The role of bioturbators in seagrass blue carbon dynamics

Alexandra Clare Goudie Thomson

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Certificate of Original Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Alexandra Thomson

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Preface

This thesis has been prepared in publication format, whereby each chapter represents a manuscript ready for submission to a scientific peer-reviewed journal. Due to this preparation, there will be a degree of repetition across chapter introductions and methodologies. All data chapters are prepared as research articles. As of yet, no individual chapter has been accepted for publication in a peer-reviewed journal.

Two papers (both submitted to journals, not yet in publication) were produced in association with this PhD, but do not form a part of this thesis. These two papers (one review paper, one research article) are attached in the appendix of the thesis. These are both formatted in the style of the particular journal they have been submitted to.

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Abstract

The ability of vegetated coastal habitats to enhance carbon (C) sequestration and sustain C stocks plays an important role in the global cycling of atmospheric CO_2. These blue carbon ecosystems (encompassing seagrass meadows, mangroves, and saltmarshes) are among the most efficient and productive environments for C storage worldwide. In fact, seagrass meadows transfer C into the sediment more efficiently than any terrestrial ecosystem. There is therefore a huge potential to capitalise on these C sinks, and understanding processes that affect the sequestration and storage of C within seagrass ecosystems is essential. There is however a major deficit in our understanding of the factors affecting C cycling in seagrass sediments, and this is how burrowing macrofauna within seagrass sediments affect the flux of C.

Benthic macrofauna ("bioturbators") are a natural component of seagrass environments. Their activity within the sediment potentially has major impacts on seagrass C sequestration, given their influence on organic matter, and relationship with sediment microbes. It is generally accepted that the effects of bioturbators are a poorly studied component of blue C ecosystems. Quantifying the effect of bioturbation on C sequestration is essential in understanding the continuing C sequestration capacity of these systems.

The overarching objectives for this thesis were two-fold; (1) to determine whether bioturbation has a net overall positive or negative effect on seagrass C sequestration; and (2) to evaluate the mechanisms behind these processes in relation to a meadows C flux. To address these objectives, this thesis took a holistic approach, following the burial and decomposition of organic matter (detritus), and investigating the extent of sediment oxygenation and microbial activity. Finally, we were able to quantify the flux of both sediment and detrital-C from the sediment. A number of species were investigated, including globally-distributed Thalassinidean shrimp ("Callianassid"), and the lugworm *Arenicola marina*. The overall findings of this thesis encompass a "scaled-up" approach to the potential impacts of bioturbators on seagrass sediment C stocks.

The results uncovered in this thesis revealed that bioturbation can have varying impacts on both seagrass C stocks, as well as C sequestration. It was shown that not only do bioturbators influence the burial of organic matter (i.e. detritus), bioturbation also

affects the degradation rate of organic matter. The results in this thesis also brought to light that bioturbation stimulated microbial degradation of sediment-bound C stocks, a process known as “microbial priming”. The results of this thesis outline that bioturbation ultimately results in favourable sediment conditions for microbial degradation of both detrital and sediment-C. The culmination of these processes may result in “hot-spots” of C loss. However, it is also evident that bioturbation has a larger scale impact on seagrass as a whole ecosystem. We conclude that bioturbation is likely to have ecologically-meaningful impacts on both Australian and global seagrass C sequestration.

Introduction

Carbon sequestration in blue carbon habitats

Between the years 2000 and 2010, more fossil fuels were combusted than ever before, resulting in a significant release of greenhouse gasses (GHG), of which more than > 60% consisted of CO_2 (IPCC 2015). As a result, ocean surface temperatures have been predicted to rise by at least 2°C by 2100, which is expected to have a significant impact on global marine biology and biogeochemical cycles (IPCC 2015). Therefore, limiting the further rise of global temperatures and promoting the mitigation of the effects of climate change is now an international policy priority (IPCC 2013, 2015).

Natural vegetated environments sequester atmospheric carbon, and as such play a key role in the overall cycling of atmospheric CO_2 (Mcleod et al. 2011), and are contributing factors to the mitigation of existing climate change. Traditionally, capitalising on vegetated habitats to absorb and store carbon (C) in an effort to both understand global C budgets and mitigate the release of CO_2 has focussed on the terrestrial biome. However, global interest is building for the possible role and importance of vegetated coastal habitats in the sequestration of organic C (Mcleod et al. 2011, Duarte et al. 2013b).

