MACROFAUNAL BIODIVERSITY-ECOSYSTEM FUNCTIONING RELATIONSHIPS IN SEAGRASS SEDIMENTS

by

© Ty J. Colvin

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Abstract

Seagrasses create structurally complex habitat that supports diverse ecological communities and functions but face numerous threats, despite their ecological importance. I investigated how eelgrass, *Zostera marina*, habitats interact with macrofaunal biodiversity to support carbon and nutrient cycling processes. I experimentally uprooted small seagrass patches and assessed benthic flux rates, macrofaunal biodiversity, and sedimentary variables from sediment cores taken periodically from the uprooted patches, undisturbed seagrass, and unvegetated sediments. Macrofaunal abundance and diversity declined significantly in disturbed treatments, but abundances recovered over three months to resemble natural seagrass and unvegetated treatments. Benthic fluxes varied more over time than among treatments, highlighting seasonal effects on ecosystem functioning. In parallel, I assessed sediment cores from artificial seagrass patches that mimicked seagrass physical structure, natural seagrass, seagrass patch edge, unvegetated habitat, and canopy control treatments. After three months, and though lower than natural seagrass treatments, macrofaunal abundance and diversity in artificial seagrass resembled unvegetated treatments, suggesting that seagrass trophic inputs support macrofaunal biodiversity. In both experiments, minimal macrofaunal diversity effects on benthic flux rates suggest a lesser role for bioturbation in nearshore sandy sediments than in other ecosystems. My results demonstrate that natural seagrass meadows help maintain biodiversity, with potential implications for seagrass conservation.

Acknowledgements

Table of Contents

List of Tables and Appendices

Table 2.1. Biological traits and their levels used to calculate functional diversity indices. Reworking modes and movements from (Queirós et al. 2013)

Appendix 2.1. Overall Bray-Curtis dissimilarities between treatments and the main contributing species from the SIMPER analysis on fourth-rooted abundances.

Appendix 2.2. Environmental Variables measured from extra cores. MGS: mean grain size; MSS: mean sortable silt (> 10 μm, < 63 μm) size; TOC: total organic carbon; TN: total nitrogen. % Mud consists of % Silt plus the clay fraction.

Appendix 3.1. Environmental Variables measured from extra cores. MGS: mean grain size; MSS: mean sortable silt (> 10 μm, < 63 μm) size; TOC: total organic carbon; TN: total nitrogen. % Mud consists of % Silt plus the clay fraction.

List of Figures

Figure 2.1. Sites (GC – Green Crab Disturbance Experiment, Chapter 2; SB – Salton's Bay, BC – Buckley's Cove, Chapter 3) and eelgrass presence within Newman Sound. Inset shows Newman Sound location on the island of Newfoundland. Eelgrass range adapted from Warren et al. 2010.

Figure 2.2. Sediment core incubation setup. Acrylic sediment cores (diameter = 6.7 cm, length = 35.6 cm) were sealed with airtight lids fitted with sampling ports and magnetic stir bars powered by small motors. Oxygen optode patches were attached using clear silicone and covered with electrical tape to avoid light degradation. Cores were placed in chilled water baths to maintain *in-situ* temperatures.

Figure 2.3. Mean (± SE) abundances of macrofauna (individuals·m^{-2}, densities were scaled up from sediment cores 0.0035 m^{-2} in area) across treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) and the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$). Arrow points to removed disturbance outlier.

Figure 2.4. Mean (± SE) taxonomic diversity indices across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) and the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. A) Species Richness, B) Simpson's Diversity, C) Pielou's Evenness, D) Shannon Diversity. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$).

Figure 2.5. Mean (± SE) functional diversity indices across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) over the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. A) Functional Richness, B) Functional Evenness, C) Functional Dispersion, D) Functional Divergence, E) Rao's Q Diversity. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$).

Figure 2.6. Mean (± SE) oxygen and nutrient flux rates across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) over the three sampling periods (time-zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. All fluxes in mmol·m^{-2} d^{-1}, scaled up from sediment cores 0.0035 m^{-2} in area. A) Oxygen, B) Nitrate, C) Ammonium, D) Phosphate, E) Silicate. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$).

Figure 2.7. 3-Dimensional nonmetric multidimensional scaling (NMDS) plot of macrofaunal communities across the treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time (◇ – time-zero – August 20th, 2020, + – six-weeks post disturbance – October 2nd, 2020, ■ – twelve-weeks post disturbance – November 13th, 2020) from Newman Sound, based on Bray-Curtis dissimilarities.

Figure 2.8. Nonmetric multidimensional scaling (NMDS) plots of **A**) multivariate biodiversity metrics and **B**) multivariate benthic flux rates between treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time ($\diamond$ – time-zero – August 20th 2020, + – six-weeks post disturbance – October 2nd, 2020, ■ – twelve-weeks post disturbance – November 13th, 2020) from Newman sound, based on Euclidean distances between standardized variables. **A**) ellipses represent treatment 95% confidence intervals. **B**) ellipses show time 95% confidence intervals.

Figure 2.9. Redundancy analysis (RDA) of multivariate benthic flux rates as a function of A) biodiversity and B) environmental variables, across treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time ($\square$ – time-zero - August 20th, 2020, + – six-weeks post disturbance - October 2nd, 2020, $\square$ – twelve-weeks post disturbance - November 13th, 2020) from Newman Sound. Oxygen and nutrient locations represent associations with higher rates of flux. Vectors represent direction and influence of explanatory A) biodiversity and B) environmental variables. Richness: species richness; Carnivore: community weighted mean of carnivores; Infauna: community weighted mean of infauna; Sand: percent sand; Phaeo: Phaeopigment concentration ($\mu g\ g^{-1}$); MeanSortableSilt: mean size of the sortable silt fraction (phi). Some variable names have been offset slightly for clarity of presentation.

Figure 2.10. Venn diagram showing the results of the partitioning of benthic flux variation by biodiversity (0.1121) and environmental variables (0.1153), as well as the overlap variation explained by both together (0.1650).

Figure 3.1. Control (A) and artificial seagrass units (B). Plastic chicken wire (0.25 inch) was attached to square 1 m^2 PVC pipe frames. We cut round holes into the chicken wire of both treatments and covered them with chicken wire trapdoors to facilitate sediment core sampling (circled in red). We additionally tied on 75 cm long strips of green plastic ribbon (600 shoots m^{-2}) for the artificial seagrass treatments.

Figure 3.2. Mean ($\pm$ SE) abundances of macrofauna (individuals·m^{-2}, densities were scaled up from sediment cores 0.0035 m^{-2} in area) across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). Letters denote significant differences between treatments (Tukey's Test, p < 0.05).

Figure 3.3. Mean ($\pm$ SE) taxonomic diversity indices across the across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Species Richness, B) Simpson's Diversity, C) Pielou's Evenness, D) Shannon Diversity. Letters denote significant differences between treatments and ***** denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, p < 0.05).

Figure 3.4. Mean ($\pm$ SE) functional diversity indices across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Functional Richness, B) Functional Evenness, C) Functional Dispersion, D) Functional Divergence, E) Rao's Q Diversity. Letters denote significant differences between treatments and ***** denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, p < 0.05).

Figure 3.5. Mean (± SE) oxygen and nutrient flux rates across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Oxygen, B) Nitrate, C) Ammonium, D) Phosphate, E) Silicate, scaled up from sediment cores 0.0035 m^{-2} in area. Letters denote significant differences between treatments and ***** denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, $p < 0.05$)

Figure 3.6. 3-Dimensional nonmetric multidimensional scaling (NMDS) plot of macrofaunal communities among the treatments (ASU, control, edge, seagrass, unvegetated), based on Bray-Curtis dissimilarities. A) Buckley's Cove, B) Salton's Bay

Figure 3.7. Nonmetric multidimensional scaling (NMDS) plots of A) multivariate biodiversity metrics and B) multivariate benthic fluxes among treatments (ASU, control, edge, seagrass, unvegetated) and between sites (◇ - Buckley's Cove, ● - Salton's Bay), based on Euclidean distances between standardized variables. A) Ellipses show treatment 95% confidence intervals, B) Ellipses show site 95% confidence intervals.

Figure 3.8. Redundancy analysis (RDA) of multivariate benthic flux rates as a function of biodiversity, among treatments (ASU, control, edge, seagrass, unvegetated) and between sites (◇ - Buckley's Cove, ● - Salton's Bay). Oxygen and nutrient locations represent associations with higher rates of flux. Vectors represent direction and influence of explanatory biodiversity variables. Richness: species richness; Fdiv; functional divergence; DetritusFeeder: community weighted means of detritus feeders. Some variables have been offset for clarity of presentation.

Figure 3.9. Venn diagram showing the results of the partitioning of benthic flux variation by biodiversity (0.0933) and environmental variables (0.0088), as well as the overlap variation explained by both together (0.0322).

Co-authorship Statement

The research described in this thesis was designed and conceptualized by T.J. Colvin, with assistance from P.V.R. Snelgrove and R.S. Gregory. P.V.R Snelgrove, R.S. Gregory, and S.C. Dufour provided advice on thesis preparation, writing, and presentation of data. All data analyses were conducted by T.J. Colvin. Authorship of thesis chapters will evolve as the manuscripts develop, recognizing contributions appropriately.

Chapter 1: General Introduction

Globally, millions of people rely on marine ecosystems and the essential goods and services that ocean functioning provides. Ecosystem functioning refers to all of the processes that help to transfer energy and matter between ecosystem components, from primary and secondary production to carbon and nutrient cycling (Naeem 1998, Cardinale et al. 2012); ecosystem services refers to the benefits these processes provide to people (Christensen et al. 1996, Hooper et al. 2005). Productive coastal habitats support fisheries production, protect and stabilize shorelines, filter and purify water, and help regulate climate (Holmlund & Hammer 1999, Duarte 2000, Townsend et al. 2018). These goods and services have immense economic value (Costanza et al. 1997, Barbier et al. 2011), with increasing recognition of their importance with Earth's growing coastal population (Neumann et al. 2015). However, anthropogenic environmental changes already significantly impact marine ecosystems; stressors including habitat destruction, nutrient pollution, and climate change, which all greatly influence functioning (Harley et al. 2006, Hooper et al. 2012). Whereas anthropogenic stressors continue to alter ecosystem processes and functioning, accumulating evidence shows similar effects on the ecosystem services we rely upon (Worm et al. 2006).

Among these anthropogenic drivers of change, the effects of global biodiversity loss on ecosystem functioning attracts particular interest. Species extinction rates today have been estimated to be up to a thousand times higher than background extinction rates in the fossil record and show no sign of slowing, despite conservation efforts globally (Butchart et al. 2010, Pimm et al. 2014). Over the past several decades, massive declines in species richness have catalyzed scientific studies on how biodiversity loss might impact ecosystem functioning (Naeem et al. 1994, Tilman & Downing 1994, Solan et al. 2004). Initial studies manipulated community

compositions and species richness, and measured the response of single ecosystem functions (Cardinale et al. 2006). They reported generally more productive and efficient multispecies assemblages than single species assemblages; however, studies were often inconsistent and the mechanisms for these patterns were unclear (Balvanera et al. 2006). Concern over the generalizability of these experiments to natural ecosystems led to in situ studies and consideration of multifunctionality over varying spatial and temporal scales (Hector & Bagchi 2007, Snelgrove et al. 2014, Duffy et al. 2017). The prevalence of biodiversity effects on functioning in natural systems led to researchers today generally accepting that biodiversity influences ecosystem functioning, potentially by enhancing the efficiency with which communities can capture and process resources, thereby increasing their stability over time, particularly following disturbance (Stachowicz et al. 2007, Cardinale et al. 2012). Studies further suggest dependence of biodiversity-ecosystem functioning relationships on the functional traits of the species present (Covich et al. 2004, Díaz et al. 2007, Danovaro et al. 2008); through complementarity, functionally diverse communities may better partition niche space, capturing and processing more of the total available resources (Loreau & Hector 2001). Niche partitioning and efficient resource use can then lead to greater overall productivity and enhancement of the ecosystem's resource cycling capacity (Hooper et al. 2005). This concept has also led some scientists to consider identity and sampling effects, where ecosystem functions rely on key species/functional groups. Thus, diverse ecosystems promote functioning because they have a greater likelihood of containing functionally significant members (Huston 1997, Loreau & Hector 2001, Cardinale et al. 2006); current consensus suggests both diversity and species identities contribute similarly to functioning (Cardinale et al. 2011). The effects of biodiversity loss on ecosystem functioning resemble other major anthropogenic drivers of change in magnitude (Hooper et al. 2012, Tilman et al. 2012); however, these other major drivers may also impact

2

biodiversity, stressing the need to understand how the interaction of future biodiversity loss with the globally changing environment will affect ecosystem functioning.

Increasingly, studies of biodiversity-ecosystem functioning relationships in marine ecosystems have focused on benthic environments, and particularly the close associations between benthic organisms and sedimentary ecosystem processes that occur over the sediment-water interface (Snelgrove et al. 2000, Strong et al. 2015). Microbes in marine sediments play a dominant role in organic matter decomposition, driving carbon remineralization and nutrient regeneration processes (Herbert 1999, Snelgrove et al. 2018), strongly mediated by the infaunal invertebrates that live within sediments and influence microbially-mediated carbon and nutrient cycling through feeding and movement activities (Aller 1994, Snelgrove 1997, Welsh 2003). Consequently, many studies have investigated the effects of infaunal biodiversity on ecosystem functioning, particularly the roles of infaunal functional groups and diversity (Emmerson & Raffaelli 2000, Waldbusser et al. 2004, Danovaro et al. 2008).

Seagrass beds offer excellent systems for studying biodiversity-ecosystem functioning relationships (Duarte 2000), creating globally distributed and highly productive habitats that support multiple ecosystem functions (Duffy 2006, Hartog & Kuo 2007). They also create structurally complex habitats that harbour diverse ecological communities (Orth et al. 1984). In Canada, the Department of Fisheries and Oceans has designated eelgrass (*Zostera marina*) – the most widespread seagrass species – as an Ecologically Significant Species because of its role in maintaining and contributing to ecosystem function (DFO 2009, Murphy et al. 2021). Despite their ecological importance, human activity threatens seagrass habitats and contributes to seagrass decline worldwide (Short & Burdick 1996, Orth et al. 2006, Waycott et al. 2009). On the island of Newfoundland, Canada, significant eelgrass decline has resulted from the European

3

green crab (Matheson et al. 2016), a recent invader that physically disturbs eelgrass while burrowing and foraging (Davis et al. 1998, Malyshev & Quijón 2011, Garbary et al. 2014). The ecological significance and elevated vulnerability of seagrass beds underscore the need to understand seagrass biodiversity-ecosystem functioning relationships, and the potential effects of seagrass habitat destruction.

Whereas previous biodiversity-ecosystem functioning research in seagrass ecosystems has considered seagrass genetic, trophic, and landscape diversity (Duffy 2006), few studies have considered the relationships between seagrasses, macrofaunal diversity, and sedimentary ecosystem processes. In my thesis, comprised of two data chapters (Chapters 2 and 3) and an overall conclusion chapter (Chapter 4), I investigate how seagrasses support macrofaunal diversity and ecosystem functioning and assess how disturbances might affect these relationships. In Chapter 2, I investigate how physical eelgrass disturbance affects macrofaunal biodiversity and ecosystem functioning by experimentally uprooting small patches of eelgrass and monitoring changes in biodiversity and benthic flux rates. I also examine macrofaunal recovery over time following disturbance and relate changes in biodiversity and environmental variables to variation in benthic flux. In Chapter 3, I use artificial seagrass units to replicate the physical habitat created by eelgrass and compare macrofaunal diversity and benthic flux rates to natural eelgrass beds; here I seek to assess the physical and biological contributions of seagrasses to diversity and functioning. I also examine macrofaunal diversity and functioning in eelgrass patch edges to determine potential edge effects accentuated by seagrass fragmentation. In Chapter 4, I compare my findings from Chapters 2 and 3 and discuss the greater significance of my results.

