contained within sediment is difficult in incubation experiments over a short amount of time. The resistant nature and composition of sediment may impede the timely observations of alterations within the experimental time frame. In contrast, more labile components of organic matter, such as those found in phytoplankton, can be used to measure changes in carbon pools.[45] Labile marine organic matter from algae or other easily degradable sources is degraded and released in the form of labile OC metabolites. The OC released can be readily utilized by heterotrophic bacteria, whereas recalcitrant OC in the sediment

pool must be broken down from more complex organic compounds into simple inorganic forms before it can be characterized.[45,46] To facilitate this breakdown, exogeneous enzymes specific for different substrates in the recalcitrant fraction can be added to prime the reaction. Obviously, molecules like proteins or lipids in the LOC, are more susceptible to decomposition and are more readily degraded than more complicated molecules in marine recalcitrant organic matter.[46]

The approach outlined here involved the use of phytoplankton, a primary contributor in the global carbon cycle, as a source of labile organic carbon which should be metabolized efficiently. Here, we tried to replicate the natural processes occurring in the estuarine area, by using both ROC (sediment) and LOC (phytoplankton) marine sources of carbon.[47,48] Marine productivity relies on the growth and ubiquity of phytoplankton, which is continuously consumed by protozoans and zooplankton.

The specific species used in our experiments is *Nannochloropsis sp*., a genus of eukaryotic microalgae belonging to the *Eustigmatophyceae* class, that possess a single chloroplast occupying most of the cell volume.[49,59,] We selected this species due to its depleted stable isotope value (-44‰), a parameter conducted personally prior to the initiation of the incubation experiments. This choice allows us to distinguish between organic matter pools, specifically discerning the recalcitrance from labile components. As a main marine organic source of nutrition, phytoplankton

engages in marine photosynthesis, and produces organic carbon at a much higher rate than terrestrial plants.[46,51,52]

Starting at the euphotic zone near the surface of the water column, phytoplankton use chlorophyll to capture sunlight and absorb dissolved carbon dioxide from seawater to create organic compounds such as hydrocarbons, proteins, and lipids. These molecules may be used by the phytoplankton, metabolized by the bacteria in the water column, or accumulate as supplied labile organic carbon (OC). Eventually, the organic matter produced will be further co-metabolized or preferentially used to break down particulate or dissolved organic matter.[53,55] Since the composition of OM is strictly shaped by environmental factors, such as nutrient levels, salinity and temperature, its specific composition can affect the bacteria and lipid production pathways and either enhance or suppress the Priming Effect. In contrast to the sediment or terrestrial plants, phytoplankton produces organic carbon at an enhanced rate, can adapt and thrive under a variety of water conditions (*i.e.,* salinity and temperature). It is important to consider how the protein content and lipid composition of phytoplankton can influence the cycling of organic matter in the marine food web.[39,54,55] The transport of bioavailable organic matter (OM) in the water column can influence the microbial degradation of pre-existing sediment, in such a way that this bioavailable OM triggers the decomposition of sediment. Providing labile organic matter may lead to the

enhanced growth of organisms that can facilitate the degradation of recalcitrant organic matter in sediment. Supplementing ROC with LOC is known as the Priming Effect (PE).[56,57]

1.4. Priming Effect

The Priming Effect results from the presence of microbial communities (in this case *Nannochloropsis sp*.[58]) present within the labile OM that generate a range of alterations in the breakdown of recalcitrant OM. For example, the presence of this bioavailable OM may stimulate the decomposition of complex organic molecules in sediment into low-molecular-weight compounds like monomeric sugars, hydrocarbons, or carboxylic acids.[56,57] The remaining organic matter (resistant to natural degradation) can be mineralized following the introduction of these carbohydrates, amino acids or organic acids, as input from the labile organic matter (LOM).[39,59, 60]

Here, *Nannochloropsis sp*. is used to prime the degradation of ROC in sediment.[58] Previous investigations of the Priming Effect primarily concentrated on terrestrial soils instead of aquatic settings.[105] For example, there was a noted enhancement in microbial mineralization when periphytic algae were involved in the process of leaf litter decomposition.[105] One experiment explored the microbial breakdown of terrestrial dissolved organic carbon, in the presence or absence of added phytoplankton due to the potential for phytoplankton-derived organic matter to aid in the mineralization of more resistant terrestrial compounds.[58,105] Figure 1.4 is an

example of an experiment performed on soil, and shows initially the increase in Dissolved Organic Carbon (DOC) concentration in both peat and soil samples when the substrate was enriched in ^{14}C. The noticeable increase of soil organic matter (SOM) can be attributed to the increase in microbial activity through the addition of labile organic carbon. Previous literature reviews defined the priming effect as an accelerated breakdown of OC that could lead to an increase in total OC, *e.g.*, terrestrial/aquatic + algae-derived.[58,59]

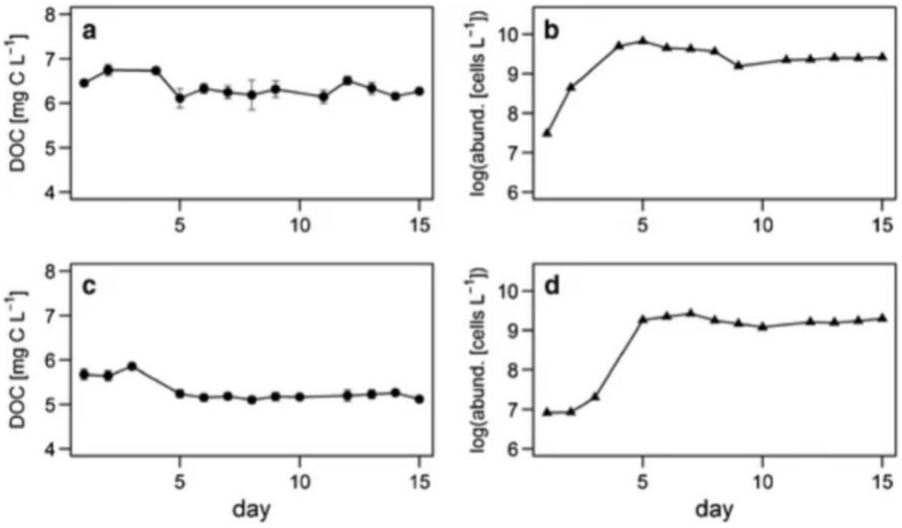

Figure 1.4. DOC concentrations and bacterial abundance in peat (a, b) and soil (c, d), mean ± standard error of the mean, $n = 3$).[105]

The sediment recalcitrance is caused by substrate limitations, where a variation of microbial dormancy, with fewer bioenergetically favourable substrates, are available to microorganisms, having implications for carbon cycling and ecosystem dynamics.[41] When LOM, such as

phytoplankton-derived material or terrestrial inputs, are introduced into the aquatic system, it can either lead to a positive or negative PE.[56] Either way, there is an augmented degradation of the recalcitrant OM because the addition of easily decomposable organic matter stimulates the microbial breakdown of more resistant organic compounds or reduces degradation of recalcitrant OM.[59,62] The interplay between these effects could have significant results for the overall carbon balance in aquatic ecosystems and highlights the intricate nature of biogeochemical processes in these environments. In this study, we propose to gather more information towards the Priming Effect concept by using biomarkers, such as hydrocarbons and fatty acids, as well as stable isotope analysis to determine whether the Priming Effect activates the dormant microbial communities that release organic carbon to the deep-sea floor. These analyses will allow us to explore the potential breakdown of resistant organic matter (OM) in comparison to a control lacking the addition of easily degradable Organic Carbon (OC). Thus, we aimed to investigate the potential presence of a Priming Effect in the aquatic system while seeking to comprehend whether this concept arises from the introduction of labile organic matter. Specifically, we analyzed different fractions: the water samples, the bulk sediment, and the extracted biomarkers for a broader view of the decomposition of OM. If this effect activates dormant microbial communities preserved in the recalcitrant sediment, it will eventually release organic carbon. We contend that the potential interactions between priming the sediment with plankton are influenced not only because of differences in stable isotope values, but also due to the interplay between abiotic and biotic factors encompassing

substrate availability, accessibility, structure and activity of the microbial community in regard to temperature, bioturbation and light availability.[63,64]

1.5. Isotope analysis and biomarkers

To trace the origin of organic matter and support the insights of naturally occurring phenomena as well as differentiating between the sources of input, biomarkers are a valuable tool for explaining the patterns of the Priming Effect.[65] In biogeochemistry and analytical chemistry, the application of isotope analysis reveals the migration pathways between different organic matter sources, since stable isotopes do not decay and are naturally present in varying abundances.[4] Among carbohydrates, proteins and lipids, the latter are ubiquitous in marine ecosystem with a great diversity and chemical stability. Fatty acids, sterols or straight chain alkanes (*i.e.,*'*normal'* alkanes; abbreviated as *n*-alkanes) are the primary lipid biomarkers used to establish a wide spectrum of organic matter sources and their dynamics.[66] The emphasis in this thesis will be on the *n*-alkanes as biomarkers for the Priming Effect to interpret and confirm the OM sources used in the experiments.

N-alkanes are non-polar, the simplest saturated aliphatic compounds, and represent the most abundant class of organic compounds detected in sediments.[67] Short chain alkanes, ranging from C15 to C19, are typically associated with aquatic algae and bacteria. Mid-length alkanes,

ranging from C20 to C25, are observed to be abundant in aquatic macrophytes.[68,69] *N*-alkanes derived from terrestrial plants, are a prevalence of odd-numbered carbon chain lengths, such as C25 to C37.[70] An *n*-alkanes signature is used to elucidate the source, since they vary depending on environments / tested locations. Using isotopic measurements and different indices based on biomarker abundance can permit tracking of the Priming Effect.

Stable isotope:

For the characterization of stable isotopes, we expressed the ratio of ^{13}C:^{12}C relative to a reference standard using the Vienna Pee Dee Belemnite (VPDB) (see Equation 2). Here the standard reference value for the $^{13}C/^{12}C$ ratio is 0.0112372.[71]

$$\delta^{13}C_{sample-standard} = 1000\left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample} - \left(\frac{^{13}C}{^{12}C}\right)_{standard}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}}\right)$$ Equation 2.

In this project, we used two types of organic matter with drastically different isotopic values to distinguish between the two sources of carbon in question. The phytoplankton cells, used as labile material, has a delta ^{13}C (δ) isotopic value, as reported in the literature, ranging between -21 to -19‰.[72,73,74] Initial measurements on the recalcitrant OM before the incubation procedure resulted in a calculated delta ^{13}C (δ) of -25‰. The labile OM was determined to be -44‰. For our

investigation, we highlight the vital role of aquatic ecosystems in the global carbon cycle and focus on OM as a fundamental component. The intricate pathway of organic carbon involves various processes and organisms, especially within the sediment pool. This difference between OM sources highlights the necessity to recognize and incorporate the features of these organic materials within the broader framework of aquatic ecosystem dynamics and the global carbon cycle. This work seeks to contribute to the understanding of the chemical pathway of organic carbon in nature, with emphasis on the aquatic ecosystem and sediment pool. The approach employed has been tested more extensively in soils than in estuaries.[57] When formulating the experimental design, a deliberate decision was made to devise an unfamiliar approach instead of drawing inspiration from existing literature. This deliberate choice was driven by the belief that utilizing this method of analyzing all the fractions involved in the incubation, would effectively assess the potentiality of PE in a way that differs from established methodologies.