Vegetated coastal habitats (seagrass, mangrove and salt marsh environments) are among the most efficient C storage systems on Earth (Mcleod et al. 2011) and are known as "Blue Carbon" (BC) ecosystems (Nellemann and Corcoran 2009). They sequester and store C in both the short and long (> thousands of years) term (Fourqurean et al. 2012) and are estimated to be 5 to 30 times more efficient in this process than terrestrial habitats (Mcleod et al. 2011, Fourqurean et al. 2012). Although seagrass C burial rates are less than that of both mangroves and saltmarshes, the significantly larger global biomass of seagrass accounts for approximately 50% of sediment C_{org} in the upper 10 cm of global marine sediment (Kennedy et al. 2010, Mcleod et al. 2011). Loss of seagrass meadows can lead to the release of stored C into the atmosphere, which can add to the accumulation of atmospheric CO_2 - loss of just one hectare of seagrass for example, would equate to the loss of 10–40 hectares of terrestrial forest in terms of greenhouse gas emissions produced as a result (Fourqurean et al. 2012). It has been reported that over 50% of global seagrass area has been lost since 1990 due to numerous

factors, namely anthropogenic activity (Waycott et al. 2009). Continued loss of seagrass will therefore lead to the loss of a highly efficient C sink. Investigating processes that both contribute to, and are detrimental to, C storage within seagrass meadows is critical to further our understanding of these mechanisms and to develop procedures to preserve these important C sinks.

Seagrass carbon cycles

Seagrass ecosystems provide a unique niche for C sequestration and storage (Fig. I.1). Carbon enters the system in two forms; firstly, as suspended particulate organic matter (POM) which becomes trapped in the seagrass, and settles on to the substrate (De Falco et al. 2000). Secondly, as CO_2 is taken up *via* photosynthesis from bicarbonate dissolved in the surrounding water (Gianguzza et al. 2002), the C is fixed within seagrass tissues, namely leaves and rhizomes. When the plant, or leaves on the plant, die they decay and are either exported to another system (i.e. "leakage"), or becomes buried in the substrate over time. Benefiting from the anoxic zones, and therefore reduced microbial degradation, in seagrass sediment, this C is stored (Burdige 2007).

Seagrass sediment constitutes a complex and dynamic ecosystem with intercalating oxic and anoxic zones, a highly diverse microbial community, burrowing macrofauna and a characteristically high C content (Guy 2010, Kennedy et al. 2010, Fraser et al. 2015). How the interactions within this environment affects sediment oxygenation, and in turn, how this affects C sequestration, is poorly understood (Jogensen and Revsbech 1985, Macreadie et al. 2014). To effectively manage seagrass ecosystems, we need to understand factors affecting both C sequestration, and the fate of buried ancient and refractory C stocks within coastal vegetated habitats. Research into the efficiency of seagrass C sequestration has historically focussed on accumulation of C; however, among the least understood processes that influence C flux within marine habitats, specifically BC ecosystems, is the impact of burrowing macrofauna i.e. crabs, decapods and polychaetes–referred to as 'bioturbators'–on sediment biogeochemistry.