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Chapter 2: The Effects of Disturbance on Macrofaunal Biodiversity-Ecosystem Functioning Relationships in Seagrass Habitats

Abstract

Seagrass beds support diverse macrofaunal communities, and collectively they influence carbon and nutrient cycles; however, we know little on how seagrass disturbance alters this relationship. In Newfoundland, Canada, the invasive European green crab *Carcinus maenas* threatens the seagrass *Zostera marina* by snipping and uprooting seagrasses while foraging and burrowing. In order to understand the effects of seagrass disturbance on macrofaunal diversity and ecosystem functioning within sediments, we experimentally uprooted small patches of seagrass and compared rates of oxygen and nutrient (nitrate, ammonium, phosphate, silicate) fluxes from sediment cores from uprooted (disturbed) patches, seagrasses, and unvegetated sediments nearby. In parallel, we assessed sedimentary macrofaunal biodiversity (taxonomic and functional) and sedimentary (granulometric properties and organic matter content/freshness) variables in all three of these treatments over a three-month period. As expected, macrofaunal abundance as well as species and functional richness declined significantly initially in disturbed cores, although this decrease had little effect on benthic flux rates. Over three months, macrofaunal colonization of the disturbed sediments resulted in abundances similar to the natural seagrass and unvegetated treatments. We also observed a change in nutrient flux rates that we attribute to seasonal shifts in regeneration pathways rather than macrofaunal community recovery, suggesting macrofaunal diversity may play a lesser role in carbon and nutrient cycling in dynamic nearshore habitats than in deeper water. Our results demonstrate the impacts of green crab mediated seagrass disturbance on macrofaunal abundance and community structure while

highlighting their potential capacity for rapid stabilization, and emphasize the overarching strength of large-scale seasonal environmental changes on ecosystem processes.

Introduction

Anthropogenic changes have resulted in significant shifts in ecosystem structure and global declines in biodiversity (Butchart et al. 2010). This biodiversity loss has led to numerous studies over the last several decades on the roles of biodiversity in supporting various ecosystem functions (Cardinale et al. 2012, Hooper et al. 2012, Strong et al. 2015) and the implications of biodiversity loss for ecosystem services (Worm et al. 2006, Paul et al. 2020). Through manipulative (Cardinale et al. 2006, Hooper et al. 2012) and observational (Plas 2019) studies across a range of ecosystems, these studies generally document positive relationships between biodiversity and ecosystem functioning, although the magnitude of these relationships depends greatly on the ecosystems and processes studied (Covich et al. 2004).

Marine scientists working in benthic environments were among the first to address relationships between biodiversity and ecosystem functioning (Duffy 2003, Solan et al. 2004), noting significant challenges in manipulating species assemblages in sediments without simultaneously altering the very ecosystem processes that benthic macrofauna potentially influence through their activities within the sediments. Infaunal feeding, movement, tube formation, and burrow irrigation all influence organic carbon distribution and sedimentary redox states (Aller 1994). Bioturbation and bioirrigation promote aerobic microbial decomposition, which drive carbon and nutrient cycles (Aller & Aller 1998, Welsh 2003, Glud 2008); indeed, previous work links macrofaunal communities to carbon mineralization and nutrient regeneration (Snelgrove 1997, Stief 2013, Snelgrove et al. 2018). The functional characteristics of infaunal organisms also play a crucial role in how they affect ecosystem processes; deposit feeders may

10

redistribute buried organic matter within oxygen-rich sediments or pump oxygen into their burrows, thereby influencing carbon cycling differently than suspension feeders that filter out organic particles drifting in the water column. Consequently, studies investigating the relationships between macrofaunal diversity and functioning report a greater influence of functional characteristics and diversity on ecosystem processes than traditional taxonomic diversity (Emmerson & Raffaelli 2000, Waldbusser et al. 2004, Danovaro et al. 2008). However, fewer studies consider how macrofaunal diversity interacts with environmental variables in regulating these processes in natural ecosystems (Godbold & Solan 2009, Spivak et al. 2009, Belley & Snelgrove 2016, Gammal et al. 2019).

Seagrass beds grow in shallow coastal waters globally and are among the most ecologically important marine habitats because of their ecosystem engineering capabilities and support of multiple ecosystem functions (Costanza et al. 1997, Orth et al. 2006). Seagrasses grow thick, above-ground canopies from dense below-ground rhizome networks, both of which provide critical habitat for many species (Laurel et al. 2003, Duffy et al. 2015). Rhizomes in particular often harbour diverse benthic macrofaunal communities (Heck & Orth 1980, Orth et al. 1984, Boström & Bonsdorff 1997). Seagrasses themselves also play important roles in carbon cycling (Duarte et al. 2005, Mcleod et al. 2011). Some species can act as significant carbon sinks (Duarte et al. 2005, Fourqurean et al. 2012) as a result of their high rates of primary production (Duarte & Chiscano 1999), resulting in much of their fixed carbon being buried or exported to the deep sea (Duarte & Krause-Jensen 2017). Seagrass canopies also attenuate water flow (Fonseca et al. 1982, Marin-Diaz et al. 2019), increasing particle sedimentation rates while decreasing resuspension (Kennedy et al. 2010). These attributes help stabilize sediments (Orth 1977), and along with direct organic inputs from rhizomes and litter from detached blades,

provide microbes with a significant source of organic matter for remineralization (Mateo et al. 2006). Increased organic carbon, as well as oxygen and nutrients exuded from their rhizomes into the sediment (Marbà et al. 2007), also contribute to nutrient regeneration. Seagrasses have a particular influence on sulfur and nitrogen cycling, in that root exudates support sulfur oxidizing and reducing microbes (Tarquinio et al. 2019, Martin et al. 2020), and stimulate high rates of ammonium production through mineralization and nitrogen fixation processes (McGlathery et al. 1998, Risgaard-Petersen et al. 1998, Welsh 2000).

Despite their importance as highly productive marine ecosystems, the coastal location of seagrass habitats results in myriad threats. Shoreline development, sediment loading, and rising ocean temperatures have contributed to worldwide declines in seagrass cover (Short & Burdick 1996, Orth et al. 2006, Unsworth et al. 2018). On the island of Newfoundland, Canada, the recent arrival of invasive European green crabs (*Carcinus maenas*, hereinafter referred to as green crab) contributes to the destruction of eelgrass (*Zostera marina*) beds. Green crab invasion on both the west (Howard et al. 2019) and east (Neckles 2015) coasts of Canada have resulted in the loss of eelgrass, the most widespread seagrass species in Canada (Murphy et al. 2021). Green crab were first discovered in Newfoundland in 2007 (McKenzie et al. 2007), where established populations on the southern coast of the island suggested colonization several years earlier (Blakeslee et al. 2010). Studies since then have attributed significant eelgrass decline to their arrival (Matheson et al. 2016), given that green crab dig and uproot eelgrass rhizomes as they burrow and forage for infaunal prey (Garbary et al. 2014) and snip eelgrass shoots (Davis et al. 1998); juveniles also graze directly on the shoot tissue (Malyshev & Quijón 2011). Large populations of foraging crabs can cause widespread habitat destruction, with serious consequences for the diverse fish (Matheson et al. 2016) and macrofaunal (Rossong 2016) communities these eelgrass habitats

support. The continuing spread of green crab across Newfoundland (Ens et al. 2022) increases the need to understand how their disruption of eelgrass habitat affects essential ecosystem functioning processes.

Because seagrasses and macrofauna both play important roles in ecosystem functioning, the destruction of seagrass habitat and alteration of their macrofaunal communities could potentially result in substantial loss of functioning. In this study, we sought to determine how disturbance of a seagrass bed not yet invaded by green crab would impact sedimentary macrofauna and macrofaunal biodiversity-ecosystem functioning relationships. To investigate how green crab invasion might impact carbon and nutrient cycling, we replicated green crab disturbance of natural seagrass beds by uprooting small patches and examining multivariate changes in oxygen, nitrate, ammonium, phosphate, and silicate fluxes across the sediment-water interface. We also examined changes in the macrofaunal community and diversity following disturbance and related these changes to variation in benthic fluxes to assess the consequences on macrofaunal biodiversity-ecosystem functioning. We also related benthic flux variation to changes in sedimentary conditions following disturbance to determine the relative influences of biodiversity and physical environment on ecosystem functioning. Finally, we assessed how biodiversity and functioning would stabilize in the months following disturbance. We hypothesized that seagrass disturbance would result in a decline in sedimentary macrofaunal biodiversity and a significant shift in sedimentary conditions, which would result in lower rates of carbon and nutrient fluxes.

Methods

Study Design and Sampling

We conducted our study in Newman Sound, a fjord in Bonavista Bay, Newfoundland, Canada (Figure 2.1) with extensive seagrass cover where green crab have not yet invaded. The selected site was located within the inner sound, where seagrass grows in dense, continuous meadows starting from ~1 m depth. Winter ice scour prevents further seagrass growth towards shore; the substrate between the seagrass bed and shore consisted primarily of medium-course grain sand. We collected sediment push cores (diameter = 6.7 cm, length = 35.6 cm) by hand from natural seagrass habitat, adjacent unvegetated habitat, and from pits created within natural seagrass designed to simulate green crab disturbance. For this latter "disturbance" treatment we uprooted seagrass by hand in small patches 0.5 m in diameter (0.196 m^2), replicating the small-scale effects of green crab burrowing and feeding in seagrass. Cores contained 10 – 15 cm of sediment and 15 – 20 cm of overlying water. For each site, we collected four replicate cores of each treatment for incubation experiments, and an additional core from each treatment for analysis of sedimentary environmental variables (36 cores in total for incubations, 9 cores for environmental variables). In order to evaluate macrofaunal community stabilization following disturbance, repeated sampling occurred over a 3-month period in 2020; immediately following disturbance on August 18th -22nd (time-zero), 6-weeks post disturbance on September 30th – October 4th, and 12-weeks post disturbance on November 11th – 15th.

Incubations

Following collection, we acclimated intact sediment cores taken at each time period for 12 – 18 hours to allow any sediment suspended during transport to settle. For the 6-week and 12-week post disturbance incubations, aquarium air pumps gently aerated near-bottom water during acclimation to avoid anoxic conditions prior to the incubation. We then incubated cores in ambient conditions for 24 hours at *in-situ* temperatures by submerging them in a cold-water bath

or placing them in a refrigerator. Incubations in complete darkness avoided any influence of seagrass photosynthesis, utilizing a green light when collecting samples during incubations, noting it is the least photosynthetically active wavelength. Cores were fully sealed with caps fitted with airtight water sampling ports and magnetic stir bars that helped to homogenize the water contained within the cores (Figure 2.2).

Nutrient and Oxygen Fluxes

In order to determine rates of nutrient flux, we collected two 50 ml water samples from each core at the beginning (T_0), midpoint (12 hours, T_{12}), and end of each incubation (24 hours, T_{24}), and replaced it with the equivalent volume of water taken from the site during core sampling. Water samples were removed through air-tight ports using acid-washed syringes and immediately frozen at -20 °C for later analysis of ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), and silicate ($Si(OH)_4$) in a Seal Analytical AAIII Segmented Flow Analyzer. We also analyzed nutrients in the replacement water taken from our site to correct for nutrient concentration changes during water replacement; measurements at T_{12} and T_{24} were adjusted to account for the nutrients removed from the cores during sampling and added during water replacement. Subsequent linear regressions of the different nutrient concentrations over time (T_0, T_{12}, T_{24}) corrected for concentrations within the replacement bottom water (nutrient concentration as a linear function of time), enabled determination of average nutrient flux rates using regression slopes. In four individual T_0 cores for which we lacked readings, we used treatment averages of initial nitrate, phosphate, and silicate values.

A PreSens Fibox 4 optical oxygen meter, in tandem with oxygen optode patches attached to the inside of each core with clear silicone, provided measurements of dissolved oxygen concentrations. Oxygen concentration measurements every four hours during the incubation

15

enabled determination of oxygen consumption rates using linear regressions, accounting for the oxygen concentration of replacement bottom water following nutrient sampling. Collectively, we refer to oxygen and nutrient flux rates as benthic flux rates.

Macrofaunal Identification and Diversity Indices

Following the 24-h incubations, we immediately sectioned the cores into 0-2 cm, 2-5 cm, and 5-10 cm layers and fixed the sections in 10% buffered formalin. Within a few weeks, samples were thoroughly rinsed over a 300-µm sieve, a mesh size chosen to collect all adult and juvenile macrofauna from within the sediments. We then transferred them into 70% ethanol for subsequent storage and identification of macrofauna to the lowest taxonomic level possible under a dissecting scope, generally to genus level. We did not retain above ground material. Using the "vegan" package within R (R Core Team 2021), we then calculated multiple species indices based on the resulting community data; species richness, Simpson's diversity, Shannon diversity, and Pielou's evenness index. To examine functional trait diversity we assigned species to five different biological traits (Table 1) following trait data compiled from the literature (Naylor & Haahtela 1966, Pavia et al. 1999, MarLIN 2006, Macdonald et al. 2010, Queirós et al. 2013, Jumars et al. 2015, Degen & Faulwetter 2019, Antczak-Orlewska et al. 2021). Fuzzy coding between 0 and 1 based on the tendency for an organism to express that particular trait level allowed species to express multiple levels of the same trait, with the total in each trait adding to 1. We then calculated functional diversity indices using the "FD" package within R (R Core Team, 2021). These indices included functional richness, functional evenness, functional divergence, functional dispersion, and Rao's quadratic entropy (Villéger et al. 2008, Laliberté & Legendre 2010). We also calculated and used the community-weighted mean values for each trait level in the analyses (Lavorel et al. 2008).

Environmental Variables

Analysis of sediment from the extra core taken for each treatment allowed us to evaluate the effects of seagrass removal on the sedimentary environment, and potential subsequent effects on the sedimentary community. For this purpose, we initially homogenized the 0-2 cm layer of the cores prior to storage in the dark at -20 °C until analysis, where we took sub-samples from this layer for separate analysis of grain size, carbon/nitrogen content, and phytopigment ratios.

We used overall mean grain size (phi) and mean of the sortable silt fraction (phi) using the Krumbein phi scale (phi = $-\log_2$(grain size in mm)), alongside percentages of gravel, sand, and mud fractions (%) to assess impacts of disturbance on physical sedimentary dynamics. Sediment grain size samples were treated with 35% hydrogen peroxide to digest any organic material, then freeze-dried for analysis. We then removed the gravel fraction (>2 mm) via sieve and weighed at ¼ phi intervals to determine the percent gravel. Analysis of the remaining sediment (<2 mm) used a Beckman Coulter LS13-320 laser diffraction analyzer to determine the percent sand (2 mm – 62.5 µm) and mud (<62.5 µm) fractions. We then determined the overall mean grain size based on all fractions and calculated the mean sortable silt (>10 µm - <62.5 µm), with higher phi values representing a higher proportion of fine silt.

Total organic carbon (TOC, mg $\cdot$g^{-1}), total nitrogen (TN, mg $\cdot$g^{-1}), chlorophyll a concentration (µg $\cdot$ g^{-1}), phaeopigment concentration (µg $\cdot$ g^{-1}), and the chlorophyll a: phaeopigment ratio enabled assessment of the impacts on organic matter freshness and accumulation over different time scales. To determine the carbon and nitrogen content of the sediment, we weighed sub-samples, dried them at 60 °C for 24 h, and then treated them with HCl fumes for 24 h to acidify and remove any inorganic carbon. Re-drying at 60 °C for another 24 h preceded transferring of 2 mg to a tin capsule and reanalysis using a Perkin-Elmer 2400 Series II

CHN analyzer for total organic carbon (TOC) and total nitrogen (TN). We could not calculate carbon: nitrogen ratios because some total nitrogen values fell below our equipment's detection limits.

