Zostera muelleri patches in New Zealand affect the sediment carbon consumption dynamics across patch boundaries into the surrounding unvegetated sediment

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Abstract

Seagrass beds are recognized for being a very efficient natural carbon sink. In the face of the climate crisis, there has been an increased interest in seagrass for its ability to reduce atmospheric carbon concentrations through biological processes. Practices that have contributed to high CO₂ levels have also negatively affected seagrass distribution in the intertidal. Urbanization has indirectly caused sedimentation and eutrophication in the oceans, which has reduced light availability, causing seagrass beds to become more fragmented, changing the landscape and patch configuration. In recent studies, scientists have worked to quantify the carbon stocks associated with seagrass beds but have failed to consider how landscape attributes may contribute variability to the results. This study works to assess how the spatial complexity of seagrass patches influences the carbon dynamics, including consumption and sequestration, across the patch boundary. Carbon consumption rates were quantified in the top 15cm of sediments in Zostera muelleri patches in New Zealand using rapid organic matter assay (ROMA). Carbon storage was measured by taking sediment samples from the top 9cm and running them through Elementar's TOC cube. Other biophysical properties, including chlorophyll a and organic matter in the sediments, were quantified in the middle, edge, outer edge, and on the outside of six patches with different perimeter-to-area ratios. The results indicate that the spatial configuration of the patch has no real bearing on the carbon dynamics. However, this research highlights the importance of the surrounding sediment, as carbon consumption and sequestration were done at similar rates in unvegetated sediment as they were in vegetated sediment. Furthermore, the rate of carbon consumption was found to have no relationship to storage rates, emphasizing the importance of measuring both factors when determining the benefits of seagrass. The results presented have real implications for seagrass conservation and restoration efforts. They suggest that seagrass has a broader impact on blue carbon stores than initially thought, as the surrounding sediment stores carbon at similar rates. While the implications of these findings are not restricted to the region and species they were found on, there is a need to expand this research to other species. Additionally, the need to conduct similar studies on patches with larger differences in their perimeter-to-area ratio has been identified to definitively state that the size of the patch has no impact on carbon dynamics.

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Contents

ABSTRACT	2
ACKNOWLEDGEMENTS	3
LIST OF ABBREVIATIONS	7
LIST OF TABLES	8
LIST OF FIGURES	9
1 INTRODUCTION	. 11
2 METHODS	. 19
2.1 Study Site	19
2.2 Patch Size	19
2.3 Pilot Study	20
2.3.1 ROMA Plate Preparation	20
2.3.2 Deployment	22
2.3.3 Data Analysis	22
2.3.4 Results of the Pilot Study	23
2.4 Main Study	24
2.4.1 Sampling Processes	25
2.4.2 Lab Analysis	26
2.4.3 Data Analysis	28
3 RESULTS OF MAIN STUDY	. 29
3.1 Patch Perimeter-to-Area Ratio	29
3.2 Carbon Consumption	29
3.2.1 Surface Layer Carbon Consumption by Patch Number	30
3.2.2 Deep Layer Carbon Consumption by Patch Number	31
3.2.3 Relationship between Carbon Consumption and Patch Perimeter-to-Area Ratio	32
3.3 Relationship Between Sediment Characteristics and Carbon Consumption Rates	33
3.3.1 Chlorophyll a	33
3.3.2 Sediment Organic Matter	34
3.3.3 Relationship Between Carbon Consumption Rate, Chlorophyll a Content, and Sediment Organic Matter	
Concentration	36
3.4 Carbon Storage in Sediments	36
3.4.1 Relationship Between Carbon Storage and Carbon Consumption	37
1 U I I I I I I I I I I I I I I I I I I	

4 DISCUSSION	
4.1 Carbon Consumption Rates	
4.1.1 Macrofaunal Activity	
4.1.2 Across Sampling Locations	
4.1.3 Across Patches	
4.2 Environmental Factors	
4.2.1 Sediment Chlorophyll a Content	
4.2.2 Sediment Organic Matter	
4.3 Stored Carbon in the Sediment	
4.3.1 Across Sampling Locations	
4.3.2 Across Patches	
4.3.3 Relationship Between Carbon Storage and Consumption	
4.4 Conservation and Management of Seagrass	
4.5 Gap in Knowledge and Future Directions	
4.6 Conclusion	
REFERENCES	

List of Abbreviations

%	percent
°C	degrees Celsius
±	plus or minus standard error
ANOVA	analysis of variance
С	carbon
chl a	chlorophyll a
cm	centimetre
CO_2	carbon dioxide
DW sed	dry weight of sediment
et al.	et alia (Latin: "and others")
etc.	et cetera (Latin: "and other similar things")
F	F-value
g	gram
HCl	hydrogen chloride
m	metre
m^2	square metre
ml	millilitre
Ν	normality
nm	nanometres
р	p-value
P:A	perimeter-to-area ratio
R^2/r^2	correlation coefficient
ROMA	rapid organic matter assay
RPM	revolutions per minute
μg	microgram
μm	micrometres

List of Tables

TABLE 1 CARBON CONSUMPTION RATES RELATED TO CHLOROPHYLL A CONTENT, SEDIMENTORGANIC MATTER, AND THE PERIMETER-TO-AREA RATIO OF THE PATCHES.36

List of Figures

FIGURE 1 SNELLS BEACH, NEW ZEALAND, WITH THE STUDY LOCATION OUTLINED IN RED. AERIAL
PHOTOGRAPH OF THE SITES, TAKEN WITH A DRONE BY THRUSH ET AL. (2022) , is shown on
THE RIGHT-HAND SIDE OF THE FIGURE. SEAGRASS BEDS CAN BE IDENTIFIED BY THE DARKER \mathbf{A})
BLUE/GREEN AND B) GREY IN THE RESPECTIVE IMAGES
FIGURE 2 LOCATION OF THE TEN PILOT STUDY SITES AT SNELLS BEACH. EACH SITE HAD THREE
ROMA PLATES DEPLOYED – ONE WITHIN THE SEAGRASS, ONE ON THE EDGE, AND ONE
OUTSIDE THE SEAGRASS PATCH
FIGURE 3 THE ROMA PLATE, INCLUDING MEASUREMENTS OF WELL PLACEMENT ON THE PLATE. 21
FIGURE 4 THE STEP-BY-STEP DEPLOYMENT OF THE ROMA PLATES: A) CREATING A WEDGE IN THE
SEDIMENTS WITH THE SPADE AND INSERTING THE PLATE VERTICALLY, WITH THE BACK
AGAINST THE SPADE, B) PUSHING THE PLATE DOWN TO HAVE IT FLUSH WITH THE SEDIMENTS,
C) REPLACING THE SEDIMENTS ON TOP OF THE PLATE GENTLY TO REDUCE THE DISTURBANCE
TO THE PLATE. IMAGE AND TECHNIQUE TAKEN FROM O'MEARA ET AL., 2018
FIGURE 5 THE AVERAGE CARBON DEGRADATION RATE IN SIX PATCHES ACROSS THREE SAMPLING
LOCATIONS, INCLUDING THE OUTSIDE, EDGE, AND MIDDLE. THE DATA IS SPLIT INTO THE
SURFACE (1CM, 3CM, 5CM) AND BOTTOM (7CM, 10CM, 15CM) OF THE ROMA PLATE FOR
ANALYSIS. THE LOWER AND UPPER FENCES REPRESENT THE 25^{TH} and 75^{TH} percentiles, with
THE LINE THROUGH THE BOX AS THE MEDIAN. THE DOTS REPRESENT OUTLIERS IN THE DATA. 24
FIGURE 6 THE SIX PATCHES SAMPLED, CIRCLED IN BLACK, WITH THEIR CORRESPONDING NUMBER
AT SNELLS BEACH, NEW ZEALAND
FIGURE 7 CONFIGURATION OF ROMA PLATES AT EACH SAMPLING LOCATION WITHIN/AROUND A
SEAGRASS PATCH. SEDIMENT SAMPLES WERE TAKEN FROM THE MIDDLE OF THE
CONFIGURATION
FIGURE 8 A) THE AREA AND PERIMETER B) USED TO CALCULATE THE PERIMETER-TO-AREA RATIO
OF EACH PATCH SAMPLED, PHOTOGRAPHED IN FIGURE 6
FIGURE 9 THE AVERAGE DEGRADATION RATE ACROSS ALL SAMPLING LOCATIONS IN THE A)
SURFACE AND B) DEEP LAYER, INCLUDING AND EXCLUDING MACROFAUNA FROM THE ROMA
plate. The lower and upper fences represent the 25^{th} and 75^{th} percentiles, with
THE LINE THROUGH THE BOX AS THE MEDIAN. THE DOTS REPRESENT OUTLIERS IN THE DATA. 30