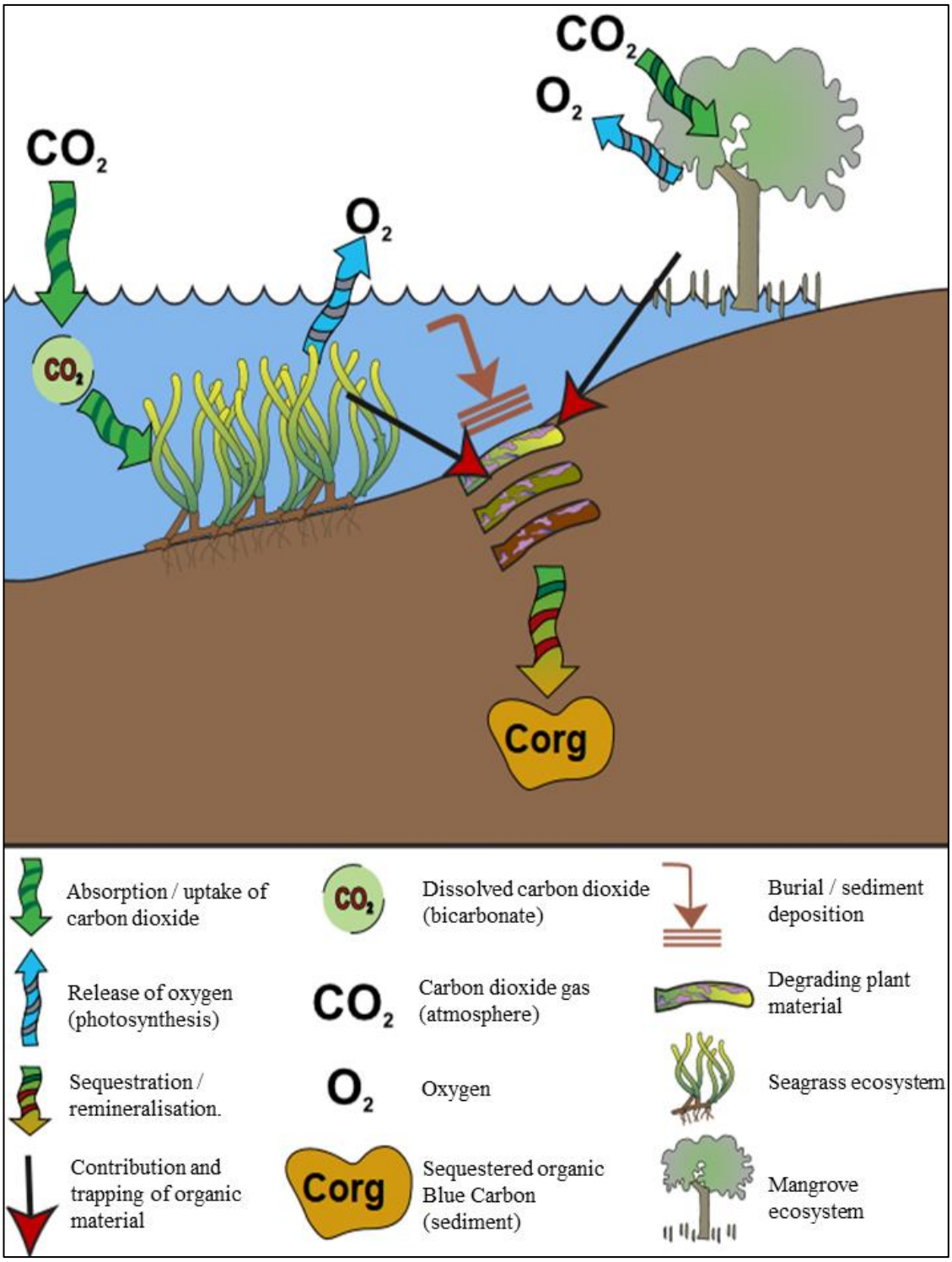

Fig. I.1: Conceptual diagram of C cycling within seagrass and mangrove ecosystems. Diagram is not to scale. Image created by A. Thomson using the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

The role of bioturbators in seagrass blue carbon cycles

Bioturbators are a natural component of seagrass ecosystems, and populations exist in a range of compositions and densities (Kristensen et al. 2012). Bioturbation is the process of macrofauna altering the sediment biology, geochemistry and physical structure and recently defined as "*...all transport processes carried out by animals that directly or indirectly affect sediment matrices.*" (Kristensen et al. 2012). These processes also include both particle re-working and burrow ventilation, known as bioirrigation (Kristensen et al. 2012). Bioturbators perform essential services within a seagrass meadow, without which seagrass would struggle to persist. For example, bioturbators play an essential role in sediment nutrient cycling and the removal of pollutants and contaminating material *via* burial and sediment oxygenation (Schaffner et al. 1997, Hedman et al. 2008); therefore promoting the growth of seagrass (and other BC) plants. However, some bioturbators (i.e. Callianassids and *Menippe* spp. stonecrab) also cause a large amount of damage to both plant and root tissue and thereby reduce the overall productivity of seagrass plants (Suchanek 1983, Townsend and Fonseca 1998, DeWitt 2009). As such, bioturbators have the potential to both positively and negatively impact both seagrass productivity, as well as BC sequestration, through their influence on the sediment and relationship with sediment microbes.

The role that bioturbators play in shaping soil ecosystem processes has long been recognised (Meysman et al. 2006b), and is accepted as being a key driving factor in the biogeochemical processes of BC habitats (Wang et al. 2010). Bioturbation changes the oxygenation of the sediment lining in constructed burrows, which in turn promotes the decomposition of organic matter (OM). This decomposition influences biogeochemical cycles and the sequestration of organic C in marine sediment. Bioturbators within the substrate are constantly re-working the sediment (Fig. I.2). By burrowing, excavating, mixing, filtering and defecating, bioturbators constantly rework and alter the sediment both physically, microbially and chemically (Kristensen et al. 2012). Bioturbation can modify the sediment particle size distribution (Papaspyrou et al. 2005), as well as the gas flux across the sediment/water interface and biogeochemical cycles (gases such as O_2, H_2S and CO_2) in the sediment adjacent to the zone of bioturbation (Bertics et al. 2010). Shifts in faunal community structure have the capacity to significantly impact biogeochemical cycling (Kristensen et al. 2014b). Importantly, these processes alter the

sediment organic matter distribution and mineralisation (Kristensen et al. 2014b), and can therefore alter the BC stock of sediment (de Vaugelas and Buscail 1990, Gutiérrez et al. 2006). It is generally accepted that the effects of these animals are a poorly studied component of BC ecosystems (Macreadie et al. 2014, Atwood et al. 2015).