We assessed the quality of organic matter over the short-term, based on phytopigment concentrations determined using a spectrophotometric assay (Danovaro 2009). Following addition of 90% acetone to weighed sediment sub-samples, we vortexed the samples for 30 seconds, sonicated them three times in an ultrasound bath in 1-minute intervals, and stored them in the dark for 24 hours at 4 ºC for pigment extraction. After centrifuging samples (800 x g, 10 mins), we measured absorbance to assess chlorophyll a concentration, and then acidified samples using 0.1N HCl prior to reanalysis to determine phaeopigment concentrations.

Statistical Analyses

In order to determine whether total macrofaunal abundance, diversity indices, and oxygen and nutrient flux rates differed among treatments and over time, we ran separate two-way ANOVAs with both "Treatment" and "Time" as fixed factors, noting independent cores that we collected and analyzed from each treatment replicate and during each time period. We assessed significant differences among treatments and over time using Tukey's tests. Q-Q plots and plots of residuals assessed assumptions of normality and homogeneous variance. Given some indication of non-normality in the residuals we applied Kruskal-Wallis tests to oxygen flux, ammonium flux, and functional richness comparisons over time, and separate Kruskal-Wallis tests for each time period separately for functional richness, using "Treatment" as a factor. We used single factor ANOVAs to assess treatment differences in oxygen and ammonium flux, given that data were normally distributed within each time period. We used Dunn's tests to assess differences among treatments and over time following Kruskal-Wallis tests, and Tukey's tests

following the one-way ANOVAs. Application of a natural logarithmic transformation to total macrofaunal abundances and species and functional richness reduced the elevated variance at higher values. We omitted an extreme outlier of macrofaunal abundance in a single time-zero green crab disturbance core that suggested an unusually dense patch of individuals more than an order of magnitude greater than any other sample, and we used a type III ANOVA in this case.

Three separate two-way permutational multivariate analyses of variance (PERMANOVA, 9999 permutations) enabled comparison of variation in macrofaunal community composition, multivariate nutrient flux rates, and biodiversity indices across treatments and time, using the "adonis2" function in R. We also compared macrofaunal community composition among treatments within each time period with single factor PERMANOVA. For community comparisons we used Bray-Curtis distances of species abundances, in contrast to Euclidean distances for standardized nutrient flux rates and biodiversity indices. We ran community and diversity analyses either including or removing species represented by single individuals and found similar results; we therefore report the analysis with rare species removed and note any differences between the two analyses. Following PERMANOVA, we verified homogeneity of dispersions using the "betadisper" function in "vegan". Non-metric multidimensional scaling (NMDS) plots visualized biodiversity patterns across treatments. Similarity percentage (SIMPER) analysis determined the species driving community differences among treatments within each time period. Given the extreme abundances of some species, we repeated SIMPER analysis on fourth-root transformed data to reduce the weighting of those species and try to elucidate community differences masked previously by the dominant species.

Two separate redundancy analyses determined the proportion of variation in rates of benthic flux explained by biodiversity indices and environmental variables, respectively, and the

explanatory variables contributing most to that variation. To avoid multicollinearity in the explanatory variables, variance inflation factor (VIF) tests removed variables with VIFs >5 stepwise. We then assessed the contributions of the remaining explanatory variables to flux variation using single variable RDAs. Furthermore, a stepwise selection process with a significance level of $p < 0.05$ determined those variables that contributed most to the explained variation. Finally, a variation partitioning analysis using both sets of explanatory variables determined the relative amount of variation in benthic fluxes explained by biodiversity indices and sedimentary variables alone, and the overlap in explained variation by both sets of variable (Legendre & Legendre 2012). We completed redundancy analyses and variation partitioning analyses in R using functions in the "vegan" package (R Core Team, 2021).

Results

Macrofaunal Abundance and Biodiversity Comparisons

Macrofaunal abundance differed significantly among treatments (Two-way ANOVA: $F_{2,26} = 22.0$, $p < 0.001$) but not over time, and we observed significant interaction between the two (Two-way ANOVA: $F_{4,26} = 4.5$, $p < 0.01$). Tukey tests to discern treatment differences indicated significantly higher macrofaunal abundance in seagrass and unvegetated treatments than in disturbance treatments at time zero (Tukey's Test: $p < 0.001$) and higher abundance in unvegetated than disturbance treatments after six weeks (Tukey's Test: $p < 0.01$, Figure 2.3). No treatments differed at twelve-weeks.

We observed a similar pattern in comparing species richness among treatments and over time, with significant treatment (Two-way ANOVA: $F_{2,27} = 67.5$, $p < 0.001$) and time differences (Two-way ANOVA: $F_{2,27} = 5.9$, $p < 0.01$); We also observed a significant interaction term (Two-

way ANOVA: $F_{4,27} = 10.7$, $p < 0.001$, Figure 2.4A). Tukey's tests showed significantly greater species richness in seagrass and unvegetated cores than disturbance cores at time zero (Tukey's Test: $p < 0.001$) and six-weeks post disturbance (Tukey's Test: $p < 0.001$). By twelve weeks, Tukey's tests did not discern differences in species richness among treatments. Significantly higher functional richness in seagrass treatments than in disturbance treatments at time zero (Dunn's Test: $p < 0.01$), contrasted no significant differences at six weeks, but significantly higher functional richness in seagrass treatments than in both disturbance treatments after twelve-weeks (Dunn's Test, $p < 0.05$, Figure 2.5A). For other comparisons of diversity, we observed significantly lower functional divergence in seagrass treatments than in unvegetated treatments at time-zero (Tukey's Test: $p < 0.01$, Figure 2.5D).

Benthic Flux Rate Comparisons

Rates of oxygen flux differed significantly over time (Kruskal-Wallis: $\chi^2_2 = 23.4$, $p < 0.001$); with Dunn's tests identifying significantly lower rates of oxygen consumption at twelve weeks than other time periods (Dunn's Test: $p < 0.001$). Analyses within each time period indicated significant differences among treatments for each time period (ANOVA: time zero: $F_{2,9} = 21.3$, $p < 0.001$, six weeks: $F_{2,9} = 8.9$, $p < 0.01$, twelve weeks: $F_{2,9,} = 12.5$, $p < 0.01$), with significantly higher oxygen consumption in seagrass treatments than in unvegetated and disturbed cores for all three time periods (Tukey's Test: $p < 0.05$), and significantly higher oxygen consumption in unvegetated treatments than disturbance treatments at time zero (Tukey's Test: $p < 0.05$), noting that non-seagrass treatments became increasingly similar over time (Figure 2.6A). When comparing nutrient fluxes, nitrate, ammonium, and phosphate differed significantly over time (Two-way ANOVA: Nitrate: $F_{2,23} = 126.9$, $p < 0.001$, Phosphate: $F_{2,23} =$

4.5, p < 0.05, Kruskal-Wallis: Ammonium: $\chi^2_2 = 26.1$, p < 0.001), but none differed among treatments.

Multivariate Community Comparisons

PERMANOVA analysis of Bray-Curtis dissimilarities showed that macrofaunal community composition differed significantly among treatments (PERMANOVA: $F_{2,27} = 6.2$, p < 0.001) and times (PERMANOVA: $F_{2,27} = 1.7$, p < 0.05), noting non-homogenous multivariate dispersion among treatments (permutation test: $F_{2,33} = 8.9$, p < 0.001). Given evidence that non-homogenous variance under balanced designs has little affect on PERMANOVA (Anderson & Walsh 2013), we accept this interpretation of the results. Separate analysis within each time period showed significant differences among communities for each time period (PERMANOVA: time-zero: $F_{2,11} = 4.6$, p < 0.001, six-weeks: $F_{2,11} = 2.9$, p < 0.001, twelve-weeks: $F_{2,11} = 1.6$, p < 0.05, Figure 2.7). Once again, we observed significantly non-homogeneous multivariate dispersion after six (permutation test: $F_{2,9} = 4.4$, p < 0.05) and twelve weeks (permutation test: $F_{2,9} = 4.8$, p < 0.05). SIMPER analysis on untransformed Bray-Curtis dissimilarities identified the abundant polychaete *Microphthalmus* sp. as the main driver of community differences between the disturbance community and the two "natural" (seagrass, unvegetated) communities at time-zero, whereas the carnivorous polychaete *Pholoe minuta* primarily differentiated seagrass and unvegetated treatments. After six and twelve weeks, *Microphthalmus* sp. contributed most strongly to community differences for all treatment contrasts, noting its high abundance in seagrass and disturbance treatments. *Pholoe minuta* and the deposit feeding polychaete *Mediomastus* sp. also contributed strongly to seagrass treatments, in contrast to the spionid polychaete *Pygospio elegans* and Unknown Naididae 2 in unvegetated cores. Following fourth-root transformation of the data, SIMPER identified several less abundant

species that characterized different treatments (Appendix 1). *Pholoe minuta* and *Mediomastus* sp.
once again contributed strongly to seagrass communities, along with the tube-dwelling amphipod
Monocorophium sp. and the small gastropod *Skeneopsis planorbis*. Lottiids, the carnivorous
nereid *Alitta succinea,* and the bivalves *Mya arenaria* and *Macoma balthica*, contributed strongly
to unvegetated communities, along with *P. elegans* and Unknown Naididae 2. *Microphthalmus*
sp. once again contributed strongly to disturbance treatments, although the fourth root
transformation removed it as the dominant driver of community differences in most cases.
Examining overall dissimilarity among treatments over time revealed increasing similarity of the
disturbance treatment to the other treatments, but especially to the seagrass treatment.

Multivariate patterns in biodiversity and benthic flux rates

Standardized biodiversity indices differed significantly both among treatments
(PERMANOVA: $F_{2,27}$ = 3.6, p < 0.001) and times (PERMANOVA: $F_{2,27}$ = 1.7, p < 0.05) when
analyzed together using PERMANOVA, noting non-homogenous multivariate dispersions across
treatments (permutation test: $F_{2,33}$ = 4.1, p < 0.05). A NMDS plot of the data separated
unvegetated treatments from seagrass and disturbance treatments (Figure 2.8A). Multivariate
benthic flux rates assessed via PERMANOVA also differed significantly differ among treatments
(PERMANOVA: $F_{2,27}$ = 2.9, p < 0.01) and times (PERMANOVA: $F_{2,27}$ = 15.0, p < 0.001), noting
homogenous multivariate dispersion across both treatment and time. A NMDS plot of core
benthic flux rates showed a distinct separation in benthic fluxes at twelve-weeks relative to time
zero and six weeks (Figure 2.8B).

Variation in Multivariate Benthic Flux explained by Biodiversity

After removing collinear variables or those with VIF values > 5, the biodiversity RDA

model explained 58.2% of the variation in benthic flux rates (adjusted $R^2 = 0.418$) and included

species richness, functional richness, functional evenness, functional divergence, and community

weighted means of carnivores, detritus feeders, suspension feeders, funnel feeders, sub-surface

deposit feeders, omnivores, up/down conveyors, biodiffusors, infauna, pelagic, and medium sized

organisms (1 – 5 cm). Species richness (14.8%), functional richness (11.2%), and community

weighted means for infauna (13.9%) and carnivores (7.6%) explained the most variation.

However, following stepwise selection, the most parsimonious set of variables chosen included

just species richness and community weighted means for infauna and carnivores, and explained

33.9% of the variation in benthic flux rates (adjusted $R^2 = 0.277$). The first RDA axis explained

18.6% of the variation in flux rates and related to all variables, whereas the second axis explained

14.4% of the variation and related mainly to species richness and community weighted means of

infauna (Figure 2.9A). When we included rare species in the analysis, functional richness

surpassed species richness as the best explanatory variable (18.6%).

Variation in Multivariate Benthic Flux explained by Environmental Factors

The initial RDA model with collinear and VIF > 5 variables removed explained 36.2% of

the variation (adjusted $R^2 = 0.280$) and included percent sand, mean sortable silt fraction,

chlorophyll a concentration, and phaeopigment concentration. Phaeopigment concentration

(17.2%) explained the most variation, whereas the explained variation from other variables fell

between 5 – 7%. Following stepwise selection of variables, the final model explained 34.2% of

the variation in benthic flux rates (adjusted $R^2 = 0.280$) and included percent sand (6.4%), mean

sortable silt (5.9%), and phaeopigment concentrations (17.2%). The first RDA axis accounted for

most of the explained variation (28.5%) and linked to all variables, whereas the second RDA axis explained just 5.2% of the variation (Figure 2.9B).

Variation Partitioning Analysis

Variation partition analysis of benthic flux rates across biodiversity indices and environmental variables revealed that both sets of explanatory variables collectively explained 49.7% of the variation (adjusted $R^2 = 0.392$, Figure 2.10). Biodiversity indices alone explained 11% of that variation whereas environmental variables accounted for 12% of the explained variation, with 17% explained by both sets of variables. Our analyses left 60.8% of the variation in benthic flux rates unexplained.

Discussion

By experimentally manipulating seagrass habitat, we determined that small-scale nearshore disturbances significantly altered macrofaunal abundance and community structure, although nutrient flux rates remained mostly unaffected, varying more with seasonal changes. This finding suggests that macrofaunal activity may play a lesser role in nutrient regeneration and carbon mineralization in dynamic nearshore habitats with coarse sandy sediments than in offshore environments characterized by finer sediments (Braeckman et al. 2014). We acknowledge, however, that we cannot conclusively draw inferences without manipulating or measuring biodiversity. By the end of the experimental period (12 weeks), we observed some recovery of the communities and abundances of disturbed patches, suggesting the potential for relatively rapid stabilization by macrofauna to the seagrass disturbance (Silberberger et al. 2016). We also found similar amounts of variation explained independently by biodiversity metrics and

environmental variables (Belley & Snelgrove 2016), emphasizing the importance of both the macrofaunal community and environmental factors for ecosystem functioning.

Macrofaunal Community in Seagrass and Unvegetated Sediments

Initially, natural seagrass and unvegetated sediments had similar macrofaunal abundances and species richness, despite differences in macrofaunal community composition. Fresh organic matter input potentially contributed to high abundances of deposit feeding polychaetes in seagrass treatments, as indicated by high chlorophyll a: phaeopigment ratios (Boon & Duineveld 1996, Morata et al. 2011). Potentially, the seagrass canopy trapped *Mytilus edulis* larvae drifting past, and provided a primary settlement surface (Bayne 1964, Newell et al. 1991) that resulted in high juvenile numbers. Abundant suspension feeders in adjacent unvegetated sediments may reflect greater flow rates and suspended particle turnover associated with skimming flow around and over the seagrass bed (Koch et al. 2006), as well as direct organic matter contributions from the seagrass bed (Duarte & Krause-Jensen 2017).

Numerous studies report higher diversity and macrofaunal abundances in seagrass beds compared to adjacent unvegetated habitats (Orth 1977, Heck & Orth 1980, Boström & Bonsdorff 1997). Seagrasses provide protection from predators (Orth et al. 1984, Reynolds et al. 2018), create complex three-dimensional habitats (Heck & Wetstone 1977, Lannin & Hovel 2011), and increase settlement of drifting organic matter and pelagic larvae (Fonseca et al. 1982, Eckman 1983, 1987). Similar numbers of species and abundances in both seagrass and unvegetated habitats in our study may result from our collecting unvegetated cores just 1-2 m away from the seagrass patch edge. However, some studies report a sharp transition in diversity and abundance over smaller spatial scales (Tanner 2005, Barnes & Hamylton 2013), and a parallel experiment

elsewhere in Newman's Sound (see Chapter 3) documented clear between-habitats differences in macrofaunal abundance at similar scales.