1.6. Thesis arrangement

This thesis is comprised of eight chapters with the first chapter dedicated to the general introduction of the main topics covered. The second chapter incorporates the sampling locations and experimental setup, while subsequent chapters delve into bulk, dissolved organic carbon and biomarker analysis. Each chapter presents a detailed account of the methods employed, ensuring an understanding of the analytical processes. The future work section provides a glimpse into

potential avenues for further investigation. Finally, the conclusion consolidates key findings and emphasizes the significance of the project's contribution to understanding the PE.

Chapter II: Materials and Incubation

2.1. Sediment sampling locations

In June 2019, a series of surface samples were collected from eight different locations, identified in Figure 1.3, to represent a spectrum of sediment types, from terrestrial to marine environments. These samples were retrieved using a box-core at stations Sag 5, 23, 21, 18.5, 18 and 16. At stations DE and G, where the sediment depth was insufficient for the box-corer, a Van Veen grab was used instead. All these sampling activities were conducted aboard the RV Coriolis II.

Prior to the incubation experiments, the frozen sediment samples were subjected to a series of sample preparation steps. Additionally, the sediment samples were homogenized to ensure uniformity for subsequent analyses. Notably, due to the substantial quantity of sediment required for the incubation process, the sediments collected from SLEG stations were mixed into one unified sample, with 100 g used per sample. In total, the experiment employed a cumulative quantity of approximately 2.5 kilograms of sediment.

2.2. Incubation experiment sample preparation

The experiment consisted of four steps, the 1) incubation of artificially controlled recalcitrant sediment material mixed with labile OM, 2) extraction of biomarkers, 3) instrumental analysis, and 4) data integration. In this section, the properties and sources of the materials are presented first, followed by the details of the incubation of OM in sediment.

The seawater employed in the experimental study was sourced from the SLEG, it was obtained during the same collection year as the sediment samples used in this investigation. The collection process involved retrieving seawater from a depth of approximately 300 meters. At this depth, seawater is known to have low Organic Carbon (OC) content. The seawater was deliberately not filtered, and this decision was made to ensure that the bacteria present in the water remained.

Approximately 200 mL of seawater was added to 100 mL of the mixed sediment to create a stable model environment. Before incubation, the samples were allowed to stabilize for three days. These sediment slurries were prepared in 500 mL polypropylene sterile bottles (Fisher Canada). The process of incubation creates a microenvironment where the labile organic material serves as a nutrient source for microbial activity.

As an enrichment agent and the primary input, we used the *Nannochloropsis sp.,* which was cultured and purchased from Reed Mariculture. The labile material, *Nannochloropsis sp.,* is a robust oleaginous (containing increased amounts of lipid) microalgae. It was utilized in the Control

Plankton and Plankton+Sediment samples and was specifically used due to its depleted isotopic signature with respect to the Vienna Pee Dee Belemnite (VPDB) standard.

To assess the impact of the Priming Effect, two time-course experiments were prepared: a single pulse (SP) and a double pulse (DP) experiment. Hence, the samples were divided into two batches with emphasis on the variations in the input of labile material. For the single pulse experiment, approximately two grams of plankton were added on the first day of incubation. For the double pulse experiment, the same amount was added on day one, with an additional and 1.5 grams on day eight, as per the calculated organic carbon content.

We decided that for each sample batch, Control Plankton and Control Sediment samples were also included for reference on each specific day of incubation. Each batch, comprising both SP and DP, encompassed a total of 36 samples in the experiment. For the Control Plankton, 2 g of plankton were incubated in seawater without sediment. For the Control Sediment, 100 g of sediment was incubated in seawater without plankton. The experiment was designed to examine the interactive effects and synergistic processes between plankton and sediment and to shed light on the intricate dynamics of the model system.

For the single pulse batch, samples were incubated for 1, 3, 5, 8, 12, and 20 days. To reiterate, the addition of plankton as an enrichment agent was implemented solely on the first day of the incubation process. For the double pulse, samples were incubated for 1, 3, 5, 8, 10, 12, 16 and 32 days. Plankton was added on the first and eighth days of the incubation.

In the incubation experiments, utmost care was taken to simulate the natural aquatic environment and samples were subjected to frequent agitation throughout. This shaking process aimed to mimic nature and facilitate the interaction between the sample and seawater. The samples were maintained at a constant temperature of 6 °C to reflect the conditions of the SLEG ecosystem.

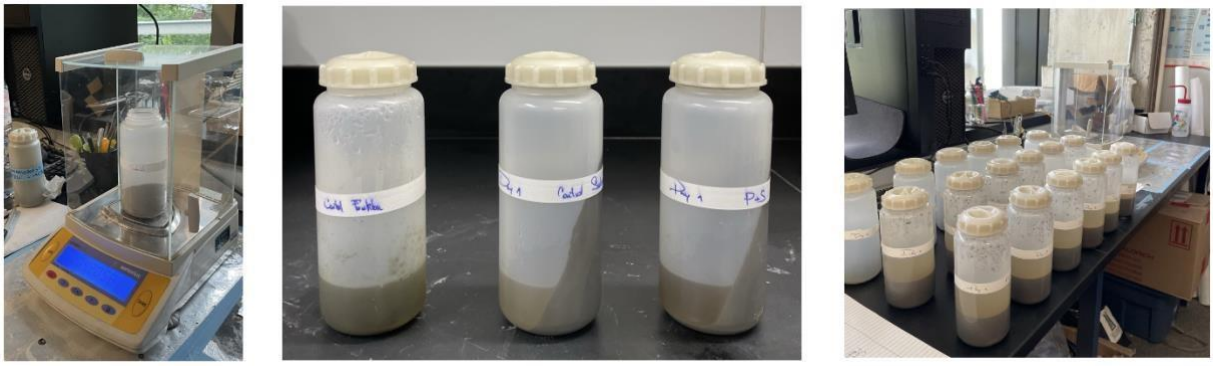

Image 1. Setup of incubation samples which included weighing the freeze-dried sediment, the stabilization part where seawater is added to each sample to acclimatize, preventing stress on bacteria and microorganisms during the incubation period.

2.3. Sample preparation

All glassware was baked in an oven at 475 °C to combust any trace Carbon. After incubation each sample was subjected to centrifugation. The liquid phase (seawater) was separated from the solid phase through filtration after the sample was centrifuged. The seawater fraction was filtered (with 0.2 μm pre-combusted GF/F filters) to remove any particulate matter and to ensure that only the dissolved organic carbon (DOC) was analyzed. The filtered seawater was acidified to stabilize the sample for DOC analysis and to preserve the sample.[75]

Chapter III: The Bulk Analysis

3.1. Bulk Sample Preparation

We have proposed to divide the analysis into three different fractions, the bulk samples, the water samples and the extracted biomarkers for a clearer distinction between how the samples were prepared and analysed.

The isotopic measurement of bulk sediment offers insights into the sources of the compounds present in the analysed samples, helping to distinguish the origins of OM. After the incubation stage, the control and mix sample fractions were freeze-dried to remove moisture and preserve the organic matter. Freeze drying involves subjecting the sample to low temperatures and pressures, causing the frozen sample to sublime (solid to vapor). This process involves sublimation, at a temperature of -58 degrees Celsius. The samples undergoing freeze-drying were maintained for at least one week to ensure complete removal of residual moisture. The freeze-dried samples were homogenized with a mortar and pestle to ensure uniformity and consistency of the material. Approximately 1.0 gram of each sample was subsampled for the EA-IRMS (Elemental Analyzer-Isotope Ratio Mass Spectrometer) analysis and the remaining portion was reserved for the extraction of hydrocarbons and lipids.[21]

3.2. Bulk analysis

The $\delta^{13}C$ signature of bulk OM in the incubation experiment was measured for each batch, (Single Pulse and Double Pulse) to corroborate the data. By analyzing the bulk samples, we were

able to understand the dynamics of the organic materials used in the experiment. This evidence was reflected through the input of *Nannochloropsis sp.* with a contrasting stable isotopic value identified in Table 1. The resulting values varied from -25.41 ± 0.12 to -44.87 ± 0.13‰ (n=4), depending on the sample. The samples were either in a more depleted range (plankton) or a more enriched range (sediment). Controls for the plankton and sediment were analyzed to assess the relationship between the controls separately and the mixed samples was analyzed in order to verify the accuracy of the analysis. Prior to the run, a calibration curve was generated showing a positive linear relationship (R^2= 0.9976) with the in-house standards (BALA and Sucrose).

Table 1. Bulk stable carbon isotope values and STDEV for Control Plankton, Control Sediment and Plankton+Sediment, Single Pulse.

Incubation day	Control Plankton δ_{13}C (‰)	Control Plankton STDEV	Control Sediment δ^{13}C (‰)	Control Sediment STDEV	Plankton+Sediment δ^{13}C (‰)	Plankton+Sediment STDEV
Day 0	-	-	-	-	-25.52	0.21849
Day 1	-44.21	0.313491	-25.41	0.127509	-30.55	0.129844
Day 3	-44.47	0.297379	-25.73	0.127969	-30.09	0.181055
Day 5	-44.47	0.12596	-25.82	0.215287	-29.41	0.205374
Day 8	-44.65	0.06718	-25.85	0.050545	-28.91	0.51478
Day 12	-44.87	0.136998	-25.95	0.128985	-28.46	0.366141
Day 20	-44.37	0.203498	-25.79	0.129462	-29.64	0.238142

Control plankton stable isotope values are relatively unchanged throughout the incubation period with the largest variation of (± 0.66) between the Day 1 and Day 12 time points (See Table 1). This is the result of the labile material not undergoing changes in the carbon source or metabolic processes during the course of the experiment.[79] The $\delta^{13}C$ values were observed to be depleted with respect to the control sediment (compare columns one and three, Table 1). The observed standard deviation (0.313491, 0.297379, 0.12596, 0.06718, 0.136998 and 0.203498, as shown in column two in Table 1) between samples at any time point is predominantly influenced by the degree of homogenization of analyzed samples. It is noteworthy that each analysis was conducted in quadruplicate, contributing to the identified standard deviation. This deviation is attributed to potential sources of error, including instrumental or manual handling factors, as the samples were manually ground using a mortar and pestle. Similar to the control plankton, the control sediment samples indicated stability in carbon composition with a variation of ± 0.54 within these samples, confirming with values from the literature that marine sediments typically have $\delta^{13}C$ values ranging from -25 to -30‰.[80] These results suggest that for the analyzed controls, the temporal factors and environmental dynamics *e.g.,* presence of seawater, confirm the isotopic value of the analyzed compounds. These two types of samples revealed that the controls are a reliable reference to which the experimental results can be compared.