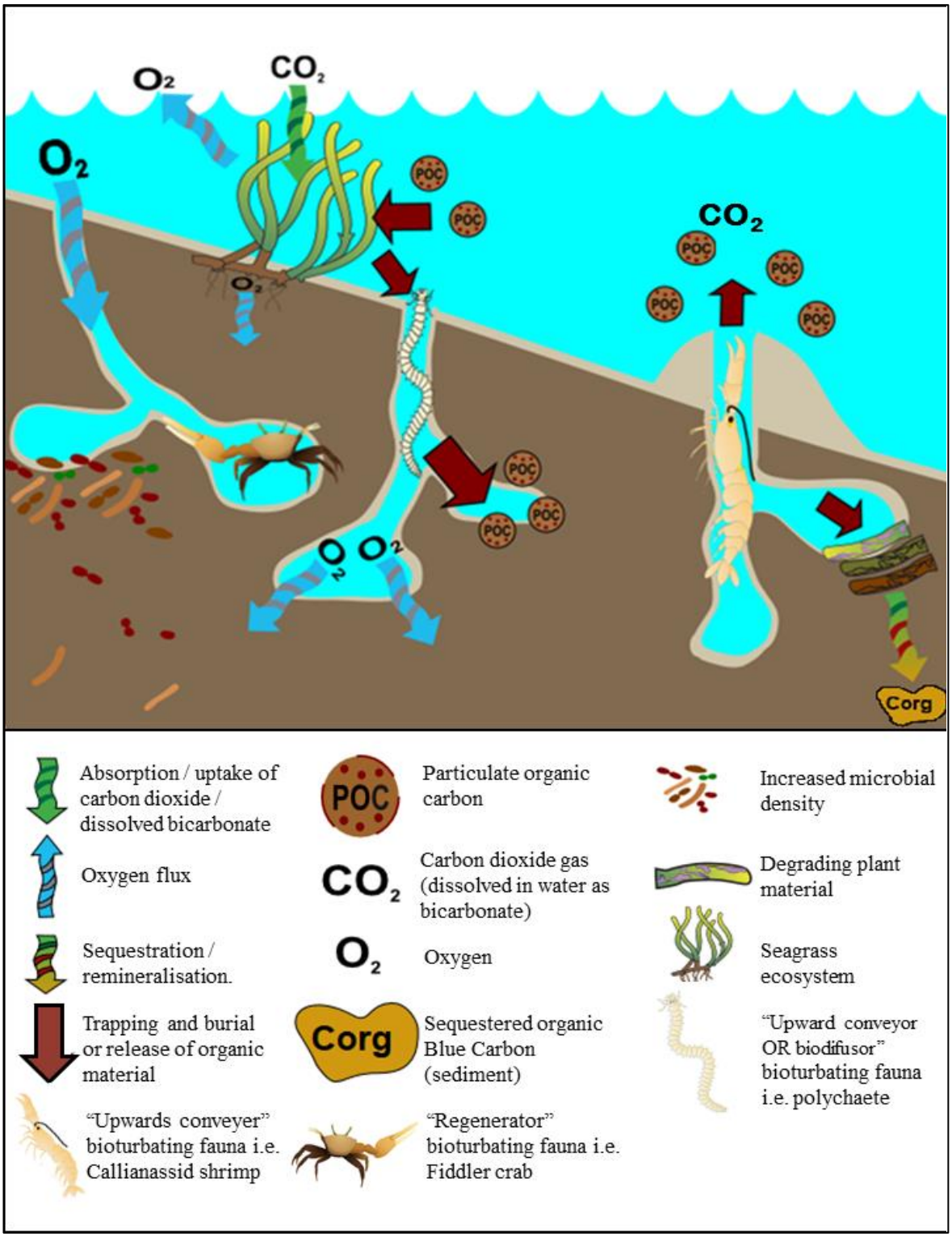

Fig. I.2: Conceptual diagram highlighting the processes within the sediment that bioturbators are known to influence. Diagram is not to scale. Image created by A. Thomson using the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

Bioturbation alters the chemical and physical properties of the sediment

There is a great deal of contention on the subject of bioturbators and their overall impact on C cycling. While in some instances bioturbators may positively affect organic matter incorporation into the sediment, they have also been shown to have the opposite effect. For example, polychaete burrows capture suspended detritus and collect settling organic particles (Aller 1983, Kristensen et al. 1985), which aids in the storage of organic C within the sediment profile (de Vaugelas and Buscail 1990, Kristensen 2008). However, it has also been shown that burrows can vastly enhance the area of sediment/water interface (burrow walls), and therefore may enhance aerobic processes (such as organic matter respiration) in otherwise anaerobic sediments, leading to loss of C from the sediment profile (Kristensen et al. 2001). In other studies, the addition of bioturbating fauna has been seen to change an ecosystem from a C sink to a C source, by altering the flux of DOC (Taylor & Allanson 1993).