Macrofaunal Community Changes Following Disturbance

As expected, we observed significantly lower macrofaunal abundances, species and functional richness, and altered macrofaunal communities in disturbance treatments resulting from seagrass and sediment removal, which persisted to six weeks post disturbance. Other seagrass removal studies reported macrofaunal decline and community shifts over similarly short timescales (Connolly 1995, Eklöf et al. 2015, Githaiga et al. 2019). An abundance of small, deposit feeding polychaetes characterized the disturbed treatment community. Similar chlorophyll a: phaeopigment ratios in disturbance and unvegetated habitats and much reduced TOC levels in disturbed pits over the entire duration of the experimental period suggest that we cannot attribute this community shift towards deposit feeders actively responding to organic matter accumulation in pits, though we cannot exclude the possibility of passive accumulation of colonizers (sensu Snelgrove 1994). Alternatively, disturbance may have altered the microbial community within the seagrass bed; although our study did not measure microbial abundance, microbes provide an essential food source for deposit feeding invertebrates (Livingston 1979).

At twelve weeks post-disturbance, macrofaunal abundances increased in disturbance treatments but simultaneously decreased in natural seagrass and unvegetated habitats, resulting in similar abundances in all treatments. The decline in seagrass and unvegetated treatments likely reflected seasonal variation in macrofaunal abundances, which reach a peak in summer following their recruitment, and decline in the fall in northern latitudes (Butman 1987, Reiss & Kröncke 2005). Other studies on long-term recovery of macrofauna in disturbed seagrass beds yielded conflicting results, with some reporting reduced abundances upwards of 13 months (Githaiga et

al. 2019), whereas others reported faunal recovery within 2 (Reed & Hovel 2006) and 10 months (Silberberger et al. 2016). Seagrass removal by Githaiga et al. (2019) resulted in a shift in the functional composition of communities to large-bodied bioturbators, which likely helped maintain the disturbance community. We did not observe such a response; rather, after twelve-weeks the disturbance community resembled the natural seagrass community more closely than the unvegetated community.

Infaunal colonization occurs primarily through pelagic larval recruitment and post-larval dispersion (Levin 1984, Smith & Brumsickle 1989). Pelagic larval stages in many infaunal species can spend considerable time in the water column and disperse great distances (Levin 1984). Pelagic larval recruitment varies seasonally with infaunal reproductive events, which depend on the life history characteristics of the species present (Levin 1984); many, but not all, species reproduce over the summer months (Whitlatch 1977). Post-larval dispersion by juveniles and adults plays a particularly important role in small-scale disturbances, lessening as patch size increases because of their limited mobility (Smith & Brumsickle 1989). Given that our study took place from late August – mid November, we likely missed much of the peak summer recruitment from pelagic larvae, noting that adult stages dominated our samples. Given the shift in the disturbance community to resemble the seagrass community, post-larval horizontal dispersion from the adjacent seagrass bed likely provided the primary source of immigrating infauna. This scenario would also explain the low abundances six-week post-disturbance; if settling larvae provided the main source of colonizers then we would have likely observed higher abundances earlier in the experiment.

Along with the dramatic shifts in the macrofaunal community and abundances, parallel changes in species and functional richness followed disturbance. This result aligns with the

positive correlation between species richness and total numbers of individuals sampled (Gotelli & Colwell 2001, Bock et al. 2007, Storch et al. 2018), noting that the capacity of a habitat to support more individuals enables more species to co-exist at stable population levels (Gaston 2000). Functional richness, the multivariate functional space taken up by each community (Villéger et al. 2008), was highest in seagrass cores, followed by unvegetated cores and disturbance cores. Again, we anticipated this result given the strong positive correlation between functional richness and species richness (Villéger et al. 2008), and we typically observed the most species in seagrass cores and the fewest in disturbance cores. However, seagrass habitats supported greater functional richness than unvegetated habitats, despite harbouring similar numbers of species throughout the experiment. This pattern suggests that the beneficial habitat-forming attributes of seagrass go beyond supporting high abundances and numbers of species, given that they also supported disproportionately greater richness of biological functions. We also observed significantly lower functional divergence in seagrass habitats than unvegetated and disturbance sediments at time zero, potentially an artifact of a higher proportion of zero values for other trait levels in those species with just one trait level, resulting in lower functional divergence in these communities.

Benthic Flux Rates Following Disturbance

Oxygen consumption rates provide a proxy for carbon mineralization (Glud 2008, Song et al. 2016, Snelgrove et al. 2018); however, this proxy overlooks contributions from anaerobic decomposition (Canfield et al. 1993, Mateo et al. 2006), an important contributor to seagrass organic matter mineralization (Jensen et al. 2007). Given similar macrofaunal abundances in seagrass and unvegetated sediments and previous studies that documented high rates of *Zostera marina* respiration (Duarte et al. 2010), we attribute the higher rates of mineralization in seagrass

cores to respiration by seagrass components during incubation, noting that we incubated our cores in darkness to avoid the confounding effect of adding oxygen via photosynthesis. Highly productive seagrasses represent net carbon sinks (Duarte et al. 2010); consequently, many studies document the carbon sequestration potential of seagrass beds and highlight their important contributions to long-term blue carbon storage (Duarte et al. 2010, Fourqurean et al. 2012, Röhr et al. 2018). We also observed seasonal changes in mineralization, with declining oxygen consumption in all treatments by mid-November. We attribute this decline to decreasing water temperature over the experimental period (time-zero = 17 $^{\circ}$C , six-weeks = 12 $^{\circ}$C, twelve-weeks = 6 $^{\circ}$C), noting many studies that demonstrate strong variation in seagrass respiration rates with temperature (Biebl & McRoy 1971, Marsh et al. 1986, Ouisse et al. 2010).

Surprisingly, we observed no significant differences in nitrogen flux among treatments, noting previous studies that document strong influences of seagrass beds on nitrogen dynamics (Caffrey & Kemp 1990, McGlathery et al. 1998, Ottosen et al. 1999). However, nitrogen assimilation during photosynthesis often outweighs other contributions to nitrogen flux in these habitats (Risgaard-Petersen et al. 1998, Risgaard-Petersen & Ottosen 2000, Hansen et al. 2000). Completing our incubations in darkness minimized seagrass nitrogen uptake for photosynthesis, potentially explaining the similarities in both nitrate and ammonium across our treatments. The absence of any clear link between nutrient cycling and macrofaunal abundance and species richness over the duration of the experiment suggests a lesser role for macrofaunal bioturbation in nutrient dynamics in this system than in other marine systems. Braeckman et al. (2014) demonstrated the variable influence of macrofaunal diversity on nutrient cycling, with a lesser role for macrofaunal bioturbation in coarse sandy sediments and overall lower rates of benthic flux than in finer sandy sediments. Mean grain sizes (MGS) in our cores ranged from fine-sand

sediment in seagrass to medium-sand in unvegetated sediments, with intermediate values in disturbance cores. Coarse sediments and the collection of cores from a high-energy shallow subtidal zone (< 1.5 m depth) could explain the negligible impact of macrofaunal diversity on benthic fluxes; greater reworking by constant wave action may overshadow macrofaunal bioturbation effects on nutrient dynamics in these permeable sediments (Koch et al. 2006).

Despite minimal effects of seagrass disturbance on benthic nutrient flux rates, seasonal changes nonetheless occurred in nitrate, ammonium, and phosphate fluxes. Nitrate flux changed from mean influx into sediments at time-zero in August to mean efflux at twelve weeks post disturbance. This pattern parallels observations by Risgaard-Petersen and Ottosen (2000) that seagrass sediments act as a sink for dissolved inorganic nitrogen in the spring and summer before becoming a net source in the fall. They attributed this change to reduced nitrate uptake with a decline in biological activity with decreasing temperatures, until nitrate release from decomposition eventually outweighed consumption (Risgaard-Petersen & Ottosen 2000); a similar process likely occurred here. Ammonium flux changed to mean influx into the sediments at six weeks post disturbance, then shifted to mean efflux at twelve weeks post disturbance. Similarly, disturbance and unvegetated phosphate flux rates shifted from net influx at time zero and six weeks post disturbance to efflux rates similar to vegetated sediments. Previous studies that reported seasonal variation in marine ammonium and phosphate fluxes in both vegetated and unvegetated habitats (Seitzinger 1987, Jensen et al. 1995, Clavero et al. 2000, Holmer et al. 2006) related changes to higher organic matter inputs and mineralization rates during summer, a trend we did not observe.

Biodiversity Indices and Benthic Flux Variation

Redundancy analysis of taxonomic and functional biodiversity indices revealed that, of the variables we measured, species richness explained the most variation in benthic flux rates, despite no significant change in benthic flux following the large decline in species richness after disturbance. We attribute this link to seasonal change in species richness rather than the decline following disturbance, noting that seasonal changes were responsible for much of the variation in benthic flux rates. Previous studies often report a greater influence of macrofaunal functional characteristics of species on rates of benthic flux (Ieno et al. 2006). Although our final biodiversity RDA model did not include metrics of functional diversity, functional richness and species richness nonetheless explained similar proportions of benthic flux variation; however, overlap in this variation resulted in removal during model selection. Furthermore, when we included rare species in the analysis, functional richness explained more variation in benthic fluxes than species richness.

Environmental Factors and Benthic Flux Variation

Of the environmental variables examined, phaeopigment concentrations explained the most variation in benthic flux, along with percent sand and sortable silt. Other studies also report phaeopigment concentrations as key drivers of benthic flux (Link et al. 2013); however, given the importance of seasonal change in benthic fluxes, seasonal shifts in these variables clearly played a role. The higher phaeopigment concentrations in seagrass cores than in unvegetated and disturbance cores throughout the experiment did not influence flux rates among treatments, suggesting that phaeopigments did not drive flux rates and instead coincided with seasonal changes in benthic flux rates (Bianchi et al. 2002), as did percent sand and mean sortable silt.

Variation Partitioning

When analyzed together using variation partitioning analysis, biodiversity metrics and environmental factors contributed equally to explain 39.2% of the variation in benthic flux rates, indicating similar impacts on ecosystem functioning, with the high overlap representing much of the seasonal variation explained by both. Other studies comparing biodiversity and environmental influences on ecosystem functioning sometimes reported similar contributions of each (Belley & Snelgrove 2016), whereas others reported a greater influence of either biological (Godbold & Solan 2009, Miatta & Snelgrove 2021) or environmental (Grace et al. 2007, Healy et al. 2008) variables. The degree to which biodiversity or the environment influences functioning thus depends greatly on the ecosystem studied, as well as the environmental variables, traits, and species considered. Our analyses left 60.8% of the variation in benthic flux unexplained, pointing to potentially important roles for unmeasured variables such as microbial abundance and diversity (Abell et al. 2013, Belley & Snelgrove 2016), noting the critical role of microbial action in organic matter breakdown and nutrient regeneration. Similarly, the comparatively few studies to date on meiofaunal contributions to ecosystem functioning indicate significant roles (Danovaro et al. 2008, Piot et al. 2014, Schratzberger & Ingels 2018), particularly through their influence on the microbial community.

Green Crab

Our study sought to determine the potential impacts of green crab invasion on the macrofaunal community and ecosystem functioning of undisturbed seagrass habitat by replicating green crab disturbance effects rather than the direct impacts of crabs themselves. Green crab both snip and tear seagrass shoots and uproot rhizomes while foraging (Davis et al. 1998, Malyshev & Quijón 2011, Garbary et al. 2014), often creating barren pits and causing significant declines in seagrass habitat (Garbary et al. 2014, Neckles 2015, Matheson et al. 2016). We emulated the

33

physical uprooting effects of green crab invasion in the absence of green crab predation on macrofauna, which can target specific macrofaunal groups, such as suspension feeding polychaetes in Newfoundland seagrass beds (Rossong 2016) and juvenile soft-shell clams (*Mya arenaria*) in Maine (Tan & Beal 2015). Given that foraging causes the primary seagrass disturbance, additional disturbance through predation would presumably have further reduced abundances in the disturbed habitat and hampered recovery. However, predation by the native rock crab (*Cancer irroratus*) also strongly affects infaunal richness and community structure in field and laboratory experiments (Quijón & Snelgrove 2005). Seagrass removal in our disturbance treatment potentially allowed increased rock crab foraging much as green crab might have, if present.

Conclusions

Seagrass disturbance similar to that associated with green crab invasion significantly impacted macrofaunal abundance, species and functional richness, and macrofaunal community composition; however, these changes did not translate into clear effects on nutrient cycling. Despite similar benthic fluxes in disturbance and non-disturbance treatments, significant declines in macrofaunal abundance and richness likely have major consequences for other aspects of ecosystem functioning, including secondary production and food-web support for higher trophic levels (Clare et al. 2022). Disturbed communities also recovered in total abundance, but not species richness, within twelve-weeks of disturbance. This pattern suggests that removal of seagrass disturbance could lead to rapid macrofaunal community stabilization and potential recovery, noting the significant challenge of eradicating green crab once established (Gehrels 2016). Measures to prevent green crab invasion and control their populations, once established,

would therefore help to protect seagrass habitats and the diverse ecosystems and functions they

support.