Stable isotope values ($\delta^{13}C$) of the mixed samples were progressively more enriched in ^{13}C (Figure 3.1). As expected, the enrichment originates from the plankton component that is gradually getting consumed and depleted in its contribution to the mix.[62] Over the incubation period, the organic matter associated with plankton in the mix, is being utilized or decomposed, leading to a shift in the carbon composition of the samples. As plankton is known to have a depleted isotopic value compared to sediment, a decrease in the contribution of plankton derived OC results in $\delta^{13}C$ values that are similar to those of the control sediment.

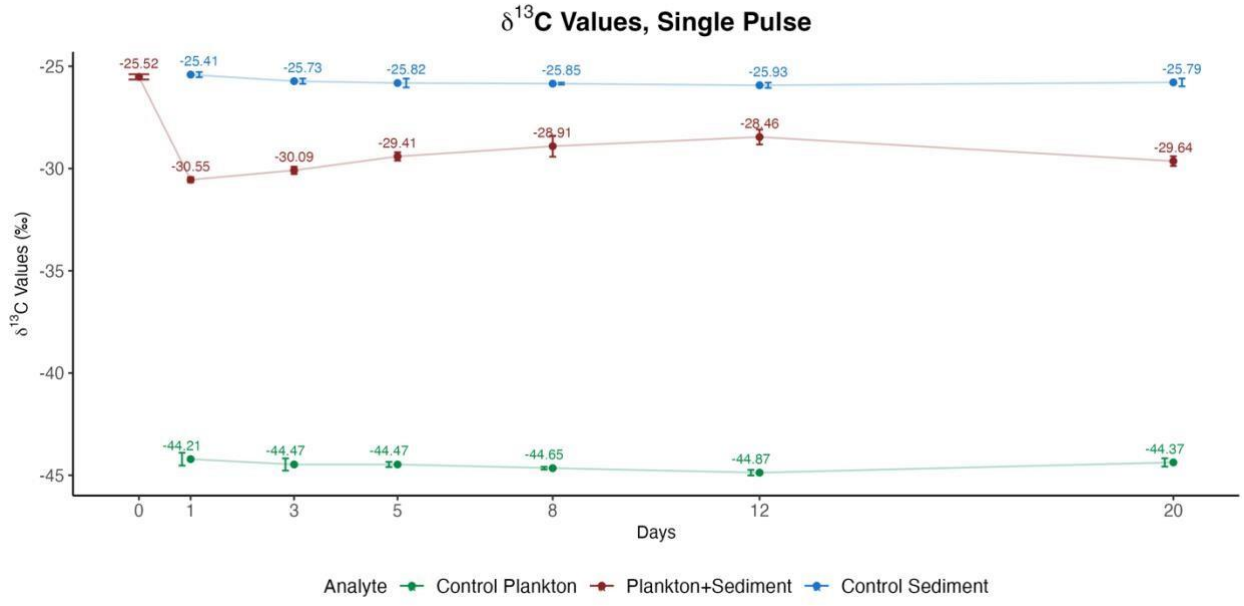

Figure 3.1. Stable isotope values for Control Plankton, Sediment and Plankton and Sediment mix, Single Pulse.

These changes in $\delta^{13}C$ values are consistent with the idea that microbial communities within the labile material release a considerable amount of CO_2 and the value is being depleted over time, while the sediment is remaining the dominant contributor to the mix's carbon

composition. The standard deviation values (Table 1) indicate several variations, but support the suggestion that plankton is being depleted and transformed over time. Through these discrepancies in the stable carbon isotopic composition, we can assess the input, considering that sedimentary organic matter exhibits a depletion in $\delta^{13}C$ between -28 to -25‰ (more negative and ^{13}C depleted), in contrast to marine organic matter with $\delta^{13}C$ values falling between -22 and -18‰.[21] The substantial input from an alternative organic matter source increases the challenges in accurately estimating the ratio between terrestrial and marine organic matter within an environmental setting. This is a difficulty that arises from mixing both LOM and ROM in a sample. Discriminating the persistence of the degraded molecules becomes challenging because their distinct rates of degradation and turnover complicate the accurate assessment of their respective contributions to the overall organic matter composition. Throughout the incubation period, plankton and sediment mix stable isotope values reached equilibrium and remained relatively stable depicting through the identified values, the presence of two different sources of OC (Figure 3.1 blue trend).

While these results show a promising direction in our ability to trace the isotopic ratio of carbon within solid samples, it is important to note that such observations do not unequivocally establish the remineralization of sediment or inherently provide clear evidence of the PE. This can potentially also be attributed to fluctuations in microbial activity, modifications in carbon sources or changes in the microbial pump, which may not be necessarily linked to the Priming Effect. The addition of labile material on day one suggests a definite shift in carbon source compared to day

0. This shift occurs because the labile material is more rapidly consumed by microorganisms. We expected for the identified values to portray the degradation of the depleted LOM with emphasis on microorganisms which metabolize the labile plankton. The microorganisms in the system preferentially use the lighter isotopes of carbon, leading to an increase in the relative abundance of heavier isotopes in the remaining carbon pool. This specific preference causes the carbon pool to display more depleted isotopic values. Along with enhanced microbial activity, additional measurements, such as carbon fluxes or specific organic compound concentrations, would add additional context to assess the potential presence of the PE since we have not been successful in identifying the PE in this analyzed system. The bulk control samples, composed of different biogenic components, resulted in different stable isotopic compositions (labile versus recalcitrant organic matter) which fell into the range described in the literature.[72,73,74] The isotopic values alone provided a limited perspective in terms of identification of plankton degradation and fractionation of more depleted material, but a comprehensive interpretation would benefit from a more detailed examination of the biological and chemical processes occurring in the set-up environment. In order to do this, we propose also to analyze the isotopic values of biomarkers to better understand the metabolic pathway of organic carbon.

Similarly, the $\delta^{13}C$ values for Double Pulse, also analyzed in quadruplicate (See Figure 3.2), confirm the information stated above. In this context, the distinctive feature lies in the stable isotopic values on days 8, 10, 12 and 16 of incubation and the second input of LOM from day

eight. For these days, the isotopic values manifested a more pronounced degree of depletion subsequent to the second addition.

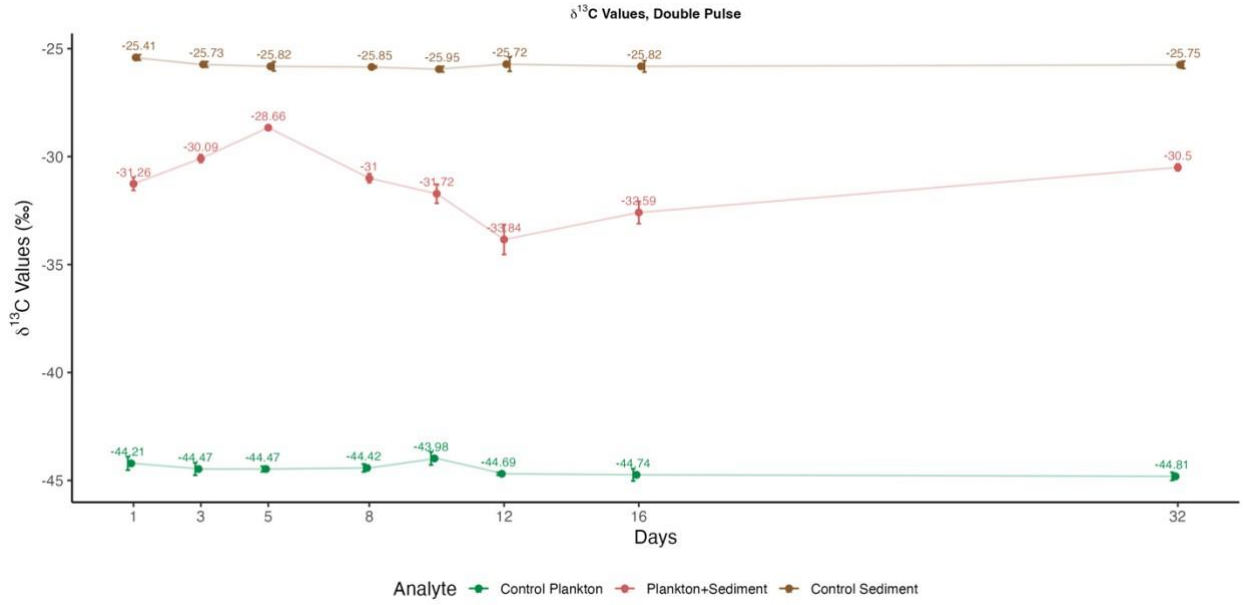

Figure 3.2. Stable isotope values of bulk sample Control Plankton, Sediment and Plankton+Sediment, Double Pulse.

3.3. Bulk sediment isotope analysis

To prepare the previously freeze dried and weighed bulk samples for the analysis of organic carbon (OC) and stable carbon isotope ratios, a decarbonation process was employed.[58] This involved exposing the samples to HCl fumes for 24 hours, to allow for analysis of the organic carbon fraction without interference from inorganic carbon species, which could affect the accuracy and interpretation of the results obtained from EA-IRMS (Elemental Analyzer-Isotope

Ratio Mass Spectrometry) analysis. Subsequently, a heating step at 40°C for 12 hours was performed to eliminate any residual water or acid before the actual analysis.[78] A small quantity of the complete sample, ranging from 20 to 100 μg of organic carbon was introduced into the EA, and rapidly combusted, producing CO_2 gas which was subsequently transported to the IRMS for analysis.

The EuroVector elemental analyzer was used in conjunction with an Isoprime 100 continuous flow isotope ratio mass spectrometer (EA-IRMS) to measure the concentrations of organic carbon (OC) and to determine the stable isotope ratios of carbon present. A calibration curve for the OC analysis was created using a β-alanine standard with known concentrations (C= 40.45%) and stable carbon isotope ratios ($\delta^{13}C$= -25.98 ± 0.23‰). The calibration curve covered a full range of masses for the instrument to detect and subsequently, the samples were analyzed in quadruplicate, with the β-alanine standard and a certified sucrose standard (IAEA-C6) with known concentrations and stable carbon isotope ratios (C= 42.10% and $\delta^{13}C$= -10.80 ± 0.47‰ respectively) at intervals of every six samples. To enhance precision and compensate for any potential drift in the instrumentation as well as to ensure that any changes or inconsistencies in the instrument performance are accounted for, intermittent standard samples were included between sets of six samples during the analysis.

Chapter IV: The DOC Analysis

4.1 DOC (Dissolved Organic Carbon) analysis

Prior to conducting the DOC analysis on the seawater samples, a standard curve was established using a potassium hydrogen phthalate (KHP) solution. This calibration involved several standard samples with known concentrations with the resulting calibration curve serving as a reference for quantifying the concentration of organic carbon compounds in the liquid sample.

Table 2. Standard curve data for Dissolved Organic Carbon analysis.