FIGURE 10 THE AVERAGE CARBON DEGRADATION PER METRE PER DAY ACROSS ALL PATCH
LOCATIONS IN SIX PATCHES IN THE SURFACE LAYER OF THE SEDIMENT WHEN A) MACROFAUNA
IS INCLUDED AND B) EXCLUDED
FIGURE 11 THE AVERAGE CARBON DEGRADATION PER METRE PER DAY ACROSS ALL PATCH
LOCATIONS IN SIX PATCHES IN THE DEEP LAYER OF THE SEDIMENT WHEN A) MACROFAUNA IS
INCLUDED AND B) EXCLUDED
FIGURE 12 THE R^2 value of carbon consumption and location relative to patch among
SIX PATCHES OF VARYING PERIMETER-TO-AREA RATIOS IN THE A) SURFACE AND B) BOTTOM
LAYER OF SEDIMENTS
FIGURE 13 THE CHLOROPHYLL A CONTENT IN THE SEDIMENTS ACROSS THE FOUR SAMPLING
LOCATIONS IN SIX PATCHES OF DIFFERENT P:A RATIOS
FIGURE 14 THE AVERAGE AMOUNT OF CHLOROPHYLL A IN THE SEDIMENTS PLOTTED AGAINST THE
A) AVERAGE CARBON CONSUMPTION AND B) PERIMETER-TO-AREA RATIO
FIGURE 15 PERCENT ORGANIC MATTER IN THE SEDIMENTS ACROSS FOUR SAMPLING LOCATIONS IN
SIX PATCHES OF VARYING P:A RATIOS
FIGURE 16 THE AVERAGE PERCENTAGE OF ORGANIC MATTER IN THE SEDIMENTS PLOTTED AGAINST
A) THE AVERAGE CARBON CONSUMPTION AND B) THE PERIMETER-TO-AREA RATIO OF THE
PATCHES
FIGURE 17 PERCENT OF CARBON WITHIN THE SEDIMENTS IN ALL SIX AT 2CM, 5CM, AND 8CM
BELOW THE SURFACE ACROSS ALL SAMPLING LOCATIONS
FIGURE 18 THE AVERAGE CARBON STORAGE IN THE TOP 9CM OF SEDIMENTS (BLUE) AND THE
AVERAGE CARBON CONSUMED BY THE ENVIRONMENT WHEN MACROFAUNAL ACTIVITY WAS
EXCLUDED (RED) IN ALL SIX PATCHES ACROSS ALL SAMPLING LOCATIONS

1 Introduction

Seagrass beds are an important part of many coastal environments as they are recognized for their high primary productivity (Terrados & Borum, 2004). Although seagrasses have low taxonomic variation, they have a wide distribution, covering all coastal regions except polar ones (Orth et al., 2006). Seagrasses enhance water quality by stabilizing sediments and accumulating essential nutrients (Greiner et al., 2013). Seagrass beds also provide several ecosystem services, including carbon sequestration, flood protection, and food provisioning through fisheries (Nordlund et al., 2016). Their wide distribution suggests that many coastal communities rely on seagrass meadows for their quality of life through the ecosystem services they provide (Unsworth & Cullen, 2010). Of major significance to the climate crisis, humans can benefit from the capability of seagrass to sequester carbon, which may help to reduce human carbon emissions in the atmosphere.

Since the UN Climate Change Conference in 2015 led to the legally binding Paris Agreement (Paris Agreement to the United Nations Framework Convention on Climate Change, 2015), there has been an urgency to decrease the amount of atmospheric carbon dioxide to mitigate climate change (Huang & Zhai, 2021). Seagrass has recently been acknowledged as a key contributor to achieving this goal because, similarly to trees, the plant consumes and sequesters carbon. However, seagrass beds are estimated to store up to 83,000 metric tons of carbon per square kilometre in the sediments beneath them, nearly triple that of forests (Fourqurean et al., 2012). Their ability to store carbon has ranked them as one of nature's most efficient natural carbon sinks, and therefore, they are instrumental in mitigating the effects of climate change (Serrano et al., 2021).

The importance of seagrass beds in the face of climate change has been well established (Gao et al., 2022; Stankovic et al., 2021). Through photosynthetic processes, seagrass fixes carbon, lending itself to play a significant role in the global regulation of carbon (Terrados & Borum, 2004). Seagrass is a disproportionately large carbon store, responsible for 12% of the carbon stored in ocean sediments while only making up 0.1% of the ocean floor (Unsworth et al., 2019a). Seagrass is known for fixating more carbon than necessary for its metabolic needs, resulting in excess carbon being translocated from its leaves to its roots and rhizomes (Mateo et

al., 2006). This is where seagrass stores the carbon and slowly releases it into the surrounding soils. Research suggests that as much as 17% of carbon fixated by seagrass leaves is exuded into the sediments within six hours (Moriarty et al., 1986). It is in the sediment where the carbon is accumulated and stored for long periods. This carbon is referred to as "blue carbon" (Singh et al., 2022).

Blue carbon is a term used to differentiate marine carbon stores from terrestrial ones, as they have many different characteristics. Since marine sediments are mainly anoxic and accumulate continuously, carbon can be stored for hundreds of years (Greiner et al., 2013). The canopy of seagrass leaves reduces the ocean wave energy, causing sediments and organic matter to accumulate and settle on the ocean floor (Agawin & Duarte, 2002). It has been proven that seagrass species with thicker canopies are likely to be more efficient in trapping sediments and, thus, storing carbon at higher rates (Prentice et al., 2020). Once settled, the roots and rhizomes of the seagrass provide stability, preventing the sediments from getting stirred up. The stability provided by the roots and rhizomes ensures the carbon is not recirculated into the water column and kept in anoxic sediment, inhibiting microbes from decomposing organic carbon into carbon dioxide (Kim et al., 2022). This is unlike terrestrial environments, which often lose carbon stocks to the atmosphere through decomposition or disturbances, such as wildfires (Greiner et al., 2013). Seagrass meadows' complex structure supports the accumulation of carbon from other associated photosynthetic organisms and macrofauna that die and are buried in anoxic sediment. Here, decomposition is slow, further contributing to the blue carbon stores associated with seagrass.

Seagrass beds also support a wide range of living fauna that contribute to the beds' carbon dynamics through respiration. The complexity of the seagrass structure makes it an ideal location to seek refuge from harsh conditions and predators, lending itself to a high abundance of organisms (Boström et al., 2006). Crustaceans, echinoderms, and molluscs are often found in seagrass meadows and increase in abundance the further into the bed they get (Vonk et al., 2010). Although seagrass provides refuge for many, it is not conducive for bioturbating species as the rhizomes can present difficulties for them (Valentine et al., 1994). However, bioturbators can be found in high abundance outside of seagrass patches, resulting in a different community

composition in the unvegetated sediment (Githaigia et al., 2019). The communities are often similarly abundant but have a different composition of fauna due to the species' needs and preferences from its environment (Bowden et al., 2001). The variation in species composition leads to differences in the carbon dynamics found inside and out of seagrass beds. While respiration occurring at different rates among species can lead to changes in carbon dynamics, bioturbators also negatively impact the carbon stored when burrowing as they remineralize the blue carbon (Thomson et al., 2019).

Blue carbon stores are not just impacted by biological and physical processes but also by the landscape configuration of seagrass patches. As landscape ecology becomes better understood, we realize the implications of changes in landscape architecture as important ecosystems are losing their coverage. Seagrass has been an essential environment for understanding landscape ecology due to its natural patchiness over various spatial scales (Robbins & Bell, 1994). Habitat fragmentation is becoming increasingly common in seagrass beds as coverage is lost through the direct effects of coastal development and the indirect impacts of decreasing water quality (Waycott et al., 2009). As seagrass coverage is lost, blue carbon stores are released into the ambient water, causing a flux in organic carbon. The amount of carbon lost is correlated to the type of disturbance, with loss due to turbidity releasing the least and loss due to thermal stress being the most (Thorhaug et al., 2017). Habitat fragmentation will result in smaller, more isolated seagrass patches (Hovel & Lipcius, 2001). This impacts the macrofauna that lives within them and the ecosystem services that seagrass provides. Fragmented beds could also affect carbon dynamics and blue carbon storage as small, fragmented meadows cannot trap sediments as effectively as large, continuous ones (Simpson et al., 2022). Fragmentation has also changed the landscape of seagrass beds by decreasing the density of their canopies (El Allaoui et al., 2016). This has implications for seagrass as canopy densities are linked proportionally to suspended sediments and their contribution to blue carbon stores (Barcelona et al., 2023). An increase in gaps within seagrass beds will increase the edge effect present in patches.