Figures
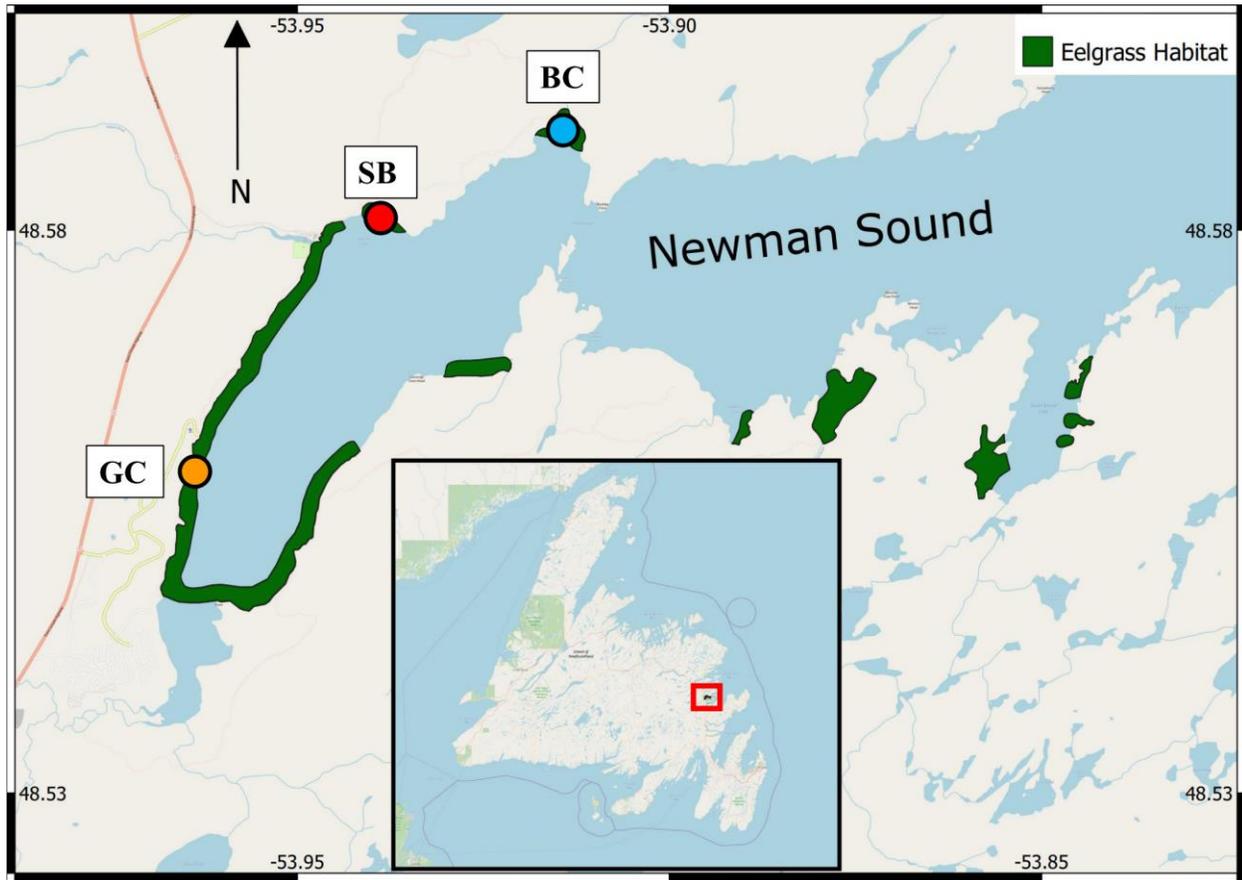
Figure 2.1. Sites (GC – Green Crab Disturbance Experiment, Chapter 2; SB – Salton's Bay, BC – Buckley's Cove, Chapter 3) and eelgrass presence within Newman Sound. Inset shows Newman Sound location on the island of Newfoundland. Eelgrass range adapted from Warren et al. (2010).
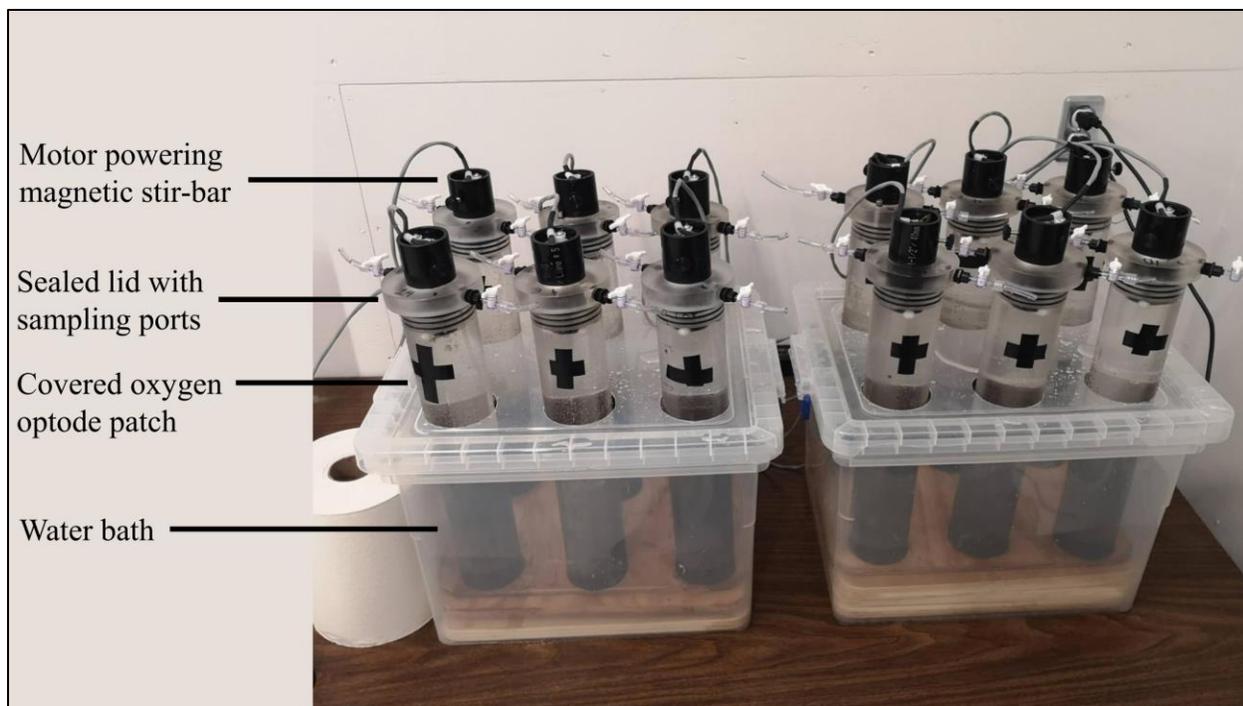
Figure 2.2. Sediment core incubation setup. Acrylic sediment cores (diameter = 6.7 cm, length = 35.6 cm) were sealed with airtight lids fitted with sampling ports and magnetic stir bars powered by small motors. Oxygen optode patches were attached using clear silicone and covered with electrical tape to avoid degradation in light. Cores were placed in chilled water baths to maintain *in-situ* temperatures.

Figure 2.3. Mean (± SE) abundances of macrofauna (individuals·m^{-2}, densities were scaled up from sediment cores 0.0035 m^{-2} in area) across treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) and the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. Letters denote significant differences between treatments (Tukey's Test, p < 0.05). Arrow points to removed disturbance outlier.

Figure 2.4. Mean (± SE) taxonomic diversity indices across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) and the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. A) Species Richness, B) Simpson's Diversity, C) Pielou's Evenness, D) Shannon Diversity. Letters denote significant differences between treatments (Tukey's Test, p < 0.05).

Figure 2.5. Mean (± SE) functional diversity indices across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) over the three sampling periods (time zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. A) Functional Richness, B) Functional Evenness, C) Functional Dispersion, D) Functional Divergence, E) Rao's Q Diversity. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$).

Figure 2.6. Mean (± SE) oxygen and nutrient flux rates across the treatments (● – seagrass, ▲ – disturbance, and ■ – unvegetated) over the three sampling periods (time-zero – August 20th, 2020, six-weeks post disturbance – October 2nd, 2020, twelve weeks post disturbance – November 13th, 2020) from Newman Sound. All fluxes in mmol·m^{-2} d^{-1}, scaled up from sediment cores 0.0035 m^{-2} in area. A) Oxygen, B) Nitrate, C) Ammonium, D) Phosphate, E) Silicate. Letters denote significant differences between treatments (Tukey's Test, $p < 0.05$).

Figure 2.7. 3-Dimensional nonmetric multidimensional scaling (NMDS) plot of macrofaunal communities across the treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time ($\Diamond$ – time-zero - August 20th, 2020, + – six-weeks post disturbance - October 2nd, 2020, ■ – twelve-weeks post disturbance - November 13th, 2020) from Newman Sound, based on Bray-Curtis dissimilarities.

Figure 2.8. Nonmetric multidimensional scaling (NMDS) plots of **A**) multivariate biodiversity metrics and **B**) multivariate benthic flux rates between treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time ($\diamond$ – time-zero - August 20th, 2020, + – six-weeks post disturbance - October 2nd, 2020, ■ – twelve-weeks post disturbance – November 13th, 2020) from Newman sound, based on Euclidean distances between standardized variables. **A**) ellipses represent treatment 95% confidence intervals. **B**) ellipses show time 95% confidence intervals.

Figure 2.9. Redundancy analysis (RDA) of multivariate benthic flux rates as a function of **A**) biodiversity and **B**) environmental variables, across treatments (green – seagrass, red – disturbance, and blue – unvegetated) and over time ($\Diamond$ – time-zero - August 20th, 2020, + – six-weeks post disturbance - October 2nd, 2020, ■ – twelve-weeks post disturbance - November 13th, 2020) from Newman Sound. Oxygen and nutrient locations represent associations with higher rates of flux. Vectors represent direction and influence of explanatory **A**) biodiversity and **B**) environmental variables. Richness: species richness; Carnivore: community weighted mean of carnivores; Infauna: community weighted mean of infauna; Sand: percent sand; Phaeo: Phaeopigment concentration ($\mu g\ g^{-1}$); MeanSortableSilt: mean size of the sortable silt fraction (phi). Some variable names have been offset slightly for clarity of presentation.

Figure 2.10. Venn diagram showing the results of the partitioning of benthic flux variation by biodiversity (0.1121) and environmental variables (0.1153), as well as the overlap variation explained by both together (0.1650).

Table 2.1. Biological traits and their levels used to calculate functional diversity indices. Reworking modes and movements from Queirós et al. (2013)

Biological Traits	Level
Feeding Mode	Carnivore
	Detritus Feeder
	Suspension Feeder
	Funnel Feeder
	Grazer
	Omnivore
	Parasite
	Scavenger
	Surface Deposit Feeder
	Sub-surface Deposit Feeder
Reworking Mode	None/Epifauna
	Surficial Modifier
	Up/Down Conveyor
	Biodiffusor
Movement	None/Fixed
	Limited Movement
	Slow Movement through Sediment
	Free Movement in Burrows
Habitat	Infauna
	Epifauna
	Pelagic
Adult Size	Small (< 1 cm)
	Medium (1 – 5 cm)
	Large (> 5 cm)

Appendix 2.1. Overall Bray-Curtis dissimilarities between treatments and the main contributing species from the SIMPER analysis on fourth-rooted abundances.

	Time-Zero	Six-Weeks	Twelve Weeks
Seagrass vs Unvegetated			
Overall Dissimilarity	48.3%	47.9%	59.0%
Main Contributors	*Pholoe minuta* – 5.8% *Lottidae* Indet. – 5.0% *Mediomastus* sp. – 4.3% *Paranais litoralis* – 3.9%	*Mediomastus* sp. – 7.1% *Monocorophium* sp. – 6.5% *Macoma balthica* – 6.2% *Mya arenaria* – 5.7%	*Mediomastus* sp. – 7.6% *Monocorophium* sp. – 7.4% Unknown Naididae 2 – 5.3% *Pholoe minuta* – 5.2%
Seagrass vs Disturbance			
Overall Dissimilarity	56.5%	45.1%	48.3%
Main Contributors	*Pholoe minuta* – 7.5% *Monocorophium* sp. – 5.7% *Skeneopsis planorbis* – 5.6% *Microphthalmus* sp. – 5.5%	*Microphthalmus* sp. – 8.2% *Monocorophium* sp. – 6.0% *Mediomastus* sp. – 5.7% *Skeneopsis planorbis* – 5.2%	*Monocorophium* sp. – 7.8% *Microphthalmus* sp. – 7.5% *Skeneopsis planorbis* – 5.8% Chironomidae Indet. – 5.4%
Unvegetated vs Disturbance			
Overall Dissimilarity	62.7%	60.3%	57.4%
Main Contributors	*Alitta succinea* – 6.0% *Polydora cornuta* – 5.6% *Lottidae* Indet. – 5.5% Bivalvia Indet. 1 – 5.0%	Unknown Naididae 2 – 7.3% *Microphthalmus* sp. – 7.3% *Macoma balthica* – 6.5% *Mya arenaria* – 6.0%	Unknown Naididae 2 – 7.5% *Microphthalmus* sp. – 7.2% *Mediomastus* sp. – 5.7% *Macoma balthica* – 5.5%

Appendix 2.2. Environmental Variables measured from extra cores. MGS: mean grain size; MSS: mean sortable silt (> 10 μm, < 63 μm) size; TOC: total organic carbon; TN: total nitrogen. % Mud consists of % Silt plus the clay fraction.

	Treatment	MGS (phi)	MSS (phi)	% Gravel	% Sand	% Mud	% Silt	TOC mg g^{-1}	TN mg g^{-1}	Chlorophyll a (μg g^{-1})	Phaeopigment (μg g^{-1})	Chla:Phaeo Ratio
Time-Zero	Seagrass	2.951	4.787	0	81.3	18.7	15.8	3.999	0.092	28.947	58.774	0.493
	Disturbance	1.839	4.667	8.2	87.4	4.4	3.9	1.613	0.199	4.384	27.239	0.161
	Unvegetated	1.818	4.637	11.3	80.4	8.3	8.2	9.349	0.410	3.616	34.191	0.106
Six-Weeks	Seagrass	2.494	4.629	0	90.5	9.5	9.1	7.137	0.437	9.272	59.017	0.157
	Disturbance	2.258	4.636	0	93.8	6.2	5.9	1.977	0.000	3.389	42.671	0.0794
	Unvegetated	1.243	4.693	23.2	69.4	7.4	6.5	16.305	0.765	2.022	26.673	0.0758
Twelve-Weeks	Seagrass	2.342	4.707	0.3	91.3	8.4	7.3	4.366	0.000	20.669	33.744	0.613
	Disturbance	2.229	4.67	0	94.3	5.7	5.1	1.348	0.000	3.691	36.199	0.102
	Unvegetated	1.568	4.853	7.4	86.3	6.3	4.8	11.710	1.021	2.477	24.769	0.100

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Chapter 3: The Role of Seagrass Physical Structure in Macrofaunal Biodiversity-Ecosystem Functioning Relationships

Abstract

Seagrass above-ground shoot canopies and below-ground rhizome networks provide structurally complex habitat that supports diverse macrofaunal communities. Seagrasses also support biodiversity through their biological activity by influencing food availability. Whereas numerous studies have demonstrated that seagrass physical and biological habitat elements influence macrofaunal diversity and community structure, we lack an understanding of how these elements interact with sedimentary macrofaunal communities to regulate ecosystem functioning processes. In order to understand how physical seagrass structure affects macrofaunal biodiversity and the processes of carbon and nutrient cycling, we deployed artificial seagrass patches that mimicked canopies and surface rhizomes, in tandem with parallel observations of natural seagrass (eelgrass, *Zostera marina*), unvegetated habitat, seagrass patch edge, and canopy control treatments. After three months we recorded rates of oxygen and nutrient (nitrate, ammonium, phosphate, silicate) flux from sediment cores, and assessed macrofaunal biodiversity (taxonomic and functional) and sedimentary (granulometric properties and organic matter content/freshness) variables to relate them to benthic flux patterns. We found significantly higher macrofaunal abundance and diversity in natural seagrass treatments compared to unvegetated, patch edge, canopy control, and artificial seagrass treatments. Aside from higher oxygen consumption in natural seagrass treatments that we attributed to seagrass respiration, we found no difference in benthic flux rates across all treatments, suggesting a lesser role for macrofaunal bioturbation in nutrient regeneration in these nearshore sediments. Our results also suggest lesser importance for the structural benefits of seagrasses than their

biological contributions for supporting macrofaunal biodiversity. Negative edge effects on macrofaunal abundance and diversity suggest potential consequences for macrofaunal communities of fragmented seagrass habitats associated with anthropogenic disturbance.

Introduction

Researchers have long recognized the essential role of physical habitat structure in assembling ecological communities and supporting diverse ecosystems (MacArthur & MacArthur 1961, Kovalenko et al. 2012). High structural heterogeneity (the number of different structural components) and complexity (the amount of a given structural component) can promote biodiversity by providing increased niche space and greater habitable surface area (Heck & Wetstone 1977, McCoy & Bell 1991, Attrill et al. 2000). However, biogenic habitats formed by living organisms interact with the environment through their physical structure and their biological activity (Edgar 1999).

Seagrasses form highly productive biogenic habitats that support abundant and diverse ecological communities, often attributed to the structural complexity they provide to otherwise bare sediments (Orth et al. 1984). Seagrasses grow dense above-ground canopies that form critical habitat for many fish (Laurel et al. 2003, Murphy et al. 2021) and epifaunal macroinvertebrate (Heck & Orth 1980) species, with abundances often varying with shoot density (Reiss et al. 2019) and above-ground biomass (Heck & Wetstone 1977). Seagrass canopies also modify the sedimentary environment; consequently, infaunal abundance and diversity also often vary with above-ground complexity (Homziak et al. 1982). Seagrass canopies attenuate waves and currents (Fonseca et al. 1982, Marin-Diaz et al. 2019), and the decreased water flow increases sedimentation of drifting organic matter (Fonseca & Fisher 1986)

and dispersing pelagic larvae (Eckman 1983), while simultaneously decreasing particle resuspension (Kennedy et al. 2010) and stabilizing the sediment (Orth 1977).