No. of samples	STDs	Mean Area	STDEV
STD 0	0.0 mg/L	0.713	0.115
STD1	19.11 mg/L	15.627	0.204
STD2	40.828 mg/L	32,343	0.480
STD3	60.124 mg/L	47,017	0.319
STD4	80.392 mg/L	62.560	0.825
STD5	100.48 mg/L	78.147	0.378

Considering that the presence of DOC in water is directly linked with the transport and exchange of nutrients with the sediment and *vice versa*, the formation of the dissolved organic carbon complex further facilitates the transfer of labile dissolved organic carbon of degraded molecules in the dissolved organic carbon pool. Measurements of DOC in marine water varied from 181.2 to 3364.7 mg/L OC, depending on samples as depicted in Figure 4.3. Considering each

sample's carbon content, Control Sediment was analysed without being diluted whereas Control Plankton and Plankton+Sediment samples were subjected to a dilution process with a dilution factor of 50 prior to the execution of DOC analysis. In this data presented, the dilution factor has been considered and calculated appropriately.

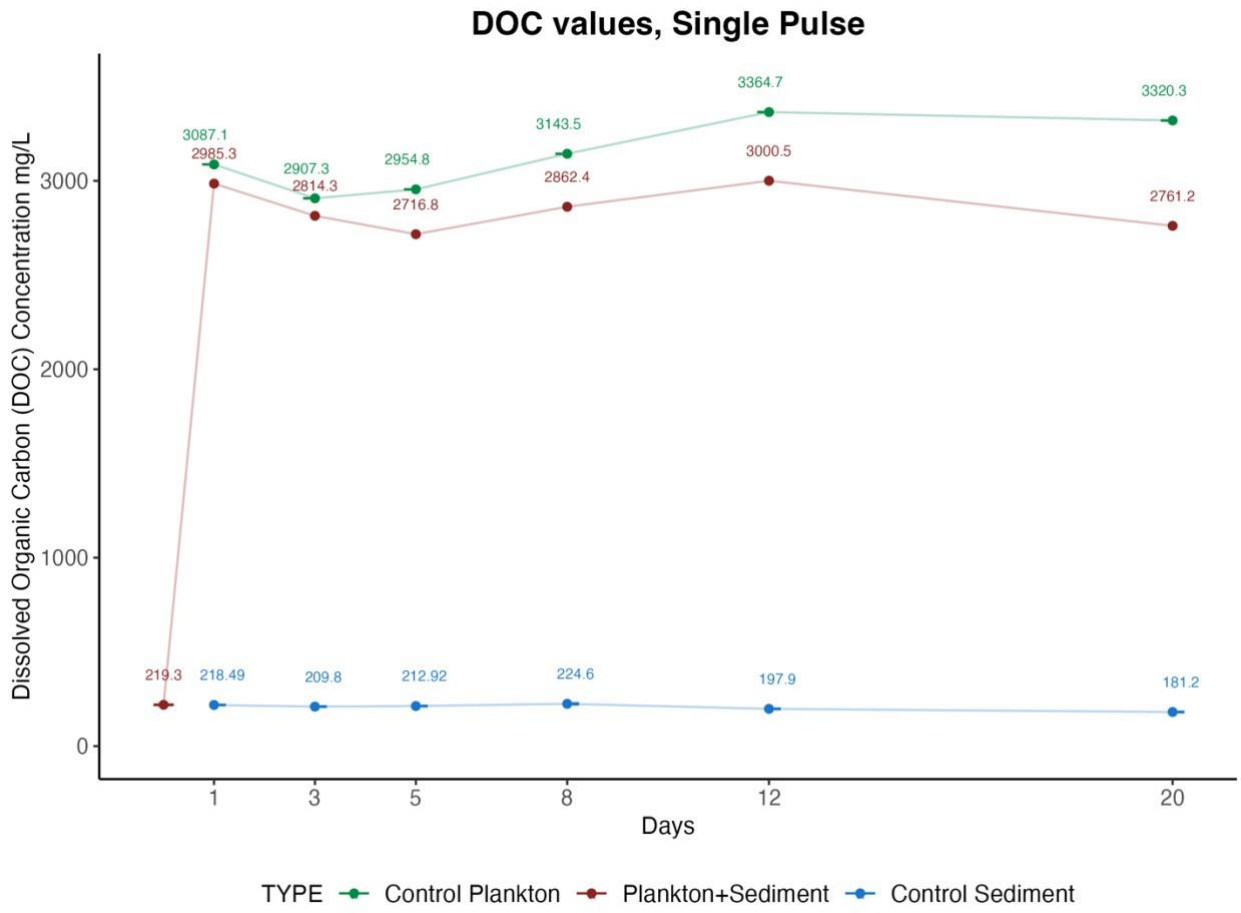

Figure 4.1. Dissolved Organic Carbon (DOC) analysis on Control Plankton, Control Sediment, Plankton+Sediment, Single Pulse.

We evaluated the nutrient transport, or release, that is identified as an enhanced nutrient supply in the water column. Each analyzed fraction, revealed distinct patterns in organic carbon concentrations. To begin with, the Control Sediment samples were consistently characterized with relatively low DOC values ranging from 181.2 to 224.6 mg C/L. Throughout the incubation phase,

these values reflected and confirmed the Control Sediment's recalcitrant state and the fact that it is constant, indicates no changes in the OC activity.[81] In contrast, the Control Plankton samples exhibit significantly higher DOC values compared to the Control Sediment. These identified values indicate a considerably higher abundance of OC in the plankton community. Thus, we have identified plankton as being an enhancer in the microbial cycle, that consumes and converts a diverse range of nutrients, influencing significantly the long-term sink of carbon.

The mix sample, Plankton+Sediment follows a similar trend to the Control Plankton, due to the influence of mineral particles and organic compounds related to the input of labile material, (Figure 4.1). The drastic increase in concentration from 219.3 to 2985.3 mg C/L from day 0 to day 1, substantiates the speed and ability of heterotrophic bacteria within this material to release enhanced amounts of OC. Due to the breakdown of organic matter introduced into the system, these values lead to a partial transfer of plankton material to the sediment through different sorption processes. In addition to the DOC values, this claim was confirmed within the stable isotope values of bulk material in Section 3.2. Over time, the disparity between the observed mixed sample and the control sample increased, which can be attributed to a progressively higher transfer of material to the sediment, or by the degradation of plankton into CO_2. Notably, the analyzed mix illustrates the feasibility of several dynamic processes occurring in the water column, involving microbial activity, and plankton dynamics within the sediment. At the same time, the solid particles that released the dissolved organic carbon sustained the microbial community by potentially mediating carbon sequestration.[82]

This fraction serves as a source for the microbial carbon pump, reintroducing consumed carbon and nutrients into the food chain to eventually trigger the release of microorganisms from the depleted material. The observed increase in values is linked with the pathway involving sinking particulate labile material which enhanced the production of DOC and bacterial pathways. This

provides a common DOC enrichment that eventually supports the sedimentary dormant microbial community. Notably, plankton contributes substantially more to the organic carbon pool than sediment alone as observed on day 8, which indicates a synergistic effect between players. These results can be supported with the stable isotope values of the seawater system, identified in Figure 4.2.

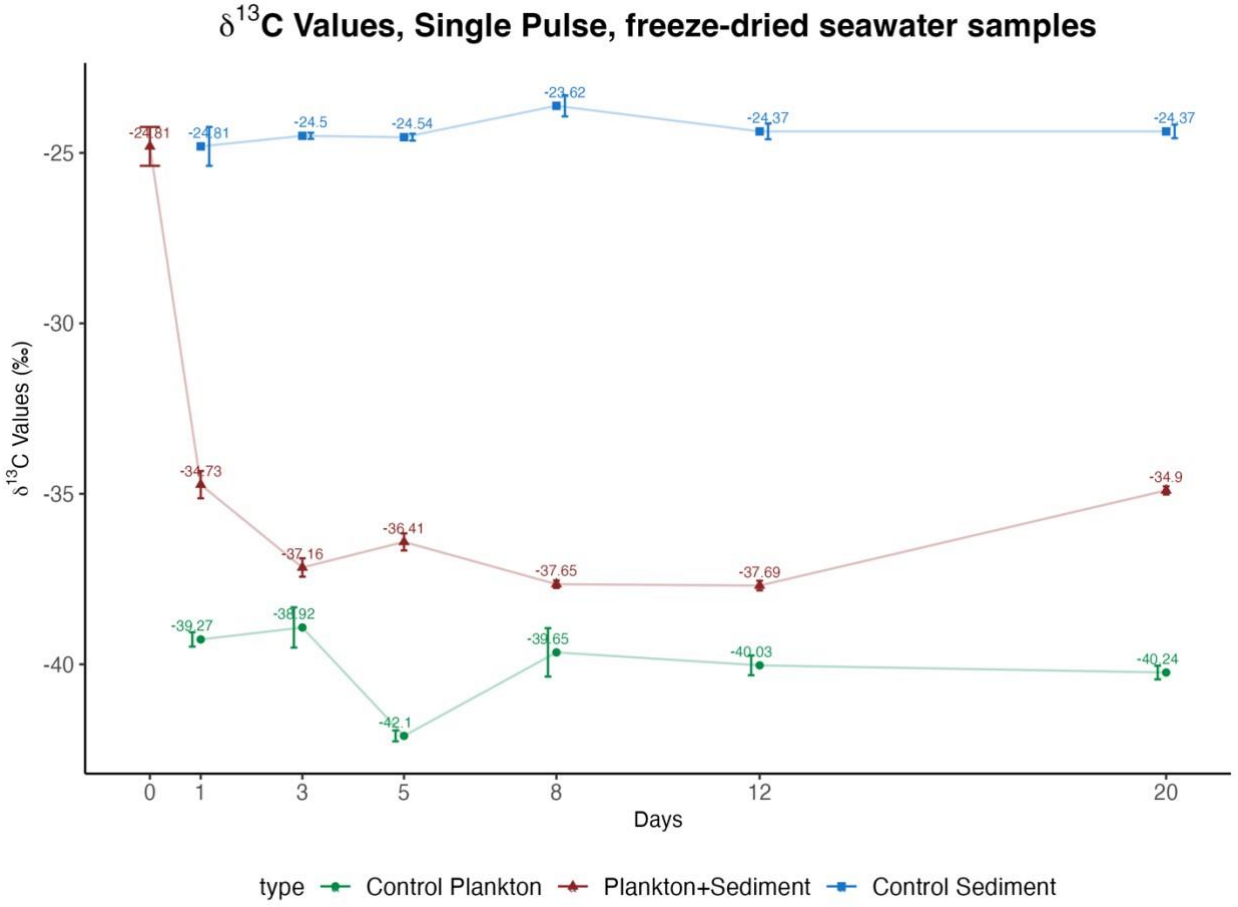

Figure 4.2. Stable isotope values for freeze-dried seawater samples, Control Plankton, Control Sediment and Plankton+Sediment mix, Single Pulse.