As macrofauna live within anoxic sediment, they must ventilate their burrows with the overlying, oxygen-rich water. The movement of water through the burrow (bioirrigation) serves several purposes: removal of metabolic waste; supply of oxygen-rich water to support faunal respiration; and filtration of organic particles from the overlying water (Gutiérrez et al. 2000). Bio-irrigation will alter the redox and oxygen penetration into burrow walls, and therefore alter the microbial community and sediment biogeochemistry (Heilskov and Holmer 2001). Bio-irrigation is not constant; for example, the polychaete *Nereis virens* is active for only about 20% of the day, cycling between short (5–10 minute) irrigation periods followed by periods of inactivity 2–3 fold longer, while the irrigation activity of *Arenicola marina* proceeds in longer cycles of up to an hour duration (Kristensen 2001). When dense populations of polychaetes are actively bio-irrigating and ventilating the sediment, the amount of oxygen entering the sediment is up to 2–3 fold that of ambient sediment (Kristensen 2001). Bioturbators are also known to influence denitrification, *via* the movement of oxygen and C from the sediment surface to deeper layers of the sediment (Bertics et al. 2012), highlighting a similar potential for C release and uptake. Furthermore, there is a higher rate of sulfate reduction (up to 3 orders of magnitude) in faunal burrows compared to the surrounding sediment (Bertics et al. 2012).

Mechanisms by which bioturbators affect C sequestration are not entirely understood (Atwood et al. 2015). Direct impacts include mechanical re-working of sediment to disperse C-rich sediment from the sediment surface into oxygenated water, whereas indirect impacts would include feeding processes that alter the microbial community within the deeper parts of the burrow (Kristensen 2008). Furthermore, the role of bioturbators in C re-mineralisation is affected by both biotic and abiotic factors (Gutiérrez and Jones 2006, Kristensen et al. 2012). Bioturbators irrigate the sediment with oxygenated water thereby increasing the oxygen in contact with the burrow wall sediment interface. On the other hand, bioturbators can incorporate a larger amount of organic material into burrow walls compared to surrounding sediment, and provide a home for microbes (with up to 11 times more microbes found within the walls of burrows than in surrounding sediment; Papaspyrou et al. 2005). In particular, bioturbation by Callianassid shimp is known to significantly alter the bacterial community composition within the sediment, having direct implications on sediment nutrient cycling (Laverock et al. 2010).

Incorporating bioturbators into our understanding of BC dynamics is indeed a challenge. In order to develop clear measures to understand and model BC systems, there is a need to undertake studies to ascertain when the presence of bioturbators can have ecologically meaningful impacts on BC sequestrations. A fundamental goal of such experiments would be to generate information that will enable generalisations to be made about the mechanisms, and the overall impact, that bioturbators have on seagrass C sequestration.

The overarching question for this thesis is whether bioturbation has a net overall positive or negative effect on BC, and more specifically, what is the impact of these processes in relation within meadows? This thesis will examine both the factors affecting sequestration and release of C from sediment in seagrass ecosystems, and aims to explore how bioturbation impacts these processes. A holistic approach to seagrass C sequestration will be taken, that will include a study of the processes and interactions influenced by bioturbation, including physical (sediment movement, burial of OM) and chemical (flux of C, C content and source) variables. To address these questions, the objectives of the thesis are described below:

1. How do bioturbators influence sediment carbon stratification? (Chapters 1 and 2)

Bioturbators play a key functional role in both the influx and efflux of OM into seagrass sediment. However, a problem arises when trying to characterise whether this contribution favours net OM storage or release. In some cases, bioturbation contributes to the initial burial of labile C (Papaspyrou et al. 2007, Otani et al. 2010), thereby contributing to BC stocks. In other cases, the mechanical exposure of refractory C to the sediment surface or water column reduces the BC pool (Kristensen 2001, Wang et al. 2010).

Even when comparing the bioturbation effects of the same species (i.e. Callianassids) in the same BC environments (i.e. seagrass) distinct differences arise as noted in the conflicting data in studies carried out by de Vaugelas and Buscail (1990), Papaspyrou et al. (2005), and Bertics et al. (2010). In two studies, Callianassid burrow walls were found to have 10–17 times more OM than surrounding sediment (de Vaugelas and Buscail 1990, Papaspyrou et al. 2005) which could potentially enhance the C stock. However, recent studies suggested that the addition of oxygen to the sediment by bioturbators could in fact, affect long-term C storage through the oxidation of refractory C (Bertics et al. 2010). While previously studies have largely focussed on the direct effects of bioturbators on sediment C (i.e. the mechanical movement of particles and OM), how bioturbation impacts indirect sediment C mineralisation (i.e. through oxygenation) also need to be considered.

While it is obvious that bioturbators influence OM within BC environments, both positive and negative contributions of OM to sediment stocks and overall flux of C are apparent (see Fig. I.2). One key issue with this flux seems to be the quality of the C affected, rather than the amount supplied (Papaspyrou et al. 2005). For example, in the situation where bioturbators are contributing labile C and storing it deep within the sediment, while mechanically exposing refractory C to the surface through burrow maintenance, only a small change in net C would be apparent. However, the quality (and stability) of the C remaining would be low, as it would largely comprise of fresh labile C. If bioturbators reach high densities within a meadow, large amounts of ancient C pools could be replaced by labile C. These observations highlight that the quality of the C–not just the total amount of C–needs to be assessed. The aims of this research are

two-fold: (1) To assess the impact of bioturbation on sediment C stratification, including the distribution of labile and refractory sediment C (Chapter 1); and (2) assess the impact of bioturbation on both ancient and young C stocks (Chapter 2). Furthermore, subsequent studies will aim to quantify the export of C *via* release of CO_2 (Chapters 3, 4 and 5).