Edge effects are characterized by changes in environmental factors at the interface of two or more habitats, such as a seagrass patch and bare sediments (Laurance & Yensen, 1991). As habitats become more fragmented, there is an increase in the proportion of the seagrass biomass

that is on the sand-to-seagrass interface. This concept has been studied extensively in the terrestrial environment. Researchers suggest it may benefit plants as it increases the amount of sunlight available and, consequently, the amount of carbon sequestered (Montoya et al., 2010). Similarly, there is ample research on the edge effect that macrofauna, such as fish and crabs, experience due to seagrass fragmentation (Smith et al., 2011; Yarnall et al., 2022). However, the relationship between seagrass landscapes and blue carbon storage has only been explored briefly, suggesting that the distance to the edge of the patch is an important determinant of carbon storage, with the innermost position recording the highest carbon stock (Ricart et al., 2015). For this reason, scientists have suggested that the proximity to the edge, rather than the age of the seagrass patch, is more telling in how much carbon will be stored in the sediments below (Oreska et al., 2017). Still, there is limited information regarding how patch size impacts blue carbon dynamics and storage. From a conservation point of view, it is essential to know how edge effects and patch size impact blue carbon to determine how to protect and restore these environments best. From an ecosystem service point of view, if this relationship is quantified, there will be a better understanding of the overall impact seagrass has on our environment.

For my study, I focus on *Zostera muelleri*, the only seagrass species endemic to New Zealand. It has very small leaves compared to other tropical species, ranging from 5-30cm in length and 0.1-0.4cm in width (Matheson et al., 2009). It is olive green to brown in colour and can be challenging to spot at low tide as it lays flat across the sediment (Matheson et al., 2009). They help support many species, including small cockles, bivalves, gastropods, small crustaceans, and polychaetes (Inglis, 2003). *Zostera muelleri* is predominately found in mid to low tidal levels on intertidal flats in estuaries and bays but can also be located on intertidal rocky shores (Jones et al., 2008). Previously, *Zostera muelleri* was found in high densities in the subtidal zone, but their presence here has been largely lost (Ha et al., 2021). This is likely due to human impacts, as dense subtidal meadows of *Zostera muelleri* can be found in pristine waters around New Zealand (Schwarz et al., 2006).

Over the years, the distribution of *Zostera muelleri* has changed drastically. There are reports of seagrass loss averaging a 50% coverage decline in several locations across New Zealand in recent years (Matheson et al., 2011; Ha et al., 2021; Zabarte-Maeztu et al., 2020; Inglis, 2003).

There are many causes for the loss of seagrass, including human effects through pollution (sediments, agricultural run-off, etc.), physical damage (Zabarte-Maeztu et al., 2021b), and native species such as the black swan (Dos Santos et al., 2012). However, in New Zealand, there is strong evidence to suggest that an increase in turbidity has been a significant threat to seagrass, and their loss has destabilized the sediments, resulting in the resuspension of fine sediments, causing a further reduction in water clarity (Lundquist et al., 2018; Matheson & Wadhwa, 2012; Park, 1999; Turner & Schwarz, 2006). With reduced water clarity, seagrass tends to have a lower nutrient removal capacity due to a reduced rate of photosynthesis (Bulmer et al., 2018). Subtidal zones, in which seagrass resides, are particularly threatened as the input of organic or inorganic matter through run-off contributes to the intensity of turbidity (Lloyd et al., 1987; May et al., 2003). This creates a negative feedback loop as higher turbidity threatens seagrass. Sediment stability achieved by the roots and rhizomes system is lost with decreasing seagrass coverage, leading to sediments being resuspended more easily, increasing the water column turbidity (Turner & Schwarz, 2006). Unfortunately, turbidity is not the only stressor that seagrass faces.

Sedimentation and eutrophication have been described as the most common cause of diminishing seagrass cover globally (Grech et al., 2012). Sedimentation rates have increased worldwide as coastal developments, deforestation, and other activities contribute significantly (Walling, 2006). Seagrass leaves can become smothered in sediments with sedimentation, inhibiting photosynthesis, as light is unavailable (Turner & Schwarz, 2006). Although Zostera meadows only suffer significantly when 50% of the plant's height is buried under sediment for three or more weeks, it is still a real threat that must be accounted for (Vermaat et al., 1998). Furthermore, sedimentation can negatively impact the porosity and permeability of the rhizosphere. This, partnered with eutrophication, can reduce oxygen availability, causing metabolic problems for aerobic microbes (Zabarte-Maeztu et al., 2021a). Eutrophication occurs through urban, industrial, and agricultural runoff, adding excess nutrients to the oceans. Eutrophication, often through excess nitrogen, stimulates algal growth, reducing light availability for seagrass, which is when it threatens coverage (Christianen et al., 2012). Although there have been areas in the Baltic Sea where Zostera meadows can thrive despite highly eutrophic waters, eutrophication and, consequently, algal blooms generally occur on a landscape scale, causing mass mortality. The meadows of Zostera in the Baltic Sea can withstand burial under sand,

which reduces light availability, having the same impact as eutrophication. The burial does not affect the seagrass in the Northern Baltic Sea's structure or functional community (Herkül & Kotta, 2009). *Zostera* is responsible for 12% of oxygen fluxes in the sediment (Frederiksen & Glud, 2006), which is thought to assist it in dealing with light limitations (Herkül & Kotta, 2009). Similar reasonings may help explain why this seagrass can withstand eutrophication. However, this has only been recorded in the Northern Baltic Sea, and eutrophication has a global impact on seagrass coverage (Krause-Jensen et al., 2021). Since it often occurs on a wide scale, the consequences can be catastrophic, meaning recovery without human intervention is highly unlikely (El-Hacen et al., 2019).

Alas, seagrass research is significantly underfunded, which makes it difficult to protect. For every 250 million USD granted to coral reef research, 1 million USD is given to seagrass efforts (Unsworth et al., 2019b). As a result, 60% of coastal habitat published research is about coral reefs, whereas salt marshes, mangroves, and seagrass meadows account for only 14% (Duarte et al., 2008). This creates a gap in the scientific knowledge, creating implications for conserving seagrass and its ecosystem services. In addition, the lack of funding has led to seagrass species often having their extinction risk classified wrong (Short et al., 2011). Due to their vast coverage, it can be difficult to map and quantify seagrass coverage properly without the appropriate funding. Whether improper status classifications lead us to believe there is more or less seagrass, such categorizations will divest efforts from the species that require it most. With increased public understanding, many of these issues could be solved, resulting in more effective management and conservation of these ecologically important ecosystems.

In New Zealand, there have been attempts to replant *Zostera muelleri* in the Manukau Harbour, where it previously existed. However, due to autumn storms, six months later, plant loss occurred (Turner, 1995). This emphasizes the importance of site selection and protection from wave action. New Zealand has been identified as a prime location for seagrass restoration as it can be partnered with existing shellfish restoration projects, providing a holistic approach to conservation (Tan et al., 2020). Oyster reefs have also proven to be ideal for seagrass restoration, specifically in the Northern Gulf of Mexico, by protecting wave energy and increasing water clarity and sedimentation (Sharma et al., 2016). Whether protection methods are natural, such as

shellfish or oyster reefs, or synthetic stabilizing devices, like seagrass mats, it is important to provide a safeguard for newly transplanted seagrass to ensure the long-term success of restoration projects (Matheson et al., 2017).

Despite the loss of seagrass in some areas, *Zostera muelleri* has been found in other regions where it hasn't been previously, without human intervention. In recent years, it has been inundating Snells Beach, now making up 0.58 km² (~40%) of the total intertidal area (Chiaroni et al., 2008). Through restoration projects and natural distributional changes, such as what has been seen at Snells Beach, seagrass densities and distributions are changing worldwide. As seagrass cover changes, it reassures the importance of understanding carbon dynamics across different landscapes.

While many studies dive deep into how much blue carbon is stored as a result of seagrass, there are limited comparisons made to the surrounding patch boundary sediments or the spatial configuration of the patch and how this relates to the consumption rates (Kennedy et al., 2010; Miyajima & Hamaguchi, 2019). Quantifying the relationship between patch configuration and carbon dynamics can support conservation and restoration, as we can understand how to get the best environmental benefit for the lowest cost, further guiding efforts. Previous studies often overlook the surrounding sediments when quantifying seagrass' blue carbon (Lavery et al., 2013; Mateo et al., 2006). Without knowing how the carbon stocks in the seagrass patches compare to those outside the patches, accurate predictions cannot be made regarding the seagrass' contribution to blue carbon. Failure to analyze the surrounding sediment stocks can limit confidence in the effectiveness of seagrass patches.

Here, I measure the carbon consumption and stores of *Zostera muelleri* to determine how patch size affects the edge effect of carbon dynamics. A study by Ricart et al. (2017) examines how the landscape configuration of *Posidonia oceanica* affects carbon density and sources, suggesting that continuous meadows have more carbon per unit area. Like Ricart et al. (2017), many studies refrain from assessing carbon stocks outside the seagrass patch, which I aim to quantify while analyzing the edge effect. The concept of edge effects describes that the higher the perimeter-to-area ratio and the smaller the patch is, the more likely an edge effect will occur (Fonseca, 2008).