From the performed analysis, the stable isotope values for the Control Plankton and Control Sediment exhibit minimal variations attributable to no temporal changes, and the slight difference

in values likely reflect measurement and instrument accuracy. The observed minimal fluctuations may be indicative of intrinsic biological and metabolic activities within the plankton community, but since there was no additional input of external material to the system, the isotope value of day 5 (-42.1‰) should be considered as an outlier. The consistency in isotopic values can also be identified in the Control Sediment that aligned with the characteristics of the recalcitrant sediment. These values provide insights into the isotopic composition of the dissolved organic carbon within seawater even in the presence of sediment, which is recalcitrant. Organic Matter leaching from the bulk sediment into the seawater is itself relatively resistant to rapid degradation.[83] Alternatively, this suggests that the sediment is not significantly influencing the isotopic composition of the seawater and any potential input from the bulk sediment is relatively constant over time.[79] In the context of this project, filtering the seawater prior to incubation raised the question regarding the potential impact on the identification of priming effect, if any. While this method is commonly used to eliminate larger contaminants or particulate organic matter, it is crucial to recognize that it may also remove smaller particles, including dissolved organic matter and microbial communities. Alternatively, for future projects, adjusting the filtration strategy by using a larger filter or omitting filtration altogether may be necessary to preserve the presence of smaller particles.

Table 3. Stable isotope values for seawater samples, Control Plankton, Control Sediment and Plankton+Sediment mix, Single Pulse.

Incubation Day	Control Plankton $\delta^{13}C$ (‰)	Control Plankton SDEV	Control Sediment $\delta^{13}C$ (‰)	Control Sediment STDEV	Plankton+Sediment $\delta^{13}C$ (‰)	Plankton+Sediment STDEV
Day 0	-	-	-	-	-24.81	0.57
Day 1	-39.27	0.21	-24.81	0.57	-34.73	0.40
Day 3	-38.92	0.59	-24.50	0.09	-37.16	0.27
Day 5	-42.10	0.16	-24.54	0.10	-36.41	0.25
Day 8	-39.65	0.71	-23.62	0.31	-37.65	0.11
Day 12	-40.03	0.29	-24.37	0.23	-37.69	0.14
Day 20	-40.24	0.20	-24.37	0.20	-34.90	0.12

The observed stability for the Plankton and Sediment samples is initiated by a substantial depletion in $\delta^{13}C$ values from day 0 to day 1 (-24.81 to -34.73 ‰), indicating a notable shift toward more negative values. This decrease suggests a distinct isotopic signature associated with the initial processes (labile OM input) during incubation that is noticeably identified in the water column, attributable to the release of dissolved organic matter (DOM).[84] The subsequent increase on day 20 is suggestive of potential equilibration processes within the system, reflecting ongoing degradation processes and isotopic fractionation.

As the plankton is inherently more labile than the sediment, it undergoes degradation resulting in a fractionating process that leaves behind a more enriched value, contributing to the overall increase in stable isotopes. The substantial difference in the stable isotope values in seawater samples between labile OM and recalcitrant OM allows one to infer that plankton, being at the base of the marine food web, release carbon into the water sample. This causes an enrichment in lighter isotopes, enriching the water column, and hence more closely matching the Control Plankton values rather than the Control Sediment values.

To assess the potentiality of a Priming Effect based on the acquired analyses reflected in the seawater samples, DOC was the most active carbon pool identified through concentration values. The DOC enrichment was controlled by specific biogeochemical conditions, such as simulation of an aphotic zone (region in the water column of persistent darkness) with a stable 6 °C temperature and frequent agitation. Given these observations, the goal of analyzing this fraction was to acquire additional information on the nutrient transport and kinetic processes of this carbon pool which is clearly an important fraction that provides DOC enrichment that ultimately supports the "deep" microbial community.[85] In the form of mixed samples, the concentrations of dissolved organic carbon increased during the first 1-2 days whereas an enhanced release of DOC is observed on day 12. The maximum release rates of DOC varied between 15.2 to 171 mg/L. This variability indicates that *Nannochloropsis sp.* released extracellular organic compounds at the beginning of incubation, confirming that labile algae is one of the essential DOM producers that persists in

cycling of dissolved organic carbon within the environment. Labile organic matter undergoes rapid respiration and concurrent degradation by stimulating primary production as nutrients are generated, identified in either concentration values (219.3 to 2985.3 mg/L, an increase of 2766 mg/L in dissolved organic carbon production) or stable isotope values (-24.81 to - 34.73, a decrease of 9.92‰). The notable difference between day 0 and day 1 can be attributed to exudation and cell lysis processes.[86] Consequently, a more substantial release of Organic Carbon into the seawater occurs, with undeniable implications for nutrient cycling and ecosystem dynamics.[86] Lower values of Organic Carbon than expected (between 2716.8 to 3000.5 mg/L for Plankton+Sediment versus 2907.3 to 3364.7 mg/L for Control Plankton) for the mix between Plankton and Sediment implies that not all plankton is dissolved, and that a portion of it may precipitate or degrade within the sediment. This can be confirmed using the stable isotope values of bulk sediment in section 3.2. Additionally, the discernible variation in isotopic values implies a concurrent degradation of labile organic matter that represents respiration by organisms. To establish if these microbial communities engage in simultaneous production, degradation and consumption of Organic Carbon while undergoing respiration, we proposed to further extract organic compounds, *e.g.,* hydrocarbons and/or fatty acids, to perform the analysis which would reflect the exudation of intracellular and extracellular material of either recalcitrant or labile material.[21]

Regarding this analyzed fraction, using the stable isotopes or the changes in nutrient concentrations provided evidence that in this system driven by plankton, the Priming Effect is not observed. We believe that in order to identify the Priming Effect, a more drastic alteration in the rate of decomposition would be required, including excluding filtration of the seawater prior to incubation experiments. Even if we proposed to combine observational approaches, to determine the PE, extended observation periods would better enable monitoring decomposition rates and microbial dynamics.

4.2. Seawater Samples and analysis

The DOC pool is the fraction with the most substantial information, displaying both the release of organic carbon, resulting from the input of organic matter and its concurrent role for storage of organic matter. In this experiment, the seawater collected on each day of incubation was subjected to filtration and acidification with 6 M HCl to achieve a pH of 2, rendering it suitable for analysis using the Total Organic Carbon (TOC) analyzer.[75] Eventually, a portion of these samples, approximately two grams were freeze-dried for isotopic measurements to determine the carbon content within the seawater sample. The process of freeze-drying operates through sublimation, where a solid undergoes direct transformation into a gas state at a temperature of -58 degrees Celsius. The samples subjected to freeze-drying were held for a minimum duration of one week to confirm the absence of residual moisture within the sample. The methods for stable isotope analysis for the seawater were performed in conjunction with the bulk samples.

The seawater fraction was initially filtered to remove any particulate matter and to ensure that only the dissolved organic carbon (DOC) was analyzed. Subsequently, the filtered seawater was acidified to stabilize the sample for DOC analysis as well as to preserve the sample by inhibiting decomposition by microbes.[75]

For DOC analysis, a 20 mL of seawater sample was extracted, and acidification was performed to prevent degradation of OC by microbes and to remove the inorganic carbon. A 5-point calibration curve was obtained using potassium hydrogen phthalate (KHP) as a primary standard. The TOC quantifies the concentration of OC compounds present in a liquid sample by combusting the sample at 700˚C where the organic carbon is converted into carbon dioxide (CO_2) through high-temperature combustion. The CO_2 produced is quantified and measured using non-dispersive infrared (NDIR) spectroscopy.

Chapter V: Biomarker Analysis

5.1. Hydrocarbon results

Previous analysis of organic matter has focused on using the gross geochemical indicators: dissolved organic carbon (DOC) analysis along with stable isotope parameters. However, these results provided a generalized overview of sources whereas the use of extracted biomarkers offers a more detailed understanding of incubation dynamics and whether the addition of labile material enhances any remineralization of the sediment.[87] Aliphatic hydrocarbons, specifically *n*-alkanes, were extracted from the bulk sample. We choose these as biomarkers due to their resilience against diagenesis (*e.g.,* physical, and chemical alterations caused by microbial activity), their unique source-specific characteristics and because *n*-alkanes are tools for tracking organic matter of different inputs.[88,89,90]

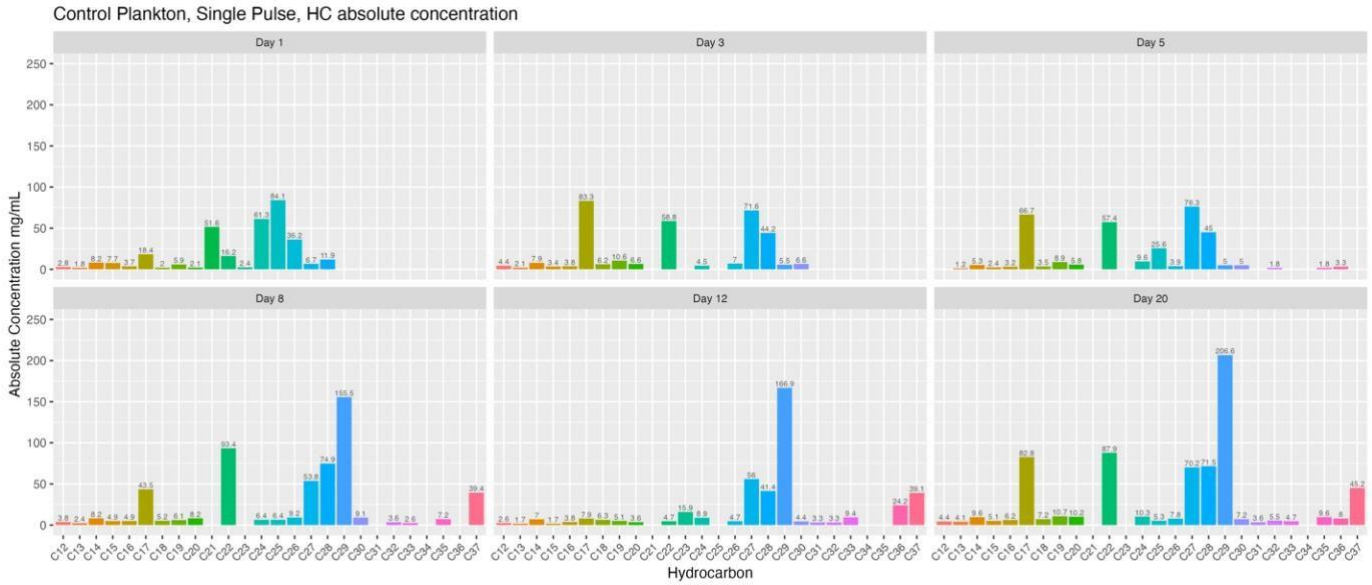

Figure 5.1. Absolute concentrations of hydrocarbon biomarkers, Control Plankton, Single Pulse.