Seagrasses also grow from dense rhizome networks that form complex below-ground habitats and protect infauna from surface predators (Reise 1978, Blundon & Kennedy 1982, Orth et al. 1984). Their tight network of rhizomes can also inhibit large burrowers and bioturbators (Brenchley 1982, Githaiga et al. 2019), often favoring small-bodied infaunal communities. Seagrasses also affect the environment through their biological activity, primarily by affecting food availability. Seagrass shoots influence epiphytic algal biomass and community structure (Pinckney & Micheli 1998, Trevizan Segovia et al. 2021), which provide an important food source for macrofauna (Bologna & Heck 1999). Direct inputs of decaying shoot and rhizome material, along with root oxygen and nutrient exudates (Marbà et al. 2007), support diverse sedimentary microbial communities that deposit feeders utilize for food (Livingston 1979, Tarquinio et al. 2019).

Through these physical and biological attributes, seagrasses also greatly influence the ecosystem processes of carbon and nutrient cycling. Highly productive seagrass beds input organic matter in the form of seagrass detritus, epiphytic algae, and accumulated suspended organic matter that all promote microbial carbon mineralization (Mateo et al. 2006). These beds act as carbon sinks (Duarte & Chiscano 1999, Fourqurean et al. 2012) with significant carbon sequestration ability (Duarte et al. 2010, Fourqurean et al. 2012, Röhr et al. 2018), following export of much of their fixed carbon or burial in anoxic sediments (Duarte & Krause-Jensen 2017). Increased organic matter sedimentation and rhizome oxygen and nutrient exudates also promote microbial decomposition and nutrient regeneration (Marbà et al. 2007), whereas the unique microbial communities harboured in the seagrass rhizosphere influence nitrogen cycling

through nitrogen fixation, nitrification, and denitrification processes (McGlathery et al. 1998, Risgaard-Petersen et al. 1998, Welsh 2000).

Alongside the influence of seagrasses on ecosystem functioning, the diverse macrofaunal communities they support closely link with carbon and nutrient cycling (Snelgrove 1997, Biles et al. 2002, Stief 2013). Infaunal movement and feeding behaviours in the sedimentary matrix alter redox states and organic matter distributions through bioturbation and bioirrigation (Aller 1994, Lohrer et al. 2004), promoting microbial decomposition and nutrient regeneration (Aller & Aller 1998, Welsh 2000, Glud 2008). Furthermore, these processes closely tie to macrofaunal community structure and species-specific functional characteristics (Snelgrove 1997, Ieno et al. 2006). Consequently, studies increasingly address relationships between macrofaunal diversity and ecosystem functioning processes (Snelgrove et al. 2014), with functional diversity metrics often explaining greater variation in functioning than traditional taxonomic diversity (Emmerson & Raffaelli 2000, Waldbusser et al. 2004, Danovaro et al. 2008).

The current global decline in seagrass habitat increases the relevance of understanding relationships between seagrasses and ecosystem functioning (Orth et al. 2006, Waycott et al. 2009, Unsworth et al. 2018). Seagrasses are particularly vulnerable to anthropogenic disturbances because their habitat requirements restrict them to shallow coastal waters; threats include sediment loading, eutrophication, shoreline development, invasive species, and rising ocean temperatures (Orth et al. 2006). These disturbances can fragment seagrass beds, which drastically affects seagrass bed structure by increasing relative proportions of edge habitat (Yarnall et al. 2022). Reduced structural complexity, seagrass biomass, and shoot densities characterize seagrass patch edges compared to patch interiors (Moore & Hovel 2010). The loss

and fragmentation of seagrass beds emphasize the need to understand how seagrass habitat interacts with macrofaunal biodiversity in regulating ecosystem functioning processes.

In this study, we investigate the role of seagrass physical structure in supporting sedimentary macrofaunal biodiversity and the relationships among seagrasses, macrofauna, and ecosystem functioning. To determine the contribution of the physical habitat provided by seagrasses to macrofaunal biodiversity and carbon and nutrient cycling, we deployed artificial seagrass units (ASUs) for three months to replicate the structural benefits provided by seagrass beds to unvegetated sediments. We then examined associated sedimentary macrofaunal diversity and community structure, and measured oxygen and nutrient fluxes across the sediment-water interface to assess carbon and nutrient cycling rates. We compared our ASU macrofaunal communities and benthic fluxes to natural seagrass (eelgrass, *Zostera marina*), unvegetated, and ASU treatments lacking a canopy to isolate the structural contributions from the biological contributions of seagrasses to biodiversity and ecosystem functioning. We also examined seagrass patch edge sediments to assess potential edge effects on sedimentary macrofaunal biodiversity and benthic flux. We hypothesized that our ASU treatments would support higher macrofaunal biodiversity and abundance than unvegetated sediments, resulting in higher carbon and nutrient flux rates. Furthermore, we hypothesized that the greater the importance of seagrass physical structure for macrofauna, the closer our ASU macrofaunal communities would resemble those from natural seagrass. We also hypothesized that seagrass patch edges would support reduced sedimentary macrofaunal diversity and abundance compared to natural seagrass treatments but higher values than those in unvegetated sediments.

Methods

Study Design and Sampling

We conducted our study in Newman Sound, Newfoundland, Canada (Figure 2.1), at two sites with clear boundaries between seagrass patches and unvegetated sediments – Buckley's Cove (BC) and Salton's Bay (SB). Both sites are moderately sheltered, with a shallow subtidal slope, and seagrass growing in distinct patches from ~ 2 m depth to the shoreline; below 2 m, seagrass grows in a continuous meadow. At each site, we collected sediment push cores (diameter = 6.7 cm, length = 35.6 cm) by hand from natural seagrass habitat, adjacent unvegetated habitat, and seagrass "edge habitat" taken from within 0.5 m of the seagrass boundary. We also collected sediment cores from artificial seagrass units (ASUs) described in detail below, referring to 1-m^2 quadrats with attached plastic blades to emulate seagrasses, as well as canopy control plots, referring to similar quadrats but with the emergent blades removed; we deployed both of these experimental treatments on unvegetated sediments. Natural treatments, ASUs, and canopy control plots were set up on August 4th – 12th, 2020 and left anchored to the sediment until sampling on October 28th – November 3rd, 2020. At each site, we collected six replicate cores of each treatment for incubation and an additional core from each treatment to analyze sedimentary environmental variables. Cores contained 10 – 15 cm of sediment and 15 – 20 cm of overlying water.

Artificial Seagrass Units

We created ASUs to assess the physical contributions of seagrass habitat for structuring macrofaunal communities. We attached plastic chicken wire (0.25 inch) to square 1 m^2 PVC pipe frames and tied on 75 cm long strips of green plastic ribbon in natural eelgrass densities found in Newman Sound (600 shoots m^{-2}, Laurel et al. 2003). We constructed canopy control treatments using the chicken wire and PVC frames without ribbon. We cut round holes into the chicken wire of both treatments and covered them with wire trapdoors to create a continuous layer of

wire and ribbon canopy that we could open to take the sediment cores during sampling. We deployed bare 1 m^2 PVC frames for each natural treatment (seagrass, unvegetated, seagrass patch edge) and anchored all treatment frames to the sediment using 30-cm long rebar hooks. When anchored flat to the sediment, the chicken wire protected infauna from surface predators much like seagrass rhizomes (Orth 1977), whereas the simulated canopy helped to buffer water flow (Fonseca et al. 1982) and thus collect drifting organic matter (Fonseca & Fisher 1986) and suspended larvae (Eckman 1983).

Incubations

We lost one ASU and one unvegetated replicate from Buckley's Cove during the course of the experiment . Following collection, we acclimated the sediment cores for 12 – 18 hours to allow suspended sediment to settle, while gently aerating the water using aquarium pumps to avoid anoxic conditions prior to incubation. We then incubated the cores for 24 hours at *in-situ* temperatures within refrigerators to assess nutrient and oxygen flux rates. We collected two 50 ml water samples from each core at the beginning (T_0), midpoint (12 hours, T_{12}), and end of each incubation (24 hours, T_{24}) for analysis of ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), and silicate ($Si(OH)_4$) concentrations, and measured oxygen concentrations every four hours using a PreSens Fibox 4 optical oxygen meter with oxygen optode patches attached to the inside of each core. We determined nutrient and oxygen flux rates from linear regressions of their concentrations over time (nutrient concentration as a linear function of time), correcting for the concentrations in the replacement water following nutrient sampling; measurements at T_{12} and T_{24} were adjusted to account for the nutrients removed from the cores during sampling and added during water replacement (see Chapter 2 Methods: Incubations for details). Collectively, we refer to oxygen and nutrient flux rates as benthic flux rates.

Macrofaunal Identification and Diversity Indices

Following the incubations, we sectioned the sediment cores, processed the sections over a 300 μm sieve, and identified all macrofauna under a dissecting scope to the lowest taxonomic level possible. We then used the "vegan" package within R (R Core Team 2021) to calculate diversity indices, including species richness, Simpson's diversity index, Shannon diversity index, and Pielou's evenness. To examine functional trait diversity, we assigned different biological traits to each species using fuzzy coding – i.e., assigning values between 0 and 1 based on the tendency for the organism to express each particular trait level (Table 2.1). We then calculated functional diversity indices using the "FD" package in R (R Core Team, 2021). These indices include functional richness, functional evenness, functional divergence, functional dispersion, Rao's quadratic entropy (Villéger et al. 2008, Laliberté & Legendre 2010), and the community-weighted mean values for each trait level (Lavorel et al. 2008, see Chapter 2 Methods: Macrofaunal Identification and Diversity Indices for details). Two canopy control replicates that contained less than three species precluded calculation of functional richness, evenness, and divergence.

Environmental Variables

To investigate how natural seagrass and our ASUs modified the sedimentary environment, we measured several environmental variables from the extra core taken from each treatment. We initially homogenized the core's 0-2 cm layer and took sub-samples for separate analysis of grain size, carbon/nitrogen content, and phytopigment ratios. We used overall mean grain size (phi), mean of the sortable silt fraction (phi), and percentages of gravel, sand, and mud fractions (%) to assess the effects of seagrass habitat structure on physical sedimentary dynamics. Total organic carbon (TOC, mg·g^{-1}) and total nitrogen (TN, mg·g^{-1}), where encompass

a wide range of organic material, allowed us to assess long-term organic matter freshness and accumulation. Noting that chlorophyll a degrades rapidly into phaeopigments, comparisons of chlorophyll a concentrations ($\mu g \cdot g^{-1}$), phaeopigment concentrations ($\mu g \cdot g^{-1}$), and chlorophyll a: phaeopigment ratios enabled assessment of freshness over the short term. We could not calculate carbon: nitrogen ratios because some total nitrogen values fell below our equipment's detection limits (see Chapter 2 Methods: Environmental Variables for details).

Statistical Analyses

To determine whether total macrofaunal abundance, diversity indices, and oxygen and nutrient flux rates differed among treatments and sites, we ran two-way ANOVAs with "Treatment" and "Site" as fixed factors noting independent cores that we collected and analyzed from each treatment and during each time period, using a type II ANOVA to handle the unbalanced data. We assessed the assumptions of normality and homogeneous variance using Q-Q plots, plots of residuals, and Levene's tests. Given the indication of non-normality in the residuals, we applied a Kruskal-Wallis test to functional dispersion and functional divergence comparisons. Application of a natural logarithmic transformation to total macrofaunal abundances reduced the elevated variance at higher values.

We compared variation in the macrofaunal community, nutrient flux rates, and biodiversity indices among treatments and sites using three two-way permutational multivariate analyses of variance (PERMANOVA, 9999 permutations), using the "adonis2" function in R. We also compared macrofaunal communities among treatments within each site using single factor PERMANOVA. We used Bray-Curtis distances of species abundances for community comparisons and Euclidean distances for comparisons of standardized nutrient flux rates and biodiversity indices. We found similar results in our community and diversity analyses with rare

species included and removed; we therefore report the analysis with rare species removed and note any differences between the two analyses. Following PERMANOVA, we verified the homogeneity of multivariate dispersions using the function "betadisper" in "vegan". We used non-metric multidimensional scaling (NMDS) plots to visualize multivariate differences among treatments. Similarity percentage (SIMPER) analysis on untransformed and fourth-rooted species abundances determined drivers of community differences among treatments.

Two separate redundancy analyses determined the proportion of variation in benthic flux rates explained by biodiversity indices and environmental variables, and the most parsimonious set of explanatory variables that contributed to that variation. Variance inflation factor (VIF) tests removed variables with VIFs > 5, followed by a stepwise selection process using permutation tests with a significance level of $p < 0.05$ to determine the variables explaining the most variation. We used single variable RDAs to determine the contributions of each variable to the overall model. Finally, a variation partitioning analysis using both sets of explanatory variables determined the relative amount of variation in benthic fluxes explained by biodiversity indices and environmental variables alone, and the overlap in explained variation by both sets together (Legendre & Legendre 2012). We completed redundancy analyses and variation partitioning analyses in R using functions in the "vegan" package (R Core Team, 2021).

Results

Infaunal Abundance and Biodiversity Comparisons

Macrofaunal abundances differed significantly among treatments (Two-way ANOVA: Figure 3.2, $F_{4,48} = 6.2$, $p < 0.001$), but not between sites (Two-way ANOVA: $F_{1,48} = 3.7$, $p >$

0.05). Tukey's Tests indicated significantly higher macrofaunal abundances in seagrass treatments compared to all other treatments (Tukey's Test: $p < 0.05$).

We also observed significant differences in species and functional richness across treatments (Figure 3.3A, 3.4A, Two-way ANOVA: species richness: $F_{4,48} = 8.9$, $p < 0.001$, functional richness: $F_{4,46} = 3.8$, $p < 0.01$) but not sites. We found higher species richness in seagrass treatments than in all other treatments and higher functional richness in seagrass treatments compared to all treatments except the canopy control treatment. We also observed significant differences in Simpson's diversity, Shannon diversity, Pielou's evenness, functional dispersion, and functional divergence between sites (Two-way ANOVA: Simpson's diversity: $F_{1,48} = 8.4$, $p < 0.01$, Shannon diversity: $F_{1,48} = 4.9$, $p < 0.05$, Pielou's evenness: $F_{1,48} = 9.4$, $p < 0.01$, Kruskal-Wallis: functional dispersion: $\chi^2_1 = 8.1$, $p < 0.01$, functional divergence: $\chi^2_1 = 5.1$, $p < 0.05$) with higher diversity in Buckley's Cove, and no significant differences among treatments (Figure 3.3, 3.4). Rao's Q also differed significantly across both treatments (Figure 3.4E, Two-way ANOVA: $F_{4,48} = 2.7$, $p < 0.05$) and sites (Two-way ANOVA: $F_{1,48} = 10.9$, $p < 0.01$), with Tukey's Tests discerning higher Rao's Q in seagrass than ASU treatments, and in Buckley's Cove compared to Salton's Bay.

Individual Benthic Flux Rate Comparisons

Rates of oxygen flux differed significantly among treatments (Two-way ANOVA: $F_{4,47} = 3.6$, $p < 0.05$) but not sites; oxygen consumption was highest in seagrass cores, followed by the other treatments (Figure 3.5A). Ammonium flux differed significantly between sites (Two-way ANOVA: $F_{1,48} = 4.7$, $p < 0.05$), although the between site variance was nonhomogeneous. We also found a significant interaction in nitrate flux across treatments and sites (Two-way ANOVA: $F_{4,48} = 2.7$, $p < 0.05$), with mean nitrate efflux in edge habitats at

Buckley's Cove in contrast to mean influx at Salton's Bay (Figure 3.5B). No nutrient flux rates differed significantly among treatments.