For this, I hypothesize a similar relationship will occur, where larger seagrass patches consume and sequester carbon faster than smaller patches, and the edge effect experienced will be proportionate to the patch size and shape as the middle and edge become less similar.

2 Methods

2.1 Study Site

This study was conducted at Snell's Beach (36.4205° S, 174.7240° E), north of Auckland, New Zealand. The beach has a tidal elevation ranging from 0.4m to 3.6m and has a mud content of 29-46% (Wei et al., 2022). My study site is located on the northern tip of Snells Beach (Figure 1). The site is beside a large and vegetated cliff with few rocks. This is a popular location for beachgoers, dog walkers, and people partaking in exercise. The seagrass beds are relatively close to shore, the closest being 83m from shore and 34m from the cliff. Aerial photographs taken by Thrush et al. (2022) confirmed that this northern part of the intertidal flat at Snells Beach has the most variation among patches with varying sizes and shapes. Photographs were taken by a drone from a 3m altitude in 1 m-by-1 m quadrats across Snells Beach and compiled into one image (Figure 1b; Thrush et al., 2022).



Figure 1 Snells Beach, New Zealand, with the study location outlined in red. Aerial photograph of the sites, taken with a drone by Thrush et al. (2022), is shown on the right-hand side of the figure. Seagrass beds can be identified by the darker **a**) blue/green and **b**) grey in the respective images.

2.2 Patch Size

Drone imaging was used to determine the size of each patch. A scale bar was identified, and the perimeter and area of each patch were outlined and quantified using the ImageJ software (Rasband, 2022). The perimeter-to-area ratio was calculated for each patch using the data from

ImageJ. Patches chosen for this study included those shown in Figure 6 and had a perimeter-toarea ratio ranging from 0.06 to 0.54.

2.3 Pilot Study

In May 2023, a pilot study was completed to test if there was a detectable edge effect in carbon consumption associated with seagrass. The results from the pilot study were used to inform the experimental design of the full study. The ten sites in the pilot study were allocated across the seagrass patches at Snells Beach (Figure 2), considering patches of different sizes and shapes. At each site, I positioned sample locations both within the middle of the patch (>1m away from sand) and at the edge of the patch (0.5m from the sand-seagrass interface). I also identified control sampling locations in bare sediments at least 1m from any seagrass.



Figure 2 Location of the ten pilot study sites at Snells Beach. Each site had three ROMA plates deployed – one within the seagrass, one on the edge, and one outside the seagrass patch.

2.3.1 ROMA Plate Preparation

ROMA (rapid organic matter assay) plates described by O'Meara et al. (2018) were used as a rapid assessment method to assess total carbon consumption rates. ROMA plates are 18cm by 9cm rectangular acrylic plates. They have three columns, 3cm apart, with five wells each, 1cm, 3cm, 5cm, 7cm, 10cm, and 15cm from the top of the plate (Figure 3). To prepare the mixture, 10g of agar powder, 13.32g of ground bran flake powder, and 21.66g of cellulose (90µm) fine powder were added to 1000ml of deionized water in a beaker. This mixture was heated on a

hotplate until it was boiling for 15 minutes. At this time, the mixture was removed from heat until it cooled to 42 degrees Celsius. The mixture was then pipetted into the 0.9ml wells without air bubbles in two layers. The wells were overfilled to reduce the risk of shrinkage. Once the mixture had cooled and solidified in the wells, the plates were wrapped in five completely soaked paper towels and placed in a chilly bin to store them overnight in a humid environment. This is important to ensure shrinkage does not occur. Three hours before deployment, a sharp blade was used to cut the excess off the plates, making the mixture flush with the surface. They were then rewrapped and stored in the chilly bin to transport them easily.



Figure 3 The ROMA plate, including measurements of well placement on the plate. For the pilot study, 30 ROMA plates were used. The ROMA plates were deployed across ten study sites for the pilot study (Figure 2). Each site included three sampling locations where one ROMA plate was deployed – one within the seagrass, one on the edge, and one outside the seagrass patch.

2.3.2 Deployment

A flat shovel was pressed into the sediment perpendicularly and pushed forward, exposing a smooth surface of sediments, and the plate was pushed in with the wells facing the sediment. The plate was pushed down until levelled, the shovel was removed, and sediments were put back in place (Figure 4).



Figure 4 The step-by-step deployment of the ROMA plates: **a**) creating a wedge in the sediments with the spade and inserting the plate vertically, with the back against the spade, **b**) pushing the plate down to have it flush with the sediments, **c**) replacing the sediments on top of the plate gently to reduce the disturbance to the plate. Image and technique taken from O'Meara et al., 2018.

The ROMA plates were collected 12 days after deployment and rinsed of sediment before the analysis was completed. The plates were stored in a chilly bin with a layer of water on the bottom, while each plate was wrapped up in damp paper towels to ensure minimal shrinkage of agar before the carbon consumption analysis was complete.

2.3.3 Data Analysis

The statistical program RStudio was used for all analyses of the data. A three-way ANOVA and post-hoc multiple comparison tests were performed to determine if there were any differences in the carbon consumption rates between patch sites, sampling locations, and depths. Linear

regressions were used to determine differences in the carbon consumption rates at the different sampling locations across the depth layers. All models were tested for linearity, normality of errors (Q-Q plot), and autocorrelation (Durbin-Watson test).

2.3.4 Results of the Pilot Study

The carbon consumption across three sampling locations was analyzed for the pilot study. Carbon consumption varied with depth (p < 0.05; Figure 5); however, post hoc testing revealed that the differences in depth occurred only between the surface layers (<5cm depth) and the deeper layers (>7cm depth). Therefore, subsequent data analysis was performed with two depth groups: surface (1-5cm) and deep (7-15cm)

The pilot study aimed to determine if an edge effect was apparent in the carbon consumption for seagrass patches to inform the sampling locations for the main study. The results suggest no statistically significant difference in carbon consumption amongst patch locations ($r^2 = 0.012$, $F_{(2,171)} = 2.011$, p = 0.137; Figure 5). However, more variability was observed in the surface layer than in the deep ($F_{(1,172)} = 22.959$, p < 0.001), which increases from outside the patch to the middle ($F_{(2,172)} = 4.42$, p = 0.013; Figure 7).



Figure 5 The average carbon degradation rate in six patches across three sampling locations, including the outside, edge, and middle. The data is split into the surface (1cm, 3cm, 5cm) and bottom (7cm, 10cm, 15cm) of the ROMA plate for analysis. The lower and upper fences represent the 25th and 75th percentiles, with the line through the box as the median. The dots represent outliers in the data.

2.4 Main Study

In June 2023, six seagrass patches of varying sizes and shapes were characterized and chosen at Snell's Beach for the full study (Figure 6). The results of the pilot study led to another sampling location being added to the experimental design to better capture variability around the edge in the main study. The new sampling location is referred to as the outer edge and was 25cm away from any seagrass patch. Additionally, a fine 64µm nylon mesh was cut and glued on top of the right row of each ROMA plate to exclude the effect of macrofauna and meiofauna and test whether the macrofauna and meiofauna were responsible for the variability amongst depths and sampling locations. Seventy-two plates were used this time, and to fill the plates, the agar solution measurements used in the pilot study were multiplied by three. Unlike the pilot study, where there was one plate per sampling location, the plates were placed in sets of three at each location to increase the replicates (Figure 7). This portion of the study also included the outer edge sampling location, equating to four sampling locations at each patch.



Figure 6 The six patches sampled, circled in black, with their corresponding number at Snells Beach, New Zealand.



Figure 7 Configuration of ROMA plates at each sampling location within/around a seagrass patch. Sediment samples were taken from the middle of the configuration.

The ROMA plates were collected 12 days later, cleaned of sediment, and analyzed for carbon consumption within the next 24 hours. ROMA plates were stored in a wet, humid environment overnight to ensure minimal shrinkage of the agar solution.

2.4.1 Sampling Processes

2.4.1.1 Carbon Stocks

To determine the carbon and nitrogen profiles, sediment cores (2cm diameter, 9cm deep) were collected from the sediment at the centre of the three ROMA plate configurations at each sampling location (Figure 7). Each core was sliced into the top 2cm to capture the oxic layer, 4-5

cm middle section, and 7-9cm to analyze the sediments below the roots. The sediments were stored at -80°C to preserve the samples.

2.4.1.2 Sediment Characteristics

At each sampling location, sediment cores, 2cm deep, were taken from the centre of the ROMA plate configurations using a 2cm diameter syringe core to determine the sediment chlorophyll a and organic content (Figure 7). Three sediment cores were pooled at each sampling location, placed into a labelled bag, and on ice. Once back at the lab, the sediment samples were frozen at -80° C.