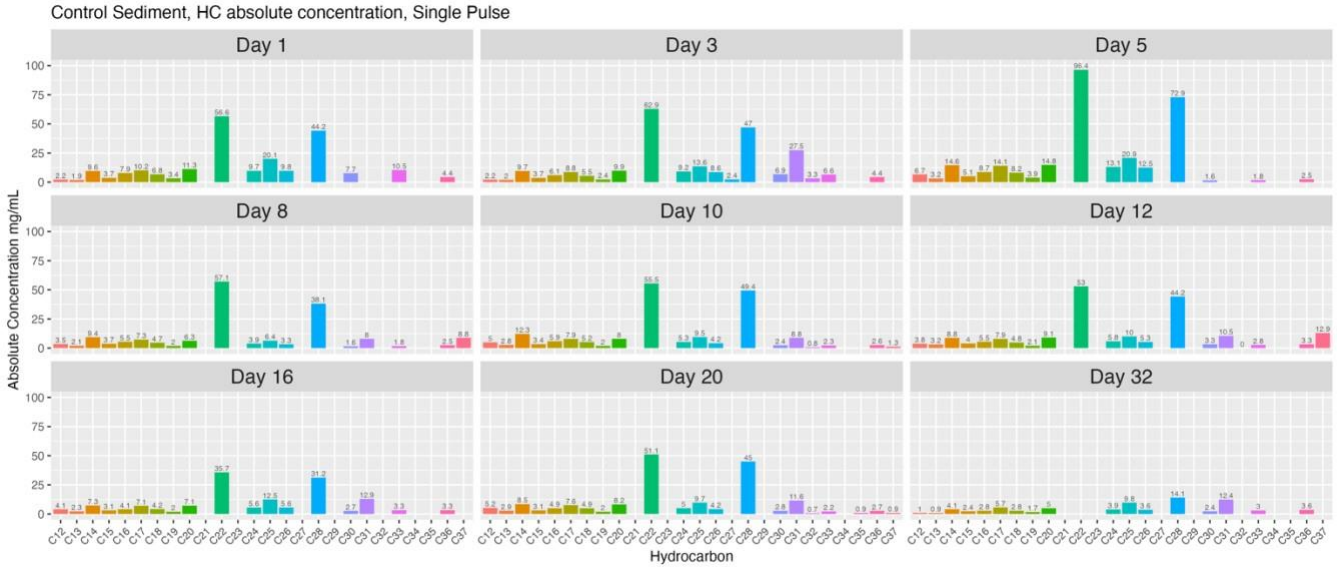

Figure 5.2. Absolute concentrations of hydrocarbon biomarkers, Control Sediment, Single Pulse.

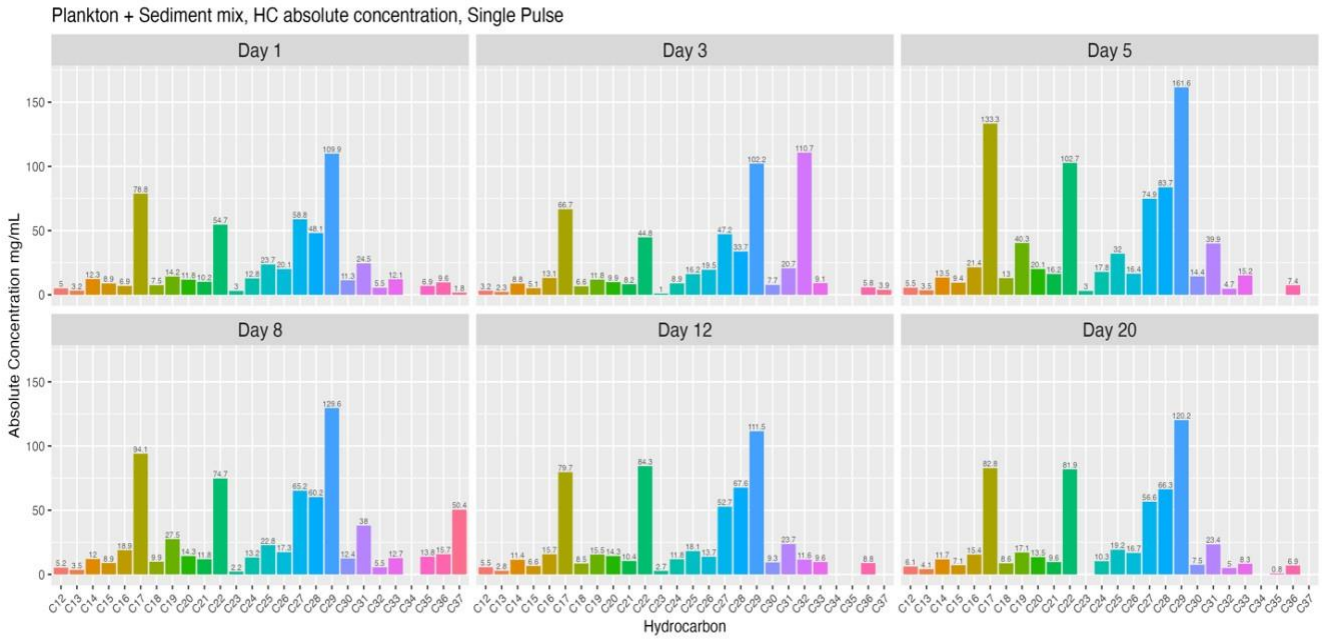

Figure 5.3. *N*-alkane absolute concentrations of Plankton+Sediment sample, Single Pulse.

An entire range of hydrocarbons were effectively extracted from the solid fraction of the samples derived through centrifugation of disrupted biomass and subsequent silica gel column chromatography. Values for plankton including sediment in the single pulse samples are depicted in Figure 5.3 while Control Plankton and Control Sediment results are graphed in Figure 5.1 and 5.2 respectively. We identified the distribution of *n*-alkanes to display the most noteworthy variations in the mixed fraction (Figure 5.3), where the recalcitrant and labile material altogether contained a wide range of concentrations of *n*-alkanes, confirming the complexity of sample compounds. The resulting *n*-alkanes encompass saturated, linear carbon chains ranging from C12 to C37 distributed between odd and even carbon length numbers, specific to autochthonous and allochthonous origin.[46,87] The concentrations varied from 0.3 to 129.6 mg/mL with emphasis on the increased concentrations of long-chain *n*-alkanes associated with input from terrestrial plants.[91]

When comparing the Control Sediment with the mix, the concentrations of *n*-alkanes in sediments are relatively low (between 0.9 to 96.4 mg/mL) expressing the recalcitrant nature of collected deep-sea OM which is highly resistant to rapid biological decomposition. Interestingly, two different *n*-alkanes (C22 and C28) had higher concentrations (C22 between 35.7 to 96.4 mg/mL and C28 between 14.1 to 72.9 mg/mL) in the Control Sediment compared to the other samples. In the literature, these are identified as contaminants or derivatives of methanogenic bacteria, and this is why in our dataset, these are in higher concentrations than the rest.[92,93] Nevertheless, considering the predominance in distribution of hydrocarbons in the Control Sediment, this study exhibits higher odd number *n*-alkanes predominance in the C12-C37 range, which is associated with hypersaline deposition.[92] If we consider the freshwater to saltwater gradient of the investigated sediment and the sources of organic matter, the geological settings of our samples correspond to these conditions. This conclusion is based on the Control Sediment that was a mixed sample from all locations illustrated in Figure 1.3 to enable the identification of a broad array of *n*-alkanes with various intensity levels. This wide range of carbon constituents puts an emphasis on the high chemical structural complexity that is recalcitrant in the absence of dormant microbes/microorganisms with specific enzymes to react to the substrate.[92]

In the context of even *n*-alkanes, the interaction between the Plankton and Sediment mix in majority did not result in an enhancement of decomposition or better said, a release of hydrocarbons from the recalcitrant sediment. This observation is drawn from the cumulative

concentrations of controls, which in our data consistently measured lower or equivalent to theoretical concentrations of plankton and sediment mix. Conversely, the analysis of odd numbered hydrocarbons revealed intriguing findings, with aggregated concentrations exhibiting higher values, indicative of a promotion of hydrocarbon production. This phenomenon is particularly relevant to microbial sources, attributed to the synthesis of fatty acids and other organic compounds derived by input of labile material. The high prevalence of odd numbered hydrocarbons *e.g.,* C15, C17, C19 and C21 has a direct link to aquatic algae (micro- and macro-algae) and photosynthetic bacteria provenience.[94] Consequently, these identified *n*-alkanes, synthesized by phytoplankton can be clearly distinguished from those found in short-chain hydrocarbons. The identified long-chain *n*-alkanes in the Plankton+Sediment samples (>C23) were quantified in higher concentrations compared to the short-chain *n*-alkanes. In terms of distribution patterns, significant input from one odd numbered *n*-alkane, specifically C21, and one even numbered *n*-alkane, C32, serve as proxies for the input of recalcitrant produced organic matter in marine environments.[95] We suggest that these identified molecules are bioactive but inaccessible to degradation as we did not identify these in the Control Sediment samples. Still, the fact that we had identified low concentrations, we suggest that these proxies are indicative of a potential remineralization in this environmental context.

Since their presence is only observed when the algal input is exposed to the recalcitrant sediment on day 1, we believe that the observed presence of these hydrocarbons suggests a

dynamic interplay within the system, by their activation relative to the added substrate. While the association of C21 and C32 with the Priming Effect is suggested, it is pertinent to inquire whether the literature provides empirical evidence substantiating the role of these as reliable indicators of PE in mixed plankton-sediment environment.[95] We arrived at the conclusion that mixing the sediment from all collected stations has introduced complexities in assessing the presence of the Priming Effect, particularly due to the diverse recalcitrance levels present at different SLEG stations. Optimal insights into this concept could be accomplished through performing an identical incubation experiment across all stations of interest within this study area, coupled with subsequent stable isotope analysis of extracted biomarkers. However, based on the acquired data for *n*-alkanes, we intend to trace the cycling of organic carbon to comprehend the effects of plankton on the structure and function of dormant bacteria within the sediment environment. We immediately observed the effects with the addition of the phytoplankton community that enhanced the abundance of *n*-alkanes which responded differently compared to our controls, in terms of concentration to the added nutrient, altering the relative abundance, and potentially the biogeochemical cycling of the assembled system. These differences in concentration could be explained by the alkane's capacity to compete for the OC intake.[96] The observed spectrum of absolute concentrations for C21 and C32, spanning from 4.7 to 16.2 mg/mL, represents a noteworthy discovery within the context of our research, particularly in relation to the microbiota composition of OM.