Multivariate Community Comparisons

The macrofaunal community differed significantly across both treatments (PERMANOVA: $F_{4,48} = 2.1$, $p < 0.01$) and sites (PERMANOVA: $F_{1,48} = 3.5$, $p < 0.01$) when we analyzed Bray-Curtis dissimilarities using PERMANOVA. However, multivariate dispersions differed significantly among treatments (permutation test: $F_{4,53} = 5.4$, $p < 0.01$). Noting the sensitivity of PERMANOVA to unbalanced datasets (Anderson & Walsh 2013), we interpreted the treatment differences with caution. Macrofaunal communities differed significantly among treatments at Buckley's Cove following our separate analysis (Figure 3.6A, PERMANOVA: $F_{4,23} = 1.9$, $p < 0.01$), although non-homogenous multivariate dispersions suggest this result may reflect the smaller dispersion around seagrass communities. At Salton's Bay, the macrofaunal communities were not significantly different (Figure 3.6B, $F_{4,25} = 1.4$, $p > 0.05$), again noting non-homogenous multivariate dispersion.

SIMPER analysis on untransformed Bray-Curtis dissimilarities at Buckley's Cove identified the abundant polychaetes *Microphthalmus* sp. and *Pygospio elegans* as primary drivers of differences among all treatments, accounting for $21 - 48\%$ of the differences. Following fourth root transformation, *Microphthalmus* sp. remained the main contributor to most treatment differences (9.2 - 16.1% contribution), aside from comparisons of unvegetated cores and edge and seagrass cores, where *Mytilus edulis* was the major driver ($9 - 11\%$ contribution). Other significant contributors included *Monocorophium* sp, *Pygospio elegans*, Bivalvia Indeterminate 1, and *Spiophanes bombyx*. Examining overall dissimilarity, we observed the *least* dissimilar communities when comparing edge and unvegetated sediments (overall dissimilarity = 60%), in

contrast to the *most* dissimilar communities in edge and eelgrass (overall dissimilarity = 79%).

Similarly, SIMPER on untransformed Bray-Curtis dissimilarities at Salton's Bay revealed

Microphthalmus sp. and *Pygospio elegans* as the dominant contributors to treatment community

differences (19.4 – 58.8% contribution). Following fourth root transformation, major

contributors included *Microphthalmus* sp., *Mytilus edulis*, *Pygospio elegans*, Bivalvia Indet. 1,

Unknown Naididae 3, and *Spiophanes bombyx*. This transformation resulted in generally lower

overall dissimilarity among treatments, ranging from 53% (edge vs unvegetated communities) to

67% (ASU vs seagrass).

Multivariate patterns in biodiversity and benthic flux rates

Multivariate standardized biodiversity indices differed significantly across both

treatments (Figure 3.7A, PERMANOVA: $F_{4,46}$ = 1.9, p < 0.01) and sites (PERMANOVA: $F_{1,46}$ =

3.9, p < 0.01) based on PERMANOVA, noting homogeneous multivariate dispersions across

both factors. However, we found no significant differences when comparing treatments within

sites separately, although Salton's Bay was nearly significant (PERMANOVA: $F_{4,24}$ = 1.5, p =

0.059). We also found no significant differences in multivariate benthic flux rates across either

treatment or site when analyzed together or by site, although we note significant differences in

multivariate dispersions between sites (Figure 3.7B, $F_{1,55}$ = 6.91, p < 0.05).

Variation in Multivariate Benthic Flux explained by Biodiversity and Environmental Factors

Following the removal of colinear variables or those with VIF values > 5, the biodiversity

RDA model explained 32.2% of the variation (adjusted R^2 = 0.086) and included species

richness, Pielou's evenness, functional richness, functional evenness, functional divergence, and

community weighted means of carnivores, detritus feeders, funnel feeders, grazers, omnivores,

sub-surface deposit feeders, up/down conveyors, limited movement, and medium-sized organisms (1 – 5 cm). Following stepwise selection, the final model chosen explained 17.4% of the variation (adjusted R^2 = 0.126) and included functional divergence (7.3%), species richness (4.9%), and community weighted mean of detritus feeders (5.5%). The first RDA axis explained 7.9% of the variation in benthic flux and was associated with high species richness, detritus feeders, and oxygen consumption. The second axis explained 7.5% of the variation and was associated with functional divergence and ammonium and silicate flux (Figure 3.8). When we included rare species in the analysis, functional richness became the best explanatory variable (7.2%). The initial RDA model using environmental variables explained just 10.4% of the variation (adjusted R^2 = 0.035) and included mean grain size, percent mud, total carbon, and chlorophyll a concentration. Following the stepwise selection of variables, the final model included mean grain size, and the single RDA axis explained just 5.5% of the variation (adjusted R^2 = 0.038) and was associated with ammonium, nitrate, and silicate flux.

Variation Partitioning

Variation partition analysis of benthic flux rates across biodiversity indices and environmental variables indicated that both sets of explanatory variables together explained 19.9% of the variation (adjusted R^2 = 0.134, Figure 3.9). Biodiversity indices alone explained 9% of that variation, whereas environmental variables alone accounted for just 1% of the explained variation, with 3% explained by both sets of variables. Our analyses left 87% of the variation in benthic flux rates unexplained.

Discussion

By comparing macrofaunal communities across a seagrass patch boundary, we observed significantly lower macrofaunal abundance and diversity in seagrass patch edges and unvegetated sediments than in seagrass patch interiors, supporting studies that associate seagrass density and complexity with macrofaunal biodiversity (Heck & Wetstone 1977, Webster et al. 1998, Rodil et al. 2021). We then used ASUs to assess the contribution of above-ground seagrass structure in supporting macrofaunal communities and observed similar macrofaunal abundance and diversity in ASU treatments as unvegetated treatments, highlighting the potential importance of below-ground complexity and biological contributions of natural seagrasses, which we did not attempt to replicate. Similar nutrient flux rates across all treatments supported our previous conclusion (see Chapter 2) that macrofaunal bioturbation might impact ecosystem functioning less in nearshore sandy sediments than in other systems (Braeckman et al. 2014).

Macrofaunal Diversity at Patch Edges

Seagrass patch interiors had higher macrofaunal abundances and species and functional richness than patch edges and unvegetated sediments, which were similar. Other studies examining seagrass edge effects yield conflicting results (Colomer & Serra 2021), with some finding similar macrofaunal densities and species richness between interior and edge habitats (Boström et al. 2006), whereas many studies report higher densities of epifauna and infauna closer to patch edges (Bologna & Heck 2002, Tanner 2005, Yarnall et al. 2022) or patch interiors (Vonk et al. 2010, Matias et al. 2013). Positive macrofaunal edge effects may result from the hydrodynamic characteristics of edges, where seagrass canopies reduce current velocities (Fonseca et al. 1982), potentially accumulating suspended pelagic larvae at patch edges (Bologna & Heck 2002). Mobile macrofauna traversing unvegetated sediments may also accumulate in edges as they seek refuge between patches (Bologna & Heck 2002). In contrast, our results align

with studies finding strong positive relationships between macrofaunal abundance and diversity with seagrass habitat density and complexity (Heck & Wetstone 1977, Webster et al. 1998, Rodil et al. 2021). Numerous studies report higher diversity and macrofaunal abundance in seagrass beds compared to unvegetated habitats (Orth 1977, Heck & Orth 1980, Boström & Bonsdorff 1997) and declining abundances with lower seagrass densities (Heck & Wetstone 1977, Webster et al. 1998). Our results align with these observations because low seagrass densities in the transition between dense seagrass and unvegetated sediments characterized our edge treatments. The negative edge effects we observed suggest that increased edge area through seagrass bed fragmentation may negatively impact macrofaunal density and biodiversity, contrasting previous research (Yarnall et al. 2022).

The NMDS plot of the communities at the unbalanced Buckley's Cove site separated seagrass from the other treatments, and the significant difference in abundance and richness in the seagrass treatments at Buckley's Cove suggests different communities. High abundances of deposit feeding polychaetes, juvenile *Mytilus edulis*, and suspension feeding amphipods characterized interior seagrass treatments; these communities closely resembled the seagrass communities described in Chapter 2, which we attributed to fresh organic matter inputs (Boon & Duineveld 1996) and seagrass canopy hydrodynamics (Eckman 1983). In this study, we observed finer sediments and higher chlorophyll a: phaeopigment ratios in seagrass treatments only at Salton's Bay (Appendix 3.1). We did not observe high abundances of suspension feeders in Buckley's Cove and Salton's Bay unvegetated communities as reported in Chapter 2. Instead, similar species comprised unvegetated, patch edge, ASU, and canopy control communities as seagrass communities, albeit in lower densities.

Macrofaunal Diversity in Artificial Seagrass

Macrofaunal abundances and species and functional richness in our ASU, unvegetated, and edge treatments were significantly lower than in natural seagrass. The majority of macrofaunal studies using ASUs focus on the role of the canopy in supporting epifauna (Lee et al. 2001, Arponen & Boström 2012, Gartner et al. 2013); fewer studies have used ASUs to examine the roles of seagrass structure on infaunal communities (Eckman 1983, Edgar 1999, Eggleston et al. 1999). Studies that solely replicate the seagrass canopy generally attribute positive relationships between infaunal abundance and canopy structure to increased larval settlement (Eckman 1983) and protection from disturbance events (Boström & Bonsdorff 2000). Those studies that manipulate the below-ground component attribute positive relationships between below-ground complexity and infaunal abundance to sediment stabilization and protection from surface predators (González-Ortiz et al. 2016).

Our results using ASUs suggest that we cannot attribute higher abundance and diversity in dense seagrass habitat compared to sparse edge habitat to the hydrodynamic benefits of the canopy. Our ASU canopies replicated the tendency of natural seagrasses to collect suspended pelagic larvae (Eckman 1983, 1987) and drifting organic matter utilized by infauna for food (Orth 1977, Fonseca & Fisher 1986). Despite much research on the roles of the seagrass canopy in supporting infaunal communities (Orth et al. 1984), other studies give little importance to seagrass above-ground structure for infauna. Boström et al. (2006) observed similar macrofaunal abundance and diversity between seagrass patch interiors and edges lacking above-ground structure, in contrast with our results on seagrass edge effects but supporting our conclusion on the relative unimportance of the canopy in supporting macrofaunal biodiversity.

Our deployment from mid-August to early November, necessitated by Covid 19-related research restrictions, potentially minimized any effect of ASU canopies on macrofaunal diversity

by possibly missing summer reproductive events; larval dispersion plays an essential role in infaunal migration to seagrass beds (Smith & Brumsickle 1989). However, in Chapter 2, we demonstrated the role of post-larval migration to recently disturbed seagrass patches over the same timeframe, suggesting that three months represents a sufficient time interval for macrofauna in adjacent habitats to migrate to our ASUs, provided they offer ideal habitat. Given that we did not see any significant post-larval migration to our ASUs with replicated above-ground structures, we suggest that the below-ground habitat and biological inputs provided by seagrasses may play a more significant role in structuring infaunal communities at our study locations than the benefits provided by the canopy.

Previous studies report higher infaunal abundances and richness with denser below-ground seagrass habitat (Orth et al. 1984), independent of above-ground density (González-Ortiz et al. 2016); previous researchers attributed this difference to the protection from surface and burrowing predators that rhizomes provide (Orth 1977, Reise 1978, Goshima & Peterson 2012). However, we suggest that direct predation by surface predators may not primarily drive these patterns, given that the dense plastic mesh in our ASU and canopy control treatments emulated the protection from predators provided by seagrass rhizomes (Orth et al. 1984, Reynolds et al. 2018). Rather, higher macrofaunal abundances in seagrass habitats may result from habitat selection by motile fauna for complex below-ground habitat, an attribute our design did not emulate. In studying seagrass epifauna, Bell & Westoby (1986) demonstrated habitat selection for dense above-ground seagrass habitat, even when excluding predators. A similar process may operate here, where infauna choose protective habitat based on below-ground complexity, regardless of the presence of actual predation.

Biological modification of the environment by natural seagrasses may also explain the low macrofaunal abundance and diversity in our ASU treatments. Although macrofauna rarely feed on seagrass detritus directly, they consume the abundant epiphytic algae that grow on shoots (Pinckney & Micheli 1998, Bologna & Heck 1999, Trevizan Segovia et al. 2021). Seagrass roots also exude oxygen and nutrients directly into the surrounding sediment (Marbà et al. 2007), and alongside increased organic matter inputs from seagrass detritus, stimulate microbial decomposition and influence the sedimentary microbial community that deposit feeding infauna rely upon (Livingston 1979, James et al. 2006, Tarquinio et al. 2019). Given that we attempted to replicate the primary benefits of seagrass physical structure and found no macrofaunal response, our results suggest that these biological contributions from natural seagrass may outweigh the physical structural benefits often ascribed to seagrass beds.

Benthic Fluxes

Higher macrofaunal abundances in seagrass cores were potentially responsible for increased oxygen consumption, given evidence that macrofauna contribute upwards of 25% to overall seagrass seafloor respiration (Rodil et al. 2020). However, in Chapter 2, we also observed higher oxygen consumption in seagrass cores than unvegetated cores that had similar macrofaunal abundances; therefore, we attribute the significantly higher oxygen consumption in seagrass treatments compared to unvegetated, edge, and ASU treatments to high rates of seagrass respiration (Duarte et al. 2010), as described in Chapter 2. The lack of difference in nutrient fluxes among treatments contrasts studies that show strong influences of seagrasses on nutrient cycles (Caffrey & Kemp 1990, Jensen et al. 1995, Holmer et al. 2006), related to their role in affecting sedimentary redox states (Aller 1994), organic matter accumulation (Mateo et al. 2006, Kennedy et al. 2010), and microbial activity (Ottosen et al. 1999, Welsh 2000, Jensen et al.

2007). However, these results align with our observations in Chapter 2, where we attributed

nutrient flux similarities to minimal seagrass activity during incubations in total darkness.

Similarly, we attribute the absence of a link between nutrient cycling and macrofaunal

abundance and diversity to a lesser role for macrofaunal bioturbation in nutrient dynamics in

coarse nearshore sediments than in other marine systems (Braeckman et al. 2014). However, as

we did not directly manipulate macrofaunal diversity while controlling for the environment, we

cannot draw definitive conclusions on the role of biodiversity alone in regulating benthic fluxes.

Biodiversity Indices and Environmental Factors Influencing Benthic Flux Variation

Noting the minimal variation in benthic flux among treatments and across sites in our

study, the low explanatory power of our RDA models comes as no surprise. Functional

divergence explained the most variation in benthic flux resulting from significantly lower

functional divergence and significantly higher ammonium influx at Salton's Bay. Similar to

Chapter 2, inclusion of rare species in the analysis elevated functional richness to the best

explanatory variable; these findings align with previous studies that report a greater influence of

functional diversity on benthic processes than taxonomic diversity (Ieno et al. 2006, Danovaro et

al. 2008). Species richness and community weighted means of deposit feeders explained similar

amounts of benthic flux variation and were associated with high oxygen consumption. We

attribute these patterns to higher species richness and deposit feeder abundances in seagrass

treatments, in which seagrass respiration resulted in significantly higher oxygen consumption

(Duarte et al. 2010). The significance of mean grain size primarily reflected the association

between higher ammonium influx and coarser sediments at Salton's Bay. The higher

contribution of biodiversity indices than environmental variables to benthic flux rates through

variation partitioning contrasts with our results in Chapter 2; however, these results align with

previous studies that also report greater biological than environmental contributions to benthic flux rates (Godbold & Solan 2009, Miatta & Snelgrove 2021).