2.4.2 Lab Analysis

2.4.2.1 Carbon Consumption

To quantify the carbon consumed during the deployment, the volume change of the mixture added to the wells in the ROMA plate was measured by using a 1ml syringe filled to the 0.9ml mark with distilled water to fill the well until it was flushed again with the surface of the plate. The volume of water remaining in the syringe was equivalent to the volume of agar in ml. The volume remaining was used to calculate the carbon consumption by using the following equation, developed by (O'Meara et al., 2018):

$$carbon \ consumed = \frac{(0.9ml \ agar - V_F) \left(\frac{0.026g \ C}{0.9ml \ agar}\right)}{12 \ days}$$

Where V_F is the volume of agar remaining in ml.

2.4.2.2 Carbon Storage

Samples were thawed overnight to prepare them to be processed. Once samples were thawed, the sediments were homogenized, and approximately one tablespoon of sediment was put into small tinfoil dishes. The sediment samples were then placed into the oven at 60°C and left to dry for three days. To determine the total nitrogen and carbon in each sample, 30mg of sediment was

weighed into a tinfoil boat and placed into the corresponding well of the metal plate. The samples were then run in Elementar's TOC cube to determine the necessary parameters.

2.4.2.3 Chlorophyll a

Sediments were homogenized and placed into a freeze drier for two days after being in the -80°C freezer for 24 hours. Approximately 1g of sediments was weighed and placed into 15ml falcon tubes wrapped in tin foil in a dark room. Then, 3 ml of 90% aqueous acetone was added to all samples before being vortexed for 30 seconds. Once all tubes were prepared, they were placed in a 4°C fridge for 24 hours. The next day, the samples were centrifuged at 3,500 RPM for three minutes before 1.8ml of supernatant from the tube was extracted into a 1-cm cuvette. The optical density for each sample was read at 664nm and 750nm. After, one drop of 0.1ml of 0.1N HCl was added and agitated into the cuvette. The sample was read at 665nm and 750nm 90 seconds after acidification.

The following equation from Lorenzen (1967) was used to determine the chlorophyll a content:

chlorophyll a content (µg Chl a g – 1 DW sed) =
$$\frac{11 * 2.43(E_{664}b - E_{665}a) * V_e}{DW \text{ sediment } (g)}$$

Where $E_{664}b$ is the corrected absorbance at 664nm before acidification, $E_{665}b$ is the corrected absorbance at 665nm after acidification, and DW sediment is the dry weight of sediment used for the extraction.

2.4.2.4 Sediment Organic Content

The organic content of the sediment was quantified by using the percentage weight loss on ignition. Foremost, empty foil dishes were placed in the furnace for four hours at 450°C and then weighed to four decimal points. Approximately one teaspoon of homogenized sediment was put into the foil dish and then into the oven at 60°C until it reached a constant weight. The dishes were then placed into a desiccator to cool and then weighed. After, the dishes were placed into the furnace at 450°C and ran for four hours. The samples were moved into the desiccator to cool and were weighed one last time. The sediment organic content was calculated using the following equation from Parker (1983):

percent of sediment organic matter =
$$100\left(\frac{D-B}{D}\right)$$

where D represents the dry weight of the sediment and B is equal to the weight of the burned sediment, excluding the weight of the dish in both variables.

2.4.3 Data Analysis

The statistical program RStudio was used for all analyses of the data. A Kruskal-Wallis test was used to compare the carbon consumption rates across the four sampling locations, as the data failed to pass the ANOVA assumptions. Furthermore, a simple linear regression model was used to compare each environmental factor to the sampling location. The seagrass environmental factors were chlorophyll a pigments, sediment organic matter content, and the carbon stored at depths. A multiple linear regression was conducted with carbon consumption rates as the dependent variable and environmental factors as the independent variables. All models were tested for linearity, normality of errors (Q-Q plot), and autocorrelation (Durbin-Watson test).

3 Results of Main Study

3.1 Patch Perimeter-to-Area Ratio

To assess the relationship between the perimeter and the area of seagrass patches, I plotted the two variables (Figure 8a). As the perimeter of each patch increased, so did the area, but not in a proportional manner (Figure 8a). Patch four has the largest perimeter-to-area (P:A) ratio of 0.547, whereas patch six has the smallest (0.063; Figure 8b). Patch two (0.238) and five (0.229) have a similar ratio, but every other patch differs from each other, and these two comparable patches (Figure 8b). The relationships between the perimeter and the area of the seagrass patches highlight the complexity of defining the spatial structure of patches in a simple metric (Figure 8b).



Figure 8 a) The area and perimeter b) used to calculate the perimeter-to-area ratio of each patch sampled, photographed in Figure 6.

3.2 Carbon Consumption

Based on the pilot study data analysis, the carbon consumption data was split into the surface (0-5cm) and deep (7-15cm) layers. For the main study, these layers were still significantly different (p < 0.001; Figure 9). It was found that the average carbon consumed, across all sampling locations, was similar when macrofauna was included (mean: 15.573 ± 1.413 grams of carbon/m²/day) and excluded (mean: 15.969 ± 1.024 grams of carbon/m²/day). The carbon consumption showed a strong relationship to the sampling location (p = 0.01) when the



macrofauna was included in the surface layer consumption (Figure 9a). However, this did not hold when there was no macrofaunal activity.

Figure 9 The average degradation rate across all sampling locations in the **a**) surface and **b**) deep layer, including and excluding macrofauna from the ROMA plate. The lower and upper fences represent the 25^{th} and 75^{th} percentiles, with the line through the box as the median. The dots represent outliers in the data.

3.2.1 Surface Layer Carbon Consumption by Patch Number

Scatter plots of the relationship between surface carbon consumption and sample location relative to the seagrass patch show inconsistent patterns (Figure 10). Most patches show carbon consumption increased towards the centre of the patch, although the slope varied between patches. But not in patch two, where the opposite pattern was apparent. Linear regression

revealed that these trends were significant in patches four and six when macrofauna was included (p = 0.031; p = 0.001) and excluded (p = 0.001; p = 0.049). The pattern in patch two was still significant in the surface layer despite being in the opposite direction to the other patches ($F_{1,10} = 9.411$, p = 0.012; Figure 10a). The macrofaunal activity in the wells increased the variation in carbon consumption overall by 63% from when they were excluded (Figure 10).



Figure 10 The average carbon degradation per metre per day across all patch locations in six patches in the surface layer of the sediment when **a**) macrofauna is included and **b**) excluded.

3.2.2 Deep Layer Carbon Consumption by Patch Number

The carbon consumption in the deep sediment layer has a low model fit ($r^2 = 0.032$). The trends produced in the deep layer were significant whether macrofauna was excluded (p = 0.024) or included (p = 0.036). Patch two exhibits a similar pattern to that in the surface layer, as it has a negative but significant trend line when macrofaunal activity is accounted for ($F_{1,10} = 26.71$, p = 0.0004, Figure 11a). However, it appears to level out and follow the trend when macrofauna are excluded from the wells (Figure 11b). Regardless of macrofaunal activity, all patches' carbon consumption occurring at depth remained consistent ($F_{1,142} = 3.582$, p = 0.061; Figure 11).



Figure 11 The average carbon degradation per metre per day across all patch locations in six patches in the deep layer of the sediment when **a**) macrofauna is included and **b**) excluded.

3.2.3 Relationship between Carbon Consumption and Patch Perimeter-to-Area Ratio

The relationship suggests that as the patch P:A ratio increases, so does the r^2 value, representing how well the model fits the data in the surface sediments (Figure 12a). However, this relationship is non-significant in both surface (p = 0.207; Figure 12a) and deep layers (p = 0.939; Figure 12b). It should be noted that the linear regression flattens out in the deep sediment layer, suggesting the model fits the data equally, regardless of patch ratio.



Figure 12 The r² value of carbon consumption and location relative to patch among six patches of varying perimeter-to-area ratios in the **a**) surface and **b**) bottom layer of sediments.

3.3 Relationship Between Sediment Characteristics and Carbon Consumption Rates

For the purpose of comparing all environmental characteristics, the surface carbon consumption rates when macrofaunal activity was excluded from the wells were used. These conditions resulted in a higher average r^2 value (Figure 12a), indicating improved model fit. Also, the surface carbon consumption samples were used for the comparisons as the sediment samples for the environmental characteristics as they were extracted from the same depths.

3.3.1 Chlorophyll a

Four of the six patches had a negative linear regression going from the outside to inside the patch, whereas patches two and six had the opposite pattern (Figure 12). Approximately 3.8% of the variation in chlorophyll a can be explained by the sampling location, suggesting that the sampling location does not have a large effect on the amount of chlorophyll a in the sediments ($F_{1,22} = 0.878$, p = 0.359).



Figure 13 The chlorophyll a content in the sediments across the four sampling locations in six patches of different P:A ratios.