The analysis of bacterial communities within the plankton-sediment system, is particularly interesting in the context of using *Nannochloropsis sp* and sediment organic matter (OM) with a specific isotopic value. This correlates with the trends which are characterized by the reproducibility and biochemical consistency of the samples in the mixture through the analysis of *n*-alkanes as biomarkers. Our control results, which in terms of stable isotopes remained practically unchanged in the absence of environmental perturbations, provided a stable baseline for understanding the dynamics of these microbial communities and a starting point for future research. The notable observation in terms of biodegradation of OM with phytoplankton-derived OM, exhibited high abundance and activity when compared to only the material sourced from the SLEG. This phenomenon is attributed to the enhanced bioavailability of phytoplankton OM, owing to its labile nature. The patterns of stability (*e.g., n*-alkane absolute concentrations) within the control results during the incubation period, emphasize the reliability of these findings, reinforcing the notion that inherent differences in OM sources influence microbial degradation rates. The traditional focus on gross geochemical indicators *e.g.,* DOC and stable isotopes, has provided a generalized overview of OM sources.[4,66] In contrast, our approach, leveraging extracted biomarkers, particularly aliphatic hydrocarbons, has enabled an increased detailed understanding of incubation dynamics. We emphasize that this approach not only distinguishes the OM sources but also would be helpful to elucidate the complex interplay between labile and recalcitrant materials within the sedimentary environment. We chose *n*-alkanes as biomarkers for their

resilience against diagenesis and unique source-specific characteristics' *e.g.,* a wide range of concentrations and carbon chain lengths specific to both autochthonous and allochthonous origins.[98] Identification of these compounds as recalcitrant or labile emphasizes the need to consider the structural complexity of OM and its resistance to rapid biological decomposition. In the context of even and odd numbered hydrocarbons, we point to microbial influences on biomarker dynamics and advise for additional analyses on *n*-alkanes, such as stable isotope analysis. Utilizing the stable isotope analysis on hydrocarbons will allow to determine the hydrocarbon origin, as various sources exhibit unique isotopic signatures. By acquiring these data, we would be able to pinpoint the transformational processes within this incubation experiment and establish the presence of PE in the system caused by the added OC source and followed by the decomposition of native organic matter.

While the interaction between these sources did not enhance the decomposition of recalcitrant sediment as expected in the bulk data analyses, the enhanced hydrocarbon values suggested that there is a microbial role in the synthesis of fatty acids and other compounds derived from labile material. We found compelling the presence of certain hydrocarbons in the mixed samples, absent in control sediment, suggests its potential attribution to the PE concept. However, the limitations of this study, including the use of mixed sediment from all stations of the SLEG, as well as filtration of seawater which removed bacteria, warrant further investigations to determine the occurrence of the Priming Effect. Future studies employing identical incubation experiments

across specific stations, coupled with stable isotope analysis on hydrocarbons and other biomarkers, would provide more reliable insights. The identified patterns contribute to a broader understanding of the complex relationships within OM sources and their response to varying inputs of organic carbon, laying important groundwork for future studies, unraveling the complexities of microbial-mediated processes in aquatic ecosystems. As opposed to control experiments, in the marine setting, the distribution of LOM could yield varied outcomes, such as a reduction in the abundance of labile molecules or an increase in intrinsically refractory molecules. Therefore, further qualitative, and quantitative characterization is necessary to elucidate the relative contributions of these two types of OM.

In the literature, these estimations explain that these findings originate from the synthesis of long-chain alkanes by cyanobacteria and eukaryotic phytoplankton, resulting in approximately 100-fold higher hydrocarbon production in the ocean compared to anthropogenic sources.[83] Despite this, these compounds are not identified in the water column or the bulk analysis. We and others hypothesize that it could be indicative of rapid biodegradation *via* co-localized microbial populations but additional follow-up analysis is necessary to confirm and substantiate our implications .[83] In conjunction with sediment interactions, the origin of these *n*-alkanes is hypothesized to stem from sediment recalcitrance.[95]

The inclusion of Figures 8.1, 8.2, and 8.3 in the Appendix, depicts the absolute concentrations of hydrocarbon biomarkers in the Control Plankton, Control Sediment and

Plankton+Sediment in the Double Pulse condition. This data serves to fortify the thesis's objective of comparing the outcomes of two distinct batches, with an anticipated result. This addition substantiates that this project was designed for a comparative analysis between two batches, affirming the expectation of comparable outcomes.

5.2. Sample extraction

Following filtration and subsequent separation of seawater, hydrocarbons and lipids were extracted from the freeze-dried sediment. The samples were sonicated in an ultrasound bath (Fisher Scientific FS60H) for 3 hours in a 1:1 mixture of acetone and hexane. [21,76] During the sonication process, at 30-minute intervals, the samples were vortexed. After sonification, 70 mL of ultrapure water was added, and the mixture was centrifuged at 9000 rpm (IEC-HN-S) for 5 minutes. The supernatant was subsequently transferred to a glass vial for further extraction. To ensure maximum recovery, the extraction step was repeated four times for each individual sample.

5.3. Hydrocarbon and lipid purification via silica gel chromatography

Following completion of the extraction process, the contents of each glass vial were subjected to evaporation using nitrogen blowdown, reducing the volume to 1 mL. This particular evaporation step served to concentrate and preserve the extracted components for subsequent analysis by yielding a smaller yet more concentrated sample.

To separate the organic phase from the concentrated sample, silica gel chromatography was carried out using six grams of silica gel as the stationary phase.[77] To eliminate any residual water present in the samples, 1.5 cm^3 of solid sodium sulfate was added atop the silica gel. The lipids were subsequently fractionated into three distinct fractions based on the increasing polarity of the mobile phase: 25% toluene in hexane, 20% ethyl acetate in hexane, and finally, methanol. For each fraction, a volume of 24 mL was used to collect the respective biomarkers. The first collected fraction (hydrocarbons) was evaporated to 1 mL and analyzed for GC-FID (Gas Chromatography-Flame Ionization Detector) whereas the other two fractions were subjected to further procedures described below.

5.4. Urea adduction

The hydrocarbon fraction, mainly comprising aliphatic compounds, was subjected to urea adduction which allows for the selective isolation of straight-chain hydrocarbons from the complex hydrocarbon mixture. Urea adduction selectively removes aliphatic hydrocarbons by forming crystalline urea inclusion complexes with the hydrocarbons, isolating the *n*-alkanes. By using a saturated urea/methanol mixture, the straight-chain hydrocarbons formed stable inclusion complexes, leading to their selective concentration. The urea/methanol mixture (1g of urea/6 mL methanol) was combined with the aliphatic sample and the mixture was then allowed to crystallize overnight at -20 °C followed by drying and a gentle wash with hexane.[77] The crystals were

dissolved in 10 mL MilliQ water, and a liquid-liquid extraction was carried out using hexane as the organic phase. The sample was then evaporated to 500 μL and stored at -20 °C until the time of analysis. This method simplified the sample and enriched it with the targeted *n*-alkanes, making subsequent isotopic analysis *via* GC-IRMS or GC-MS more precise and sensitive to variations when the isotopic ratios were required to be evaluated.

5.5. Hydrocarbon analysis

The quantification of the hydrocarbon fraction was performed using an Agilent 689N gas chromatograph equipped with a flame ionization detector (GC-FID). An Agilent DB-EUPAH column (60 m x 0.25 mm x 0.25 μm) was used, and the alkane calibration mix was achieved using C_8-C_{40}, Supelco ASTM D2887 as the external calibration standard.[77]

The samples were analyzed under constant flow using helium as the carrier gas. The method involved maintaining the initial oven temperature at 40 °C for 10 minutes, followed by a gradual increase of 6°C per minute to 230°C and a subsequent increase to 320°C at 20 °C per minute.

Chapter VI: Future Work

In the context of future work for this project, we propose to isolate the fatty acid fraction from our samples, focusing on bacterial-specific fatty acids. It is important to note the multiple challenges until one reaches the analysis part. The unique fatty acid profiles of bacteria would serve as distinctive markers for their presence in the sample. Through the isolation and analysis of bacterial-specific fatty acids, we could monitor the shifts in microbial community composition, or the specific microbial groups implicated in the Priming Effect. Similarly, since certain bacterial fatty acids are potential bioindicators of PE, their notable presence or absence will be indicative of the Priming Effect impact on this project.[104] By changing the attention to bacterial-specific fatty acids as biomarkers for this system and acquiring their isotopic values, it would allow for elucidating whether Priming Effect is present in aquatic systems. This future data would enhance the precision of tracing how OM sources respond to the introduction of additional carbon sources while also revealing the specific sources of organic matter undergoing decomposition.

However, compared with *n*-alkanes, the fatty acid extraction employs additional steps which include saponification and derivatization steps. As a last part of our project, we intended to extract and analyze the fatty acids from this incubation experiment. Due to our inability to successfully identify the fatty acids, we draw attention to challenges encountered during the derivatization step. This step is employed to enhance volatility for analysis. Due to a lack of precision in controlling humidity during this stage and exposing the sample to a hydrous

environment, the samples did not undergo complete derivatization. Consequently, the GC-MS preliminary results revealed low signals for both derivatized and underivatized fatty acids (*e.g.,* derivatized iC15:0 and underivatized iC15:0). In response to this issue, we attempted the same step three times, compromising the method's quality. The repeated exposure to heat hindered our ability to achieve identification of these biomarkers.

We raise the additional issue where filtration was employed as a preliminary step before conducting the incubation. It is important to acknowledge the potential influence of this step on the identification of PE which might have inadvertently altered the microbial communities present, as certain bacteria could have been eliminated. Though this consideration, the choice of a filter with larger pore size is necessary when the objective of the project is to ensure that the microbial communities are preserved during the filtration process, allowing for an accurate assessment of the PE in subsequent experiments.

This proposed analysis is meant to assist in discerning whether the presence of bacterial-specific fatty acids (*e.g.,* i-C15:0 and a-C15:0) is associated with plankton or sediment stable isotope values.[104] This distinction will enable us to link the observed components, C21 and C32 with the stable isotope values of both hydrocarbons and fatty acids and conclude if these are indicative of the Priming Effect.

6.1. Saponification

After silica gel chromatography, the extracted neutral and polar lipid fraction underwent a drying process under a gentle N_2 stream until complete dryness was achieved and subjected to saponification using a 10 mL solution of methanol: potassium hydroxide at pH 14 in a sand bath maintained at a temperature of 100°C for a duration of three hours.[21] To extract the neutral lipids (di- and triglycerides, alkanols and sterols) from the saponified mixture, a liquid-liquid extraction method was employed using a mixture of 6 mL of dichloromethane (DCM) and 3 mL of 5% sodium chloride. The resulting neutral lipid fractions from the organic phase were preserved for future analysis.

The fatty acid fraction underwent acidification by the addition of 6M hydrochloric acid to lower the pH to below 1. The protonated fatty acids were subjected to liquid-liquid extraction using 3 mL of DCM. This extraction process was repeated three times to ensure thorough separation and extraction of the desired components. Subsequently, in order to ensure the complete removal of water, the obtained free fatty acid fractions were dried using glass columns packed with sodium sulfate. The organic phase was subsequently evaporated to dryness in preparation for derivatization.