Quantification and assessment of OM accounts for the presence of other compounds (i.e. nitrogen, phosphorus, sulfur) within a particular particle. In some cases within this thesis, OM will be referred to rather than C_{org} when elemental analysis of C is not directly measured. However, throughout this thesis it is assumed that the OM we are analysing is characteristically C-based.

2. How does bioturbation affect the burial of organic matter? (Chapters 1 and 3)

OM burial (i.e. detritus) is one of the important elements determining C storage in seagrass ecosystems (Duarte et al. 2013a). Particle re-working occurs during burrow construction and maintenance, as well as ingestion and defecation of OM, resulting in sediment mixing. Mixing causes OM and associated microorganisms to be displaced vertically and laterally within the sediment (Kristensen et al. 2012). In this way, the composition of the sediment profile is altered: surface sediment is incorporated into burrows, and pushed further into deeper sediment layers, while the older (deeper) sediment is excavated out of the burrows, and left on the surface where it may become oxidised. In this way, bioturbator activity alters the physical structure of the sediment profile around the burrow, and this in turn alters the biogeochemistry as well as the microbial community (Gribsholt et al. 2003). However, specific types of bioturbating fauna will affect the sediment profile differently. For example, a sedentary feeder, such as the polychaete *Arenicola marina*, will consume sediment at the base of its burrow and pass faeces to the surface. These bioturbators can overturn sediment down to 40 cm (Kristensen 2001). Free-living polychaetes, such as *Nereis* spp., actively move throughout the sediment profile in search of food. This feeding practice is haphazard and results in less sediment movement than *A. marina* feeding (Kristensen 2001), and is therefore problematic for providing a comparative measure of bioturbation impacts.

Bioturbating macrofauna can be broken down into several functional categories based on how their burrowing and ventilating activities impact sediment and particle re-working. Kristensen et al. (2012) proposed that species that actively re-work the sediment be divided into 4 main categories (a concept originally proposed by François et al. 1997); (1) biodiffusers, that randomly mix sediment particles; (2) upwards conveyers, that consume and force deep sediment particles on to the surface; (3) downward conveyers, that consume and mix surface particles into deeper sediment; and (4) regenerators, that constantly burrow and transfer sediment. For example, some bioturbators such as upward conveyer-belt feeding polychaetes preferentially feed on smaller sediment particles, leaving large particles at the bottom of the burrow, while depositing smaller digested particles at the surface. *Pestarella tyrrhena* for example, is capable of sorting sediment grains, preferentially incorporating fine grained particles into burrow walls. This results in grain-size stratification and therefore influences gas exchange and diffusion through the burrow wall (Papaspyrou et al. 2005). Changes to the grain size stratification within the sediment profile will alter the movement of pore-water (and oxygenated water into the anoxic sediment), as well as to the surface area for bacterial colonisation.

It has been shown that both allochthonous (i.e. drifting macroalgae captured in the seagrass canopy) and autochthonous sources of OM (i.e. detritus) trapped in seagrass beds may often represent a significant fraction of the substrate available for benthic mineralisation (Gacia et al. 2002, Bouillon et al. 2004a, Bouillon et al. 2004b, Kennedy et al. 2010). Having access to a high-energy C source, an upwards conveyer species may process this C into deeper layers of the substrate, thereby changing the C stock (Boon et al. 1997, Rowden et al. 1998). Assessing how burial and degradation of OM, including both sediment and detrital sources of OM, is influenced by bioturbation is needed to make accurate assessments of C sequestration. However, as rate estimates of these processes do not currently exist, it is difficult to predict the relative importance of bioturbators on sediment stratification within the capacity of BC flux across a seagrass meadow. The aims of this research are to establish the impact of bioturbation on the burial of OM, including the burial and degradation of detritus.

3. How does bioturbation influence the microbial community and overall import and export of carbon? (Chapters 3, 4 and 5)

Within seagrass ecosystems, labile dissolved organic C (DOC) released directly from the rhizosome, or through microbial hydrolysis of particulate organic C (POC), is rapidly degraded by microbial activity within the sediment (Guy 2010). As such, microbial growth efficiency directly influences the amount of BC that is added to C stocks as bacterial biomass. This is significant, as bacterial cells make-up around 30% of living C and over 8% of total organic C (TOC) in seagrass sediments (Danovaro et al. 1994).