The chlorophyll a in the sediments was plotted against the amount of carbon consumed (Figure 14a) and the perimeter-to-area ratio of the patches (Figure 14b). As the carbon consumption rates increase, the chlorophyll a in the sediments does, too. However, this relationship is statistically insignificant (H(21) = 19.6, p = 0.547; Figure 14a). A slightly less positive, and still

insignificant, relationship resulted from plotting chlorophyll a content against the patch P:A ratio (H(5) = 9.12, p = 0.1044; Figure 14b).



Figure 14 The average amount of chlorophyll a in the sediments plotted against the **a**) average carbon consumption and **b**) perimeter-to-area ratio.

3.3.2 Sediment Organic Matter

Inconsistent patterns between sediment surface organic matter concentrations and location across the seagrass patch were apparent (Figure 15). The sampling location had no direct correlation to how much organic matter was found in the sediments at any patch location (p = 0.23). Nevertheless, the linear regression results suggest that the sampling location and P:A ratio of the patch can help explain 18.78% and 19.22% of the variation, respectively, whereas the rest is unexplained (p = 0.508; Figure 15).



Figure 15 Percent organic matter in the sediments across four sampling locations in six patches of varying P:A ratios.

The trendline suggests that the percent of organic matter in the sediment increases with the amount of carbon consumed (Figure 16a). The linear regression confirms that this is not a strong relationship, and there is no direct correlation between the two variables (p = 0.428). Similarly, an insignificant relationship was found between the patch P:A ratio and the organic matter content (p = 0.674; Figure 16b).



Figure 16 The average percentage of organic matter in the sediments plotted against **a**) the average carbon consumption and **b**) the perimeter-to-area ratio of the patches.

3.3.3 Relationship Between Carbon Consumption Rate, Chlorophyll a Content, and Sediment Organic Matter Concentration

The environmental factors and carbon consumption rate data were normalized in order to compare them on the same scale. Multiple linear regression was used to identify if, in combination, the sediment parameters and patch P:A ratio influenced carbon consumption. Overall, the test reveals that the combination of these factors did not significantly influence carbon consumption ($R^2 = 0.179$, $F_{(3,20)} = 0.3401$, p = 0.257; Table 1). The model coefficients were 0.118 for chlorophyll a content, 0.306 for organic matter, and 0.161 for the perimeter-to-area ratio, indicating a positive correlation between the environmental factors and carbon consumption (Table 1). Organic matter content appears to have the largest influence on the model due to the larger coefficient, whereas chlorophyll a content has the smallest.

Table 1 Carbon	consumption rates	related to ch	ılorophyll a	content,	sediment	organic	matter,	and
the perimeter-to-	-area ratio of the p	atches.						

Partial R ²						
Variable	P:A Ratio	SOM	Chlorophyll a	Consumption	Coefficients	р
Chlorophyll a				.040	.118	0.636
SOM			.070	.132	.306	0.161
P:A Ratio		.022	.007	.061	.161	0.362
					Intercept $= .340$	
Mean	.318	.508	.331	.517	$R^2 = .179$	

3.4 Carbon Storage in Sediments

Carbon storage with depth in the sediment was highly variable across the six seagrass locations. Although there is no distinct relationship between the location and depth of carbon storage (p = 0.882), it is important to note that across most patches and sampling locations, a 5cm depth is where the most carbon can be found in the sediments (Figure 17). Carbon storage varied between patches (p = 0.033), with the two that have the highest perimeter-to-area ratios (patches two and four) storing statistically larger amounts of carbon in their sediments than the others (p = 0.016; Figure 17.2, 17.4).



Figure 17 Percent of carbon within the sediments in all six at 2cm, 5cm, and 8cm below the surface across all sampling locations.

3.4.1 Relationship Between Carbon Storage and Carbon Consumption

The purpose of determining the carbon stored at depth was to see how it relates to the carbon consumed by the sediment ecosystem. To determine the relationship between carbon storage and consumption, the average storage across all depths and the carbon consumed in the surface layer, with no macrofaunal activity in the wells, were compared. Multiple linear regression reveals that the relationship between carbon consumption rate, the carbon stored (%) in the sediments, and the sampling location is insignificant ($R^2 = 0.019$, $F_{(2, 21)} = 0.204$, p = 0.817; Figure 18). Adding patch P:A ratio to the regression model did not substantively change this relationship ($R^2 = 0.114$, $F_{(2, 21)} = 0.857$, p = 0.480).





Figure 18 The average carbon storage in the top 9cm of sediments (blue) and the average carbon consumed by the environment when macrofaunal activity was excluded (red) in all six patches across all sampling locations.

4 Discussion

My research shows there is little evidence to suggest that the patch P:A ratio impacts the carbon dynamics. The results demonstrate how carbon consumption data can vary over depth and sampling location, with more carbon consumed in the seagrass patch's surface layer. When the macrofaunal activity was accounted for, there was an increase in variation among the consumption data, regardless of the depth. Additionally, it was found that carbon is stored in sediments outside the patch and along the patch boundary in similar quantities to those stored within the patch. No relationship was found between the amount of carbon consumed and the carbon stored in the sediments. Furthermore, the surface and deep layer determined in the results were consistent with the oxic layer's depth in the sediments visually observed at Snells Beach during sampling.

4.1 Carbon Consumption Rates

4.1.1 Macrofaunal Activity

There was a large difference in the variation between consumption rates when macrofaunal activity was and was not accounted for, resulting in different trendlines when these treatments were applied. This was expected and aligns with results from O'Meara et al. (2018). The variability in carbon consumption between replicate samples was higher when macrofauna was included in the wells than when they were absent, regardless of location. The variation when macrofauna was present, irrespective of the sampling location, could indicate similar macrofaunal communities across locations. Macrofaunal communities within seagrass and adjacent sandflats can be similar in composition and abundance (Lundquist et al., 2018). A study of macrofauna in Otago found that fragmented habitats of *Zostera muelleri* had a similar abundance and diversity, regardless of small (1-5m²) or large (100-200m²) patch size. The patch location in the intertidal (i.e., high vs low) was more important than patch size, suggesting macrofaunal abundance could be similar across patches of differing sizes in a similar geographical position (Mills & Berkenbusch, 2009).

4.1.2 Across Sampling Locations

The carbon consumption rates did not vary significantly across seagrass patch sampling locations, which is likely influenced by the metabolic activity of the sediment microorganisms. Similar consumption rates, regardless of sampling location, are crucial findings in seagrass research as they show that the surrounding areas are equally important for carbon consumption. Seagrass environments are known for supporting higher microbial biomass than unvegetated sediments as a result of the increase in organic matter and habitat they provide (Jankowska et al., 2015). *Zostera muelleri* supports a wide range of microorganisms as it can provide multiple microenvironments within a patch, including leaves, shoots, rhizomes, and sediments (Ugarelli et al., 2017). A study done on *Zostera marina* in the Northern Hemisphere found more than 20,000 unique microbial taxonomic units on the surface of the seagrass, which were distinct from the microorganisms found on the roots surface and surrounding sediments (Fahimipour et al., 2017). Since the species biomass is higher in vegetated sediment, the metabolic needs of the differing microbial communities must be responsible for similar consumption rates in the vegetated and unvegetated sediment.

Microbes experience reduced carbon-use efficiency in higher temperatures, resulting in a large shift in carbon fluxes (Dijkstra et al., 2011). Dijkstra et al. (2011) examined the impact of changing temperatures from 4 to 20 degrees Celsius over two hours. They found that respiration rates increased nearly ten times when temperature increased, suggesting temperature significantly impacts metabolic processes. This is similar to temperature changes experienced in unvegetated sediments between high and low tide. In New Zealand, sediments can experience an increase in temperature by 10 degrees Celsius as the tide goes out (Mortensen & Dunphy, 2016). This temperature shift experienced in the bare sediments suggests that the microorganisms outside the patch would have a spike in metabolic needs. The carbon flux that Dijkstra et al. (2011) observed in two hours would likely occur during low tide for the microbial community in the unvegetated sediments, accounting for a larger portion of carbon consumed outside the patch. Overall, the microbes in vegetated sediments are thought to experience temperatures 4 degrees Celsius lower than those in unvegetated sediments, even at high tide, due to the shade seagrass provides (Rutherford et al., 1997). This suggests that regardless of high or low tide, microbial communities will experience a higher metabolic rate, resulting in high consumption rates, in

areas that lack seagrass. However, the combination of low tide and bare sediments should produce a high carbon flux. The increase in metabolic needs as a result of temperature fluxes explains why the consumption rates across sampling locations were similar, regardless of the middle location supporting a higher abundance of organisms and seagrass biomass.