6.2. Derivatization

The derivatization step involved methylation of the free fatty acids utilizing a 0.5 mL solution of boron trifluoride-methanol (BF_3-MeOH, Supelco CAS 373-57-9). The mixtures were heated to 90°C in a sand bath for two hours to ensure complete conversion into methyl esters. Subsequently, the free acid methyl esters (FAMES) were liquid-liquid extracted using 1 mL of hexane and 1 mL of 5% sodium chloride solution and the organic phase was passed through a sodium sulfate drying column.[76] The resulting samples were dried using a N_2 stream and further concentrated to a volume of 0.5 mL using DCM and stored until analysis with GC-MS and GCIRMS.

Chapter VII: Conclusion

Studying the Priming Effect and the synergies between plankton and the aquatic environment is a burgeoning area of research, especially when one is aware of how a wide range of distinct functional groups are playing different roles in shaping aquatic biogeochemistry.[63] This is an intricate factor which arises from the dynamic nature of all species and consequently functional groups that are subject to temporal variation.[99] Because we had chosen to have a relatively short incubation time, and that the aquatic environment is one of the most complex systems to perceive, we must be cautious at interpreting our results.

The approach used in this research was to incubate the ubiquitous marine bacteria, *Nannochloropsis sp.* as the labile material (Control Plankton), the recalcitrant sediment (Control Sediment), and the mix between these two (Plankton+Sediment), these being collected from the SLEG. We deliberately used one depleted material as a carbon source to comprehend whether the $\delta^{13}C$ signature of the "food" source would exhibit a Priming Effect, correlated to their biomarker analyses. Notably, the labile fraction is one vital component of marine organic matter, serving as a critical food source and carbon export to the aquatic column.[100, 101] Its input released more labile forms of carbon which eventually would exhibit a domino effect into releasing previously stable carbon compounds into the system.[102] From this experiment, we cannot assume that the subsequent conversion of labile organic matter into labile dissolved organic carbon results in the formation and release of refractory DOC.[82] Despite the relatively well-established knowledge of the total

carbon content and global carbon stocks, there is a limited understanding of the dynamics involved in carbon cycling.[1,41,59] Sedimentary organic matter, as one major reservoir of OC on Earth, serves as a source of carbon substrates and energy for microorganisms.[52,103] These microorganisms, in turn, play a significant role in steering benthic (the bottom of a body of water, sediment floor) biogeochemical processes, influencing the supplied quantity of dissolved organic matter.

Our experimental assessment of the reactivity of ROM revealed a relatively low level of reactivity in the bulk fraction. Typically, with the introduction of labile OM into sediment we expected to induce immediate changes in the mineralization rate of the initially present sediment, leading to increased carbon levels and nutrient content.[7,64] However, in our case, such modifications were not *de facto* observed. Monitoring the respiration process in this context was another method. The composition of DOC in the water column has proven to be highly diverse, encompassing various compounds derived not only from external terrestrial sources (allochthonous) but also from internal sources within the water body (autochthonous).[92] We identified the diverse molecular weight *n*-alkanes produced in our setup and realized that the DOC and bulk values depicted a slight efficacy of microbial mineralization. However, we highlight the need to refine the accuracy of the experimental set-up, including filtration strategies and derivatization steps. With this in mind, we maintain that additional experiments must be carried out to understand if the Priming Effect can be reflected in biomarkers and the used indices (*e.g.,* stable isotopic values) or if it is only a concept related to soil environment.

A critical aspect is the exploration beyond hydrocarbons. To delve deeper into the isotopic composition of these hydrocarbons, the urea adduction method was conducted concurrently with the hydrocarbon analysis presented in the current study. Future analysis using GC-IRMS on these samples is required, unfortunately the instrument is out of order at this time. Despite this setback, we still obtained useful information using the stable isotopic values of the bulk material and determining the absolute concentrations for the *n*-alkanes.

For future work, we are interested in analyzing the bacteria-specific fatty acids, specifically the i-C15:0 and a-C15:0 fatty acids.[21] Moreover, the identification of isotopic values on i-C15:0 and a-C15:0 fatty acids would aid in the identification of bacterial types involved in the priming effect and the dynamics of the set-up system. Despite trying to perform this analysis, we encountered complexities which implied identifying underivatized compounds, which compromised the suitability of the samples for stable isotope analysis. Once we would analyze and identify the bacterial fatty acids for their isotopic signature, we could conclude whether our findings presented in our results are correlated to the remineralization of sediment.

Chapter VIII: References

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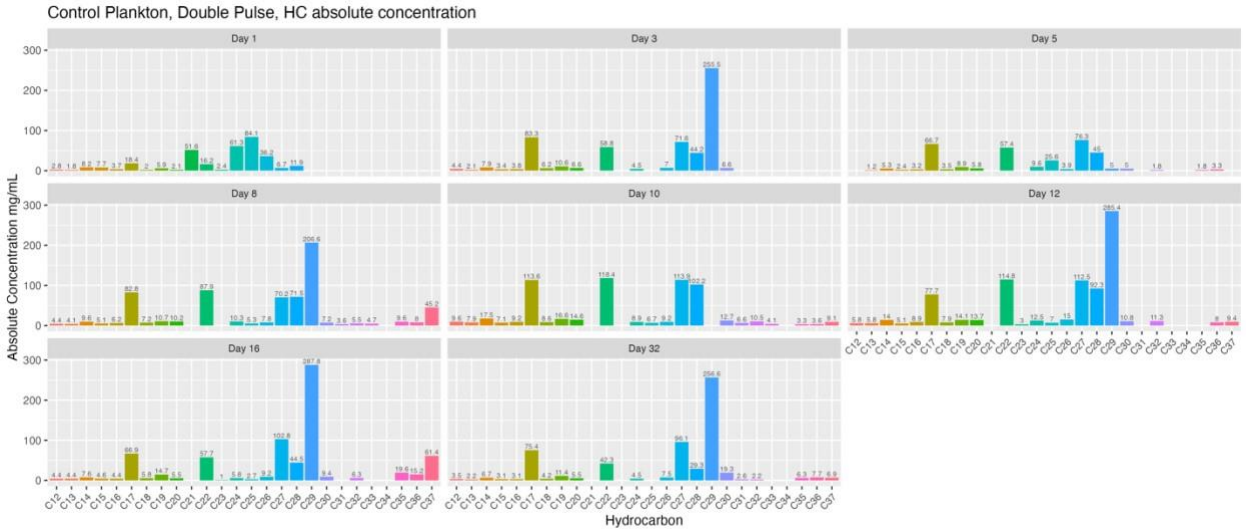

Figure 8.1. Absolute concentrations of hydrocarbon biomarkers, Control Plankton, Double Pulse

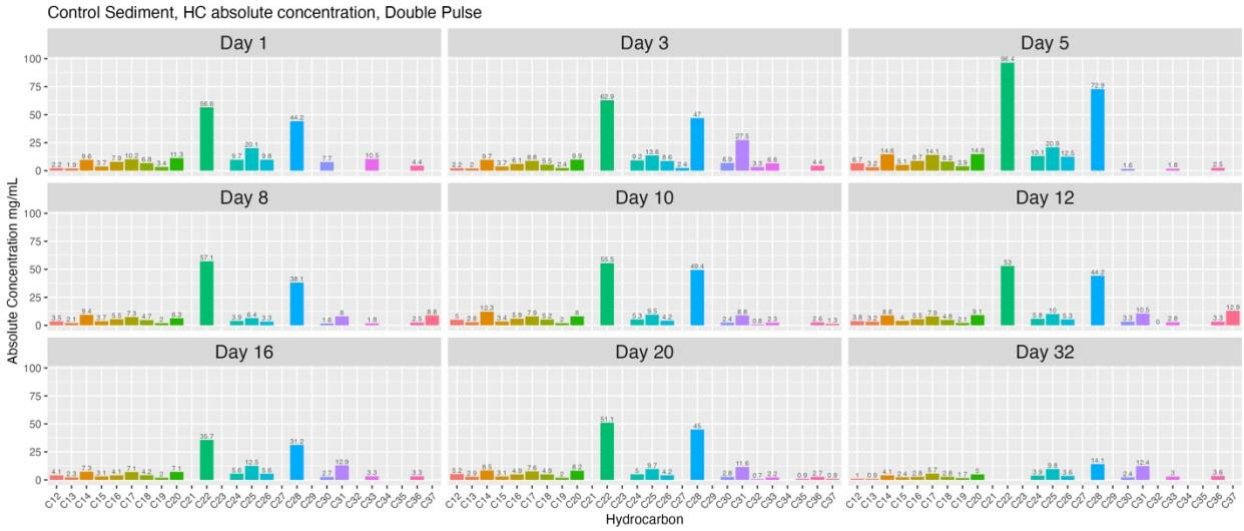

Figure 8.2. Absolute concentrations of hydrocarbon biomarkers, Control Sediment, Double Pulse

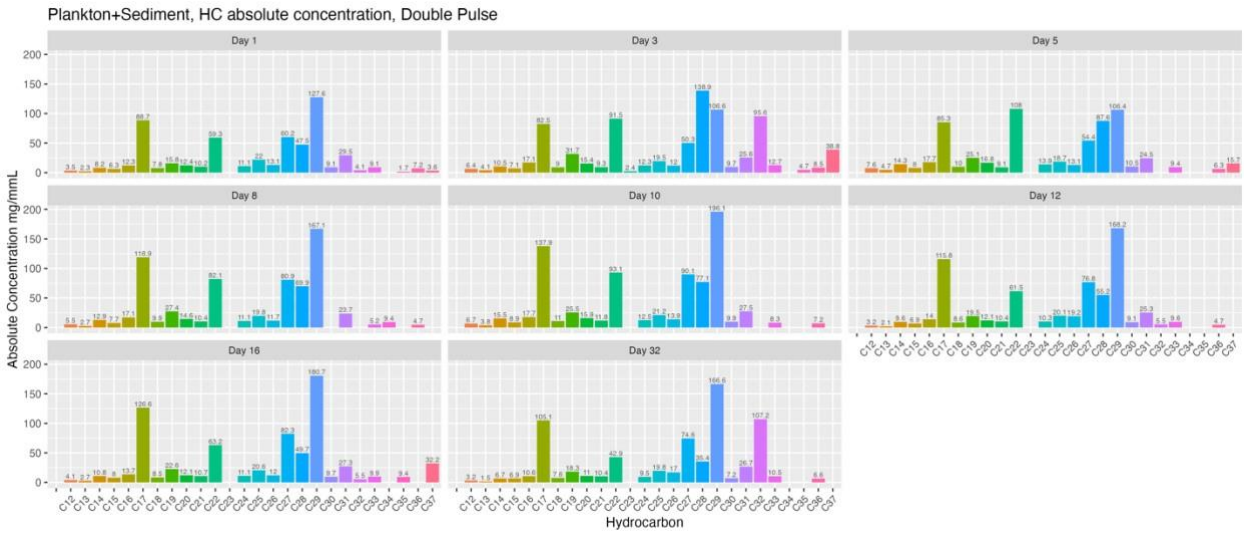

Figure 8.3. Absolute concentrations of hydrocarbon biomarkers, Plankton and Sediment mix, Double Pulse