A clear positive relationship between bioturbator activity, microbial abundance and community complexity have been described for a number of bioturbators (Papaspyrou et al. 2005, Bertics and Ziebis 2009). Burrowing bioturbators often incorporate seagrass detritus, which enriches the burrow walls with organic material. Allochthonous sources of C that become settled on the sediment surface, including phytoplankton and microphytobenthos, are known to contribute up to 50% of sediment-bound C (Kennedy et al. 2010), and may additionally be incorporated into sediment during burrowing activities. The organically enriched burrow walls in turn support a larger microbial community that can be subsequently grazed by the bioturbators. Active selection of smaller grains (most often organic particles) for consumption, leads to the production of faeces that are high in OM, and thus the burrow walls (enriched with faeces) support a greater abundance of microbes (Dworschak 2001, Papaspyrou et al. 2004).

Bioturbators could trigger re-mineralisation of refractory C in deep sediment layers is *via* 'microbial priming' (Kuzyakov et al. 2000, Fontaine et al. 2003). Microbial priming occurs when moderate changes in environmental conditions (e.g. physical disruptions) 'prime' microbes into causing leakage (efflux) of large amounts of stored C (i.e. *via* mineralisation) from the sediment out into the surrounding water, which equilibrates with the atmosphere, as CO_2 (Kuzyakov et al. 2000). Microbial priming has been widely examined in terrestrial systems; however, it has been largely ignored in reference to blue carbon environments. Within terrestrial environments, microbes within the sediment have been shown to be essentially "dormant" without a fresh C source (Fontaine et al. 2007). However, if a vector (i.e. a bioturbator) was to provide dormant marine microbes, with high-energy labile C, this may stimulate microbial priming.

Labile C sources are readily abundant in seagrass meadows (i.e. vegetative detritus), and if the input of oxygen and labile C is indeed, resulting in the loss of carbon as CO_2, an increase in bioturbator populations could be destructive to seagrass (and other ecosystems) BC stocks.

In order to establish how bioturbation in seagrass ecosystems may induce microbial priming, experiments centre around four aims; specifically to: 1) Identify whether the mixture and subsequent burial of different sources of organic matter (i.e. macroalgae and seagrass) by bioturbation stimulates changes to the rate of re-mineralisation (Chapter 3); 2) Determine if the burial of OM (i.e. vegetative material) stimulates microbial activity, including anaerobic re-mineralisation (Chapter 4); 3) Assess whether the integration of seagrass detritus, a fresh source of labile C, by bioturbation, stimulates the release of sediment C stocks *via* microbial priming (Chapter 5); and 4) Quantify the export of C (i.e. microbial re-mineralisation) *via* release of CO_2 (Chapters 3 and 5). The following five experimental chapters aim to address all of these objectives, and place the results within the context of seagrass C sequestration. By following the impact that bioturbators make on the burial, degradation, and remineralisation of seagrass C, it is hoped that we can gain a better understanding of the processes that drive seagrass BC sequestration.

Chapter 1:

Fiddler crab gender affects carbon sequestration of a seagrass ecosystem

Abstract

Globally, seagrass meadows store significant quantities of carbon below-ground. Intertidal seagrass meadows are home to bioturbators such as fiddler crabs (*Uca* spp.). Fiddler crabs have the potential to be one of the most significant organisms that affect seagrass carbon stocks due to their global distribution and active sediment disruption. We sampled fiddler crab (*Uca signata*) burrows in a *Zostera muelleri* dominated seagrass meadow on Curtis Island (Queensland, Australia) to test whether their bioturbation negatively affected the carbon sequestration capacity of seagrass meadows. We discovered male and female fiddler crabs had different impacts on seagrass sediment carbon. The total amount of organic carbon at greater burrow depths was higher in female burrows than in male burrows and undisturbed (i.e. no bioturbator control) sediment. Males however, had no detectable impact on sediment carbon. Although female burrows contained a larger amount of fresh carbon compared to male burrows, we propose that female fiddler crabs have a long-term impact on buried sediment carbon by creating favourable conditions for rapid carbon remineralisation. These findings imply that fiddler crab gender is a determinant of the carbon sequestration capacity of seagrass meadows. This is the first study to report varying impacts of animal gender on the carbon sequestration capacity of a coastal vegetated habitat. We would recommend considering incorporating gender specific behaviours and morphology into bioturbation classifications where applicable.

Introduction

Coastal seagrass habitats are among the most efficient organic carbon (C_{org}) storage ecosystems worldwide (Mcleod et al. 2011). The ability of these "blue carbon" environments to sequester atmospheric carbon (in the form of CO_2) is important in the global carbon (C) cycle, and therefore a contributing factor in mitigating climate change. There are many environmental factors that affect blue carbon ecosystems, and their capacity to capture and store C, including the species and biomass of seagrass, biogeomorphic conditions and anthropogenic activity (Mateo et al. 2006, Mcleod et al. 2011, Fourqurean et al. 2012). Animal activity can also affect C sequestration (Atwood et al. 2015). Within seagrass ecosystems, burrowing macro-fauna ("bioturbators"), such as crabs, shrimp, and polychaetes are common, appearing in a range of densities and community compositions (Woods and Schiel 1997, DeWitt 2009, Kneer et al. 2013, Garbary et al. 2014, Govers et al. 2014). The burrowing and associated activity of these animals have the potential to both positively and negatively impact C sequestration (Kristensen et al. 2012, Atwood et al. 2015). Furthermore, with pressures such as coastal development and changes to predator populations threatening to de-stabilise bioturbator populations (Atwood et al. 2015), it is essential to quantify their impact on marine C sequestration.