4.1.3 Across Patches

Even though none of the patches experienced a large difference in consumption across locations, patch two experienced higher rates of carbon consumption outside of the patch than within. This patch is of the same perimeter-to-area ratio as patch five. On this basis, it is unlikely that the spatial configuration of the patch played a role in its differing consumption rates. However, the geographic location of the patch may. Patch two is the closest patch to the cliff face and coastline at Snells Beach. With the sun setting on the west coast, the cliff face provides shade in the afternoon, causing patch two to be the first patch to lose sunlight each day. In winter, patch two could receive as little as five hours of sunlight (Wellington School of Architecture, 2024). Acclimatized or shaded plants often hit their light saturation rate at lower intensities and fix carbon more efficiently than those in the sun (Reich et al., 1998; Touchette & Burkholder, 2000). Light availability is a limiting factor in most seagrass patches, which results in the translocation of oxygen from their leaves to belowground tissues, where they conduct aerobic respiration. However, once sunlight is lost, their tissues can sustain anaerobic respiration for extended periods, allowing seagrass to be efficient in carbon fixation (Touchette & Burkholder, 2000). The seagrass in patch two likely has the lowest respiration rate as it acclimates to lower light conditions. However, for photosynthesizing organisms with higher light compensation points, there is no need to have a low respiration-to-photosynthesis ratio, indicating these organisms have a lower carbon efficiency (Craine & Reich, 2005). Since the surrounding sediment is not shaded as early in the day, the organisms likely have a higher light saturation point, resulting in less efficient carbon fixation and the opposite regression seen in the results.

Furthermore, my results showed that the carbon consumption rates in the patch with the largest perimeter-to-area ratio correlated most to the sampling locations. The patch with the highest perimeter-to-area ratio consumed more carbon at the middle sampling location than the others, driving the significant trendline. Patch four (the highest P:A ratio) has the smallest area and the

lowest seagrass biomass. Although this was unexpected, this finding is consistent across previous studies. Research suggests low seagrass biomass leads to higher net community production (Egea et al., 2023). Egea et al. (2023) used patch area as a proxy for seagrass age and found that younger seagrass export significantly more dissolved organic carbon than older ones. The younger seagrass community likely has higher net production as they are trying to colonize surrounding sediments and have lower biomass of non-photosynthetic tissues (Egea et al., 2023). Previous studies suggest that carbon uptake will be the highest in patches younger than two years old and steadily decrease after that (Barrón et al., 2004). It is thought that carbon consumption rates are less correlated to patch size the larger they get, as self-shading becomes more prominent, causing a 25-35% reduction in gross primary production (Pérez & Romero, 1992).

4.2 Environmental Factors

4.2.1 Sediment Chlorophyll a Content

The chlorophyll a content negatively correlates to the amount of carbon consumed from the ROMA plate. The relationship between chlorophyll and carbon consumption has yet to be explored with the same detail as stored carbon and chlorophyll a (Anderson et al., 1981; Ganguly et al., 2017). Chlorophyll a in the sediment is commonly used as an index for microphytobenthos biomass (Kelly et al., 2001). For this reason, it was expected that chlorophyll and carbon consumption would be positively correlated because the more microphytobenthos in the sediment, the more organisms are available to consume the carbon (McAlister, 1939), yet the opposite was found in this study. In photosynthetic organisms, the ratio of chlorophyll to carbon does not need to be 1:1. However, it is widely understood that the more carbon consumed, the more chlorophyll is required to fix carbon (Ireland et al., 1984). Findings from Steele and Baird (1962) suggest that the ratio of chlorophyll to carbon can be as low as 1:213 in the winter and as high as 1:47 in the summer in photosynthetic organisms. It is thought the ratio decreases in the winter as there is limited light available, and it is at low intensities (Steele & Baird, 1962). With sampling occurring in the winter, it was expected for chlorophyll a to be low but still positively correlated. Previous research suggests microphytobenthos are regulated by ammonium rather than carbon, which may contribute to the negative relationship produced in the results (Risgaard-Petersen et al., 2005). If ammonium levels were high in the sediment, there would be less

microphytobenthos consuming the carbon available. However, since ammonium was not sampled for this study, conclusions regarding this cannot be made.

In addition, the sampling location was found to have no impact on the chlorophyll a content, suggesting no edge effect was present. Despite no statistical difference, four patches had approximately 1µg more chlorophyll a (33%) in the unvegetated sediments than in the seagrass. A study done on *Zostera marina* in Virginia, USA, found that lower aboveground biomass led to higher light availability on the seafloor, leading to an increase in microphytobenthos biomass, which is directly correlated to chlorophyll a content (Reidenbach & Timmerman, 2019). This suggests that unvegetated sediments would have the highest light available for microphytobenthos. Since seagrass patches support a higher abundance of microbes, it was thought that chlorophyll a would be highest inside the patch. However, the increased light availability may be contributing to the unexpected insignificant effect found amongst sampling locations, as more organisms are outside the patch in the winter when light intensity is low.

4.2.2 Sediment Organic Matter

The organic matter found in the sediments produced a positive regression line from outside the patch to within in all the patches but two and three. Patch number two has also deviated from the trends in carbon consumption and chlorophyll a content, suggesting something different is occurring there than any other patch. As previously mentioned, since patch two has a similar P:A ratio to patch five, it is unlikely the spatial configuration is the reason for this, but rather the proximity to the cliff face. It is thought that the sediment surrounding patch two is where eroded sediment with high organic content settles (Petsch et al., 2000). With wave energy being slowed by the seagrass, eroded sediments will likely settle close to the cliff face, leaving the outside sampling locations rich in organic content (Bradley & Houser, 2009). The difference in organic matter outside to inside of patch three was less than 0.1% and was deemed insignificant. However, it is possible that the cliff's erosion contributed to similar concentrations of organic matter as patch three is also close by.

The sediment organic matter was also positively correlated to the carbon consumption rates. There are limited studies that compare consumption rates to sediment organic matter in seagrass

environments, so it is unknown if this result is widespread. However, many studies dive into the relationship between stored carbon and organic matter, which state a similar relationship (Rahayu et al., 2019; Ricart et al., 2017). In seagrass patches, approximately 29% of the organic matter is derived from seagrass through photosynthetic processes (Rahayu et al., 2019). This would lend itself to the positive correlation seen between the two environmental factors, as carbon fixation is required for photosynthesis. Seagrass-derived organic matter in the sediments is also linked to higher organic carbon in both the seagrass shoots and surrounding sediment, implying a positive feedback loop between the consumption rates by those tissues and sediment organic matter (Papadimitriou et al., 2005).

Additionally, the sediment organic matter was positively correlated to the patch P:A ratio, suggesting that the patches with the largest ratio have higher organic matter in their sediments. It is assumed by many scientists that patches with higher perimeter-to-area ratios are younger and are trying to colonize the surrounding sediments more than the patches with a lower P:A ratio (Egea et al., 2023). It has been found that sediments enriched with organic matter alter the microbial composition and increase enzyme expression in seagrass species, thus having a positive impact on the above-ground biomass (Fraser et al., 2016). The change in biochemistry has been found to enhance root branching and sediment stability, positively impacting growth (Fraser et al., 2016). The younger seagrass may be producing more organic matter through photosynthetic processes to assist in the growth and colonization of the surrounding sediments. Thus explaining why sediment organic matter is positively correlated to the P:A ratio.

4.3 Stored Carbon in the Sediment

4.3.1 Across Sampling Locations

The carbon stored in the sediments was similar across sampling locations, regardless of the patch. This finding is consistent with research done worldwide that illustrates blue carbon stores are only slightly higher in vegetated sediment than they are in unvegetated sediment (Prentice et al., 2020). This is likely because organic carbon is exported from seagrass meadows to neighbouring, unvegetated sediments, leading to higher concentrations of stored carbon outside the patch (Kennedy et al., 2010). Additional research suggests that approximately 50% of sediment organic carbon stored in seagrass beds is from non-seagrass sources, such as algal and

phytoplankton carbon contributions, with the fraction decreasing with the distance from the patch edge (Oreska et al., 2018). This provides a substantial explanation for why the patch edge has similar amounts of carbon stored, even though it conceptually has less seagrass cover.

Currently, there are only a few unambiguous comparisons between vegetated and unvegetated areas in proximity to each other. For example, an Australian literature review analyzed the blue carbon stores in vegetated and unvegetated patches in temperate coastal environments and found that stores in vegetated sediments were significantly higher than those in unvegetated sediments (Mazarrasa et al., 2021). However, this review did not consist of patches directly beside each other, suggesting there is no overbearing effect of nearby vegetation. Seagrass beds are known to impact the carbon content of the sediments nearby by directly supplying detached plant material, resulting in carbon spillover, and by reducing water flow velocity, resulting in suspended particulate matter being deposited in adjacent, unvegetated sediment (Ricart et al., 2015). This rationalizes the importance of seagrass, not only for the blue carbon stores within the patch but outside of it as well. Mazarrasa et al. (2021) emphasize the issue of losing seagrass cover, as unvegetated sediments cannot sequester and store carbon well without the seagrass's influence. The consistency between the studies presented further highlights the importance of surrounding sediments to blue carbon estimates in seagrass environments. Unfortunately, as seagrass cover is lost, sediment carbon stocks erode, reversing the climate mitigating effect (Marbà et al., 2015).