Conclusions

Interior seagrass habitat supported higher macrofaunal abundance and diversity than the sparse edge and unvegetated habitat, suggesting a positive relationship between seagrass density and macrofaunal abundance and diversity (Heck & Wetstone 1977). These negative edge effects have important consequences for macrofaunal communities in fragmented seagrass beds; the increased proportion of edges in fragmented habitats may significantly negatively impact seagrass macrofaunal biodiversity. However, the results from our ASU treatments suggest that these relationships between seagrass density and macrofaunal diversity do not result from canopy hydrodynamics or protection from predators, the primary physical benefits seagrasses provide. Rather, we suggest that the biological influence of natural seagrasses on food availability and macrofaunal habitat selection for below-ground rhizome density potentially explain the positive relationship often observed between seagrass density and macrofaunal diversity. Our results illustrate the value of protecting continuous, natural seagrass beds for providing the greatest benefits for maintaining their diverse macrofaunal communities.

Figures

Figure 3.1. Control (A) and artificial seagrass units (B). Plastic chicken wire (0.25 inch) was attached to square 1 m² PVC pipe frames. We cut round holes into the chicken wire of both treatments and covered them with chicken wire trapdoors to facilitate sediment core sampling (circled in red). We additionally tied on 75 cm long strips of green plastic ribbon (600 shoots m⁻²) for the artificial seagrass treatments.

Figure 3.2. Mean (± SE) abundances of macrofauna (individuals·m^{-2}, densities were scaled up from sediment cores 0.0035 m^{-2} in area) across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). Letters denote significant differences between treatments (Tukey's Test, p < 0.05).

Figure 3.3. Mean (± SE) taxonomic diversity indices across the across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Species Richness, B) Simpson's Diversity, C) Pielou's Evenness, D) Shannon Diversity. Letters denote significant differences between treatments and * denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, $p < 0.05$).

Figure 3.4. Mean (± SE) functional diversity indices across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Functional Richness, B) Functional Evenness, C) Functional Dispersion, D) Functional Divergence, E) Rao's Q Diversity. Letters denote significant differences between treatments and * denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, $p < 0.05$).

Figure 3.5. Mean (± SE) oxygen and nutrient flux rates across treatments between Buckley's Cove (blue ●) and Salton's Bay (red ▲). A) Oxygen, B) Nitrate, C) Ammonium, D) Phosphate, E) Silicate, scaled up from sediment cores $0.0035\ m^{-2}$ in area. Letters denote significant differences between treatments and * denotes significant site differences between Buckley's Cove and Salton's Bay (Tukey's Test, $p < 0.05$).

Figure 3.6. 3-Dimensional nonmetric multidimensional scaling (NMDS) plot of macrofaunal communities among the treatments (ASU, control, edge, seagrass, unvegetated), based on Bray-Curtis dissimilarities. A) Buckley's Cove, B) Salton's Bay

Figure 3.7. Nonmetric multidimensional scaling (NMDS) plots of A) multivariate biodiversity metrics and B) multivariate benthic fluxes among treatments (ASU, control, edge, seagrass, unvegetated) and between sites (◇ - Buckley's Cove, ● - Salton's Bay), based on Euclidean distances between standardized variables. A) Ellipses show treatment 95% confidence intervals, B) Ellipses show site 95% confidence intervals.

Figure 3.8. Redundancy analysis (RDA) of multivariate benthic flux rates as a function of biodiversity, among treatments (ASU, control, edge, seagrass, unvegetated) and between sites (◇ - Buckley's Cove, ● - Salton's Bay). Oxygen and nutrient locations represent associations with higher rates of flux. Vectors represent direction and influence of explanatory biodiversity variables. Richness: species richness; FDiv; functional divergence; Detritus.Feeder: community weighted means of detritus feeders. Some variable names have been offset slightly for clarity of presentation.

Figure 3.9. Venn diagram showing the results of the partitioning of benthic flux variation by biodiversity (0.0933) and environmental variables (0.0088), as well as the overlap variation explained by both together (0.0322).

Tables

Appendix 3.1. Environmental Variables measured from extra cores. MGS: mean grain size; MSS: mean sortable silt (> 10 μm, < 63 μm) size; TOC: total organic carbon; TN: total nitrogen. % Mud consists of % Silt plus the clay fraction.

	Treatment	MGS (phi)	MSS (phi)	% Gravel	% Sand	% Mud	% Silt	TOC mg g^{-1}	TN mg g^{-1}	Chlorophyll a (μg g^{-1})	Phaeopigment (μg g^{-1})	Chla:Phaeo Ratio
Buckley's Cove	Artificial Seagrass	2.123	4.789	0	98.8	1.2	0.9	11.352	1.342	3.697	28.079	0.132
	Control	2.068	4.805	0.4	98.2	1.4	1.1	15.273	1.772	5.568	28.211	0.197
	Edge	2.109	4.83	0	98.8	1.2	0.8	8.650	0.758	5.928	27.557	0.215
	Seagrass	2.02	4.72	2.9	95.5	1.6	1.2	0.640	0.000	6.319	35.082	0.180
	Unvegetated	2.143	4.791	0	98.9	1.1	0.8	0.937	0.174	4.432	27.294	0.162
Salton's Bay	Artificial Seagrass	1.656	4.773	6.3	92.4	1.3	1.1	4.611	0.487	2.589	35.655	0.073
	Control	1.671	4.859	5.9	92.8	1.3	0.9	23.297	3.064	0.000	33.180	0.000
	Edge	0.568	4.86	35.5	63.5	1.0	0.7	22.932	2.454	1.966	27.351	0.072
	Seagrass	1.931	4.832	0	98.8	1.2	0.9	10.865	1.517	4.380	27.395	0.160
	Unvegetated	1.871	4.809	1.3	97.7	1.0	0.7	10.970	1.119	3.109	27.221	0.114

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Chapter 4. Conclusion

Through this thesis, I investigated biodiversity-ecosystem functioning relationships in seagrass meadows to explore the potential roles played by seagrass habitat and macrofaunal diversity in ecosystem functioning. In Chapter 2, I examined potential effects of seagrass disturbance on diversity and functioning by experimentally uprooting small seagrass patches. I found that seagrass disturbance significantly impacted macrofaunal abundance and community structure but not benthic flux rates, aside from a decline in oxygen consumption I attributed to seagrass respiration. Over three months, disturbed macrofaunal communities recovered in abundance, in contrast to undisturbed seagrass and unvegetated abundances that declined seasonally to the point that all treatments were similar. Although nutrient cycling appeared largely unaffected by macrofaunal diversity decrease, I attributed changes in nitrate and phosphate flux over the experimental period to seasonal environmental changes. In Chapter 3, I used artificial seagrass to assess the relative roles of habitat structural complexity or biological components of natural seagrasses in supporting macrofaunal diversity. Macrofaunal abundance and diversity in artificial seagrass treatments were similar to those in unvegetated sediments after three months, suggesting a lesser role for above-ground seagrass structural habitat for macrofaunal communities than trophic inputs, such as microbial and epiphytic food sources. Low macrofaunal abundance and diversity in seagrass patch edges compared to patch interiors point to significant consequences of seagrass bed fragmentation. Comparing differences and similarities in the results of Chapters 2 and 3 offers an opportunity to highlight overarching patterns and discuss the overall significance of the research.

In Chapter 2, I observed similar macrofaunal abundances and species richness in seagrass and unvegetated treatments but different dominant species; in contrast, in Chapter 3 I observed

significantly higher abundance and richness in Buckley's Cove and Salton's Bay seagrass treatments compared to unvegetated treatments, but with similar dominant species in terms of community composition. Many studies examining macrofaunal communities report greater abundance and diversity in seagrass beds compared to adjacent unvegetated sediments (Orth 1977, Heck & Orth 1980, Boström & Bonsdorff 1997), aligning with the results from Chapter 3. Differences in macrofaunal response between chapters may reflect environmental differences between sites. Wong (2018) linked higher macrofaunal secondary production in seagrass habitats than in unvegetated sediments to surrounding environmental conditions, noting greater differences in secondary production between habitats at more exposed sites with high percent sand. Salton's Bay and Buckley's Cove sediments in Chapter 3 had higher percent sand than the disturbance site in Chapter 2 and landscape differences may have left sediments more exposed. At the disturbance site in Chapter 2, seagrass grows in a continuous bed starting at ~1 m below the low tide line; winter ice scour prevents further growth shoreward. In contrast, the more gradual subtidal slope at Buckley's Cove and Salton's Bay results in patchy seagrass distributions. The seagrass meadow in Chapter 2 may have offered inshore macrofauna some protection, whereas the patchy landscape at Buckley's Cove and Salton's Bay may have left unvegetated sediments more exposed, resulting in lower macrofaunal abundances and a greater disparity between unvegetated and seagrass sediments. Site-specific characteristics may also explain differences in benthic flux rates when comparing Chapters 2 and 3 at a similar time-point. In Chapter 2, I observed net nitrate and ammonium efflux from the sediments in November, whereas in Chapter 3, I generally observed nitrate and ammonium influx at that time.

The two experiments generally produced similar results. Throughout Chapters 2 and 3, benthic flux varied little among treatments, with only oxygen flux consistently differing; oxygen

consumption was significantly higher in seagrass cores than in other treatments. I found this

pattern consistent across both experiments regardless of macrofaunal oxygen consumption. The

significant portion of belowground seagrass biomass rooted in anoxic sediments contribute to

high rates of respiration in seagrasses in darkness (Duarte et al. 2010, Rasmusson et al. 2017);

this large biomass requires substantive dissolved oxygen uptake in the absence of oxygen from

photosynthesis (Borum et al. 2007). Differences in microbial organic matter decomposition may

also have contributed to higher rates of mineralization; higher chlorophyll a: phaeopigment

ratios, and thus a higher proportion of fresh organic input in seagrass cores create an expectation

of higher rates of aerobic microbial decomposition and remineralization (Danovaro 1996).

However, higher rates of microbial decomposition generally result in higher rates of nutrient

release from sediments (Herbert 1999), which I did not observe.

The consistent absence of any clear nutrient flux rate differences among treatments

regardless of seagrass density, habitat disturbance, or macrofaunal diversity, along with the clear

shifts in nutrient fluxes over time in Chapter 2, suggest a greater role for large-scale seasonal

processes in driving nutrient flux than macrofaunal bioturbation in sandy sediments for both

experiments. Previous research has demonstrated the variability and context-dependent influence

of bioturbation on ecosystem processes (Braeckman et al. 2014, Bernard et al. 2019), with a

lesser role for macrofaunal sediment mixing in coarse sandy sediments than in studies of finer

sediments. Physical differences may explain the absence of a link between macrofauna and

functioning across all treatments and sites. However, the reduced importance of bioturbation in

coarse sediments potentially only explains the benthic flux rates in Buckley's Cove, Salton's

Bay, and unvegetated sediments in Chapter 2, whereas inhibition of bioturbation by the dense

belowground rhizome material influenced flux rates in finer seagrass sediments (Bernard et al.

2014). Overall, seasonal changes drove the majority of variation in benthic flux, likely linked with changing biological activity associated with declining water temperatures (Jensen et al. 1995, Risgaard-Petersen & Ottosen 2000).

Chapter 3 offered insight into some of the underlying processes that structure macrofaunal communities in seagrass beds and thus some aspects of macrofaunal response to disturbance in Chapter 2. As expected, macrofaunal abundance and richness declined immediately following disturbance, and abundances recovered to resemble those in undisturbed seagrass and unvegetated treatments after just twelve weeks. In the absence of regrowth in the disturbed seagrass, sediments were largely bare aside from some residual belowground rhizome material. My disturbance patches left gaps in the canopy that exposed sediments to predators, yet macrofauna quickly recolonized from the adjacent seagrass bed. Given that the artificial seagrass and canopy control treatments designed to replicate the canopy and surface rhizomes respectively had no effect on macrofaunal abundance or community structure, I suggest that these structural habitat elements were not primarily responsible for elevated macrofaunal abundances and diverse communities in seagrass beds. Instead, I suggest that seagrasses modified the environment through food inputs (Bologna & Heck 1999, Edgar 1999) and potential habitat selection by infauna for belowground structure irrespective of direct predation. Chapter 2 results support these conclusions; the disturbance treatments lacked the structural benefits of seagrasses, but the remaining belowground material in the disturbance pits nonetheless potentially attracted immigrating macrofauna. Similarly, although I deployed the artificial seagrass and canopy control treatments ~1 m from a seagrass bed, the location of disturbance pits within a seagrass bed may have enabled trophic inputs from natural seagrasses that were not present in artificial seagrass treatments.

My findings have implications regarding seagrass habitats across Canada, particularly those threatened by green crab invasion. Whereas the significant decline in macrofaunal abundance and diversity following disturbance had little effect on carbon and nutrient cycling processes, such a loss would significantly affect secondary production and trophic transfer (Clare et al. 2022), with further consequences for many commercial fish and invertebrate species (Matheson et al. 2016). The rapid recovery of infauna over just three months suggests that macrofaunal communities can quickly stabilize following small-scale disturbances, potentially depending on remaining belowground seagrass biomass. While this interpretation provides a positive outlook for recovering seagrass beds, I deliberately chose a disturbance size to reflect small-scale green crab disturbance, and thus cannot extend conclusions regarding macrofaunal recovery to heavily invaded or degraded seagrass beds experiencing large-scale losses. Large-scale removal of seagrass habitat produces a greater impact on epifaunal abundance than small disturbances (Reed & Hovel 2006) and presumably affects infauna by limiting post-larval horizontal migration (Smith & Brumsickle 1989). Green crab invasions begin with small-scale disturbances by individual crabs but can progress to massive cumulative loss of habitat that would presumably limit macrofaunal community recovery if left unchecked (Garbary et al. 2014, Neckles 2015, Matheson et al. 2016). Measures to prevent green crab invasion and enhanced rapid response to initial colonization may help in maintaining diverse macrofaunal communities in healthy seagrass beds.

Future studies considering macrofaunal-biodiversity ecosystem functioning relationships in seagrass habitats should expand on the environmental variables considered here; in particular, microbial abundance may significantly contribute to my unexplained variance in benthic flux (Belley & Snelgrove 2016). Similarly, assessing root and rhizome belowground biomass in

natural seagrass, edge, and disturbance treatments could help explain the patterns of macrofaunal abundance in my study (Orth et al. 1984, González-Ortiz et al. 2016). Replicate cores for determining environmental variables would also enable statistical testing of how seagrasses modify the sedimentary environment. Future studies using artificial seagrass patches might assess macrofaunal community structure and benthic fluxes while replicating seagrass belowground habitat to fully isolate the structural contributions of natural seagrass from its biological inputs. Artificial seagrass patches that take place over extended periods of time and during peak infaunal reproductive events might also capture more effectively how seagrass structure affects macrofaunal colonization.

Given increasing anthropogenic threats to seagrass habitats with Earth's growing population punctuates the importance of understanding how seagrasses interact with macrofaunal diversity in supporting crucial ecosystem functions and services upon which humanity depends. My study clarifies the roles played by seagrass physical structure in supporting diverse macrofaunal communities. I also demonstrated potential effects of habitat disturbance on macrofaunal communities in altering the relationships between macrofaunal diversity and ecosystem functioning. The trend toward community recovery suggests that halting drivers of seagrass disturbance may help return altered macrofaunal communities to resemble their pre-disturbance states. Maintaining productive seagrass meadows should remain a conservation priority to maximize biodiversity and promote ecosystem functioning.

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