4.3.2 Across Patches

The two seagrass patches with the highest perimeter-to-area ratio had the highest percentage of carbon in their sediments. This contradicts previous studies that state a lower perimeter-to-area ratio may indicate patch fragmentation, which corresponds to higher carbon stores (Gullström et al., 2018). However, a study analyzing habitats dominated by *Thalassodendron ciliatum* aligns with the findings presented in this research and attributes their results to the patches facing toward the open sea (Asplund et al., 2021). The explanation regarding the patch location stated by Asplund et al. (2021) is likely to fit the high sedimentary carbon stores in patch two. Patch two experiences more hydrodynamic exposure as it is closest to the cliff face. Hydrodynamics are associated with increased carbon stocks in temperate seagrass (Dahl et al., 2020). Since patches two and five are the same size, this suggests that the patch size is not responsible for the

higher proportion of carbon in the sediments. As for patch four, it has the highest perimeter-toarea ratio, leading to most of the seagrass biomass being near the edge. Researchers have proven that edge proximity strongly correlates to the amount of carbon buried (Oreska et al., 2017). With most of patch four having its biomass near the edge, this relationship can help explain the high percentage of carbon in their sediments.

4.3.3 Relationship Between Carbon Storage and Consumption

No relationship was found between storage and consumption, regardless of the patch's perimeter-to-area ratio. Depending on various factors, such as size and location in the intertidal, some seagrass patches can support more organisms than others (Boström et al., 2006; Mills & Berkenbusch, 2009). Different abundances of organisms result in carbon being consumed at different rates from patch to patch. This suggests that even if sequestration in different patches occurs at similar rates, the relationship will not be quantifiable if carbon is consumed differently. However, carbon storage also occurs through multiple processes that can result in inconsistent amounts of carbon sequestered per patch (Duarte et al., 2013). Not only does excess carbon from fixation get transported down to the roots and rhizomes for storage (Duarte & Cebrián, 1996), but the seagrass leaves trap sediments from the ambient water, accumulating more carbon, which can occur at different rates in a small geographical area (Mateo et al., 2006, Syvitski, 2003). Seagrass closer to a sediment source will experience higher sedimentation rates, trapping more carbon in their sediments. The accumulation of sediments is responsible for the high variation in carbon stored and should be accounted for when making carbon estimates (Ricart et al., 2020). Although seagrass roots are found to reduce erosion rates in sandy sediments, this effect is density-dependent (Infantes et al., 2022), and the coverage at Snells Beach is likely too low to make a difference.

4.4 Conservation and Management of Seagrass

The high variability in the carbon storage data may impact our ability to characterize seagrass's contribution to climate change mitigation accurately. Without a real pattern that explains the drivers of the heterogeneity, it is difficult to quantify how much carbon is stored for every square kilometre of seagrass coverage. Scaling up single carbon storage measurements to heterogeneous areas is fraught due to the clear heterogeneity in carbon consumption and storage measured

across areas, which will lead to calculations overestimating and underestimating seagrass's contribution. The results also have implications for seagrass conservation, as they suggest that the patch size is not directly correlated to the percentage of carbon stored beneath them. Thus, larger patches may not be the most important to protect. Unfortunately, the lack of explanation for the heterogeneity is just one of the many challenges in the management and conservation of seagrass.

Despite being a global resource, seagrass and its contribution to human well-being are not universally recognized (Cullen-Unsworth et al., 2014). Seagrass is less aesthetically pleasing than coral reefs or mangrove systems, which has major implications as the general public, politicians, and stakeholders are less concerned with protecting it (Duarte et al., 2008). Furthermore, due to its coverage in the intertidal zone, many residents see it washed up on the shore, thinking it is unattractive and strong-smelling, or feel it tangled in their feet when swimming, contributing to the negative perception of seagrass (Triviño et al., 2016). There have been reports of hotels removing seagrass from their beaches because guests do not appreciate it, which has threatened seagrass in the Maldives (Wageningen University, 2023). With increased societal recognition, seagrass restoration projects could occur more regularly, and the meadows could be properly managed with the appropriate funding.

Conservation and restoration efforts in seagrass habitats worldwide have proven successful (Van Katwijk et al., 2016). However, many of these studies lack long-term monitoring, meaning the success is inconclusive (Matheson et al., 2017). Restoration projects can be costly and timely, which is why trials happen on a small scale (Pazzaglia et al., 2021). A meta-analysis of seagrass replanting efforts suggests that the survival and population growth rate depend on the number of seeds planted. This may explain why the survival rate of seagrass seeds during replanting efforts is only 37%, as most studies occur on a small scale (Van Katwijk et al., 2016). Large-scale plantings increase the range of environmental conditions that seagrass would experience, increasing the likelihood of optimal growth conditions being found and colonization occurring more successfully (Van Katwijk et al., 2016). However, the findings presented in this study suggest that leaving unvegetated sediment around the seagrass restoration area could be beneficial. Since surrounding sediment was proven to be equally valuable to the carbon

dynamics as the vegetated areas, leaving bare sediments would help to decrease the costs and time associated with replanting, all whilst producing similar benefits. In addition to the scale of the project, scientists have advised that replanting seagrass species at former sites is likely to increase success rates. Nonetheless, it is important to reflect on the factors that caused plant loss in the first place and to understand how these sites continue to be impacted by these threats prior to replanting (Van Katwijk et al., 2009).

4.5 Gap in Knowledge and Future Directions

The findings presented in this study have implications for seagrass conservation worldwide. Carbon consumption and storage at high rates outside seagrass patches suggest that blue carbon stocks have been underestimated and should be re-evaluated. However, it is difficult to definitively quantify the distance of the ripple effect as outside samples were only taken one metre away. Future studies need to quantify the distance in which seagrass affects the carbon consumed and sequestered in the surrounding unvegetated sediment to make more definitive estimates. The relationship regarding patch size should be further evaluated to contribute to the accuracy of blue carbon assessments. The patches analyzed in this study are relatively small and similar compared to other patches worldwide (Jelbart et al., 2006). It is possible that the difference between the patches needed to be larger to impact the relationship identified, and larger patches need to be analyzed to determine this.

Furthermore, to test the generality of the results, the geographical range should be extended to analyze different species and locations. Unfortunately, many seagrass studies still occur on a small scale compared to forestry studies (Boström et al., 2006), however, a large-scale study, consisting of more replicates, would assist in making the results more definitive. Increasing the scale would also help explain the deviations in the results, but it was not possible with the time and funding restrictions for this study. Nonetheless, this research highlights the importance of seagrass and how its coverage is necessary for a healthy, long-lasting ecosystem.

4.6 Conclusion

Although the spatial configuration of the seagrass patch had no real bearing on the carbon dynamics, it is important to consider the results identified between sampling locations. Despite

no relationship being found, the results underline the complexities of carbon dynamics in seagrass patches and should promote the evaluation of both when analyzing the ecosystem services seagrass provides. This research also demonstrates how the surrounding sediments are equally important for blue carbon stores as consumption and sequestration occur in the surrounding areas at rates similar to the seagrass patch. The findings of this study have wider implications than the geographical area and biological species they were found on and should be considered regardless of the location. The surrounding unvegetated sediments should be included when evaluating seagrass's contribution to blue carbon, which has been well studied. However, it is unknown how far past the seagrass patch these benefits go, and additional research would need to be conducted before making any conclusions regarding the radius.

The complexity and heterogeneity of carbon dynamics and the spatial configuration of seagrass patches have been established through this research. Although the surrounding sediment has similar amounts of carbon buried, it is because of seagrass that sedimentation is enhanced. Without the presence of seagrass, the sediments would remain suspended and not contribute to blue carbon stores. In the face of climate change, seagrass coverage is increasingly important but is under threat due to anthropogenic effects (Waycott et al., 2009). With coastal communities being hotspots for human activities, sedimentation and eutrophication are occurring at high rates, negatively impacting global seagrass coverage (Grech et al., 2012). Although replanting efforts have been successful, they are costly and carry the risk of failure (Pazzaglia et al., 2021; Turner, 1995). While it is a great method to restore seagrass biomass, the best seagrass is that which exist naturally. Conservation efforts, including public education, increased funding, and continuous research, will help preserve this vital ecosystem. Seagrass provides fundamental fisheries habitat, microenvironments for microbes and algae, coastal protection, and more (Terrados & Borum, 2004). Although the information presented provides insight into why this invaluable ecosystem must be managed and protected for its carbon dynamics, seagrass contributes in many ways to the well-being of the environment and must be safeguarded for all its ecosystem services.

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