Seagrass not only traps allochthonous OM, but also provides an accessible labile C_{org} source commonly used by excavating bioturbators as both a food source (de Vaugelas and Buscail 1990) and during the construction of burrows as a burrow lining (Papaspyrou et al. 2005). Within coastal environments, bioturbator-facilitated processes, i.e. the active capture (Newell and Koch 2004) and excretion of organic material (OM) (de Vaugelas and Buscail 1990), aids in the storage of C_{org} within the sediment profile. Bioturbation provides a variety of essential maintenance services in seagrass meadows including soil aeration, detritus incorporation, sediment turnover and reduction of potentially toxic sulfide levels (DeWitt 2009). Through these activities, the persistence of a healthy C_{org} accruing system is conserved. However, the rate of C_{org} degradation has been found to be greater in sediment affected by bioturbation, than undisturbed sediment (Fanjul et al. 2007). It has also been shown that bioturbator burrows can vastly increase the area of sediment/water interface resulting in enhanced aerobic processes across the surface micro-layer (such as microbial respiration of C_{org}), leading to loss

(remineralisation) of C_{org} from the sediment profile (Kristensen 2001). A clear assessment of the role of bioturbators in marine C_{org} sequestration is therefore important for understanding C budgets in coastal ecosystems, such as seagrass meadows.

Previously, bioturbators have been divided into functional groups (e.g. biodiffusers, conveyers, regenerators) in regard to their effect on sediment movement (Kristensen et al. 2012). These divisions are largely based on differences in faunal morphological and behavioural characteristics, and their relationship with the sediment. Division of species by gender however, has not previously been considered. One prevalent bioturbator in intertidal seagrass systems is the fiddler crab (*Uca* spp.). With over 80 species worldwide and occupying an array of coastal environments (Salmon and Zucker 1988), fiddler crabs are often seen in coastal areas in high densities (> 100 burrows m^{-2} for *Uca uruguayensis*) and can rework > 0.5 kg of sediment per m^2 per day^{-1} (Botto and Iribarne 2000). Mature male *Uca* spp. display one large dominant claw (hindering their burrowing activity) and show strong territorial behaviour, whereas females (and juveniles) have 2 smaller claws, and spend more time in the terminal chambers of their burrows (Christy 1982, Salmon and Zucker 1988).

In this study, we examined the sediment composition of male and female fiddler crab burrows. Our goal was to determine if different gender traits of male and female fiddler crabs (*Uca signata*) alter burrowing activity and structure, and whether any observed alterations affected C sequestration in seagrass sediments**.** Specifically, we looked for variations in sedimentary organic carbon (C_{org}) stocks among male and female *Uca signata* burrows within an Australian *Zostera muelleri* dominated meadow. The source and quality of the C_{org} were further examined using stable isotopes, solid-state nuclear magnetic resonance (NMR) spectroscopy, and thermogravimetric (TGA) analyses, respectively.

Methods

Study Site

This study was conducted within intertidal seagrass meadows at Pelican Banks, on the southern end of Curtis Island, Queensland, Australia (-23.76E, 151.31N). The seagrass meadow (approximately 607 ha) was dominated by *Zostera muelleri*, interspersed with *Halophila ovalis* and *Halodule univernis. Z. muelleri* cover varied between 30 – 90% m^{-2} across the meadow (McKenzie et al. 2001). The meadow supported a large population of burrowing macro-fauna, including Thalassinidean shrimp and fiddler crabs (*Uca* spp.).

Core collection and processing

Syringe-cores (60 mL, 10 cm in length, 3 cm in diameter) were collected at low tide from Pelican Banks around the burrows of male and female fiddler crabs (*Uca signata*). Only active burrows (those with crabs seen in, and/or returning to the same burrow a minimum of 3 times) were sampled, with the gender of the crab within the burrow being identified before sampling. Gender was assigned as a treatment factor (n = 5), and was determined by the presence of a dominant claw, as seen in male *Uca* spp. Surrounding sediment, that appeared bare and undisturbed by bioturbation, was also sampled for comparison (control treatments; n = 5). The syringe-coring method enabled collection of the burrow opening (0.5– 0.7 cm), entire burrow, and the immediately surrounding sediment (both surface and deeper) with minimal compaction to maintain natural stratification of the sediment and burrow structure. Cores were frozen (-10 ˚C for 24 hours) to allow for easy slicing and removal of any fauna present. Sediment was extracted from cores and sliced into 9 depth horizons from the surface: 0–0.5; 0.5–1; 1–1.5; 1.5–2; 2–2.5; 2.5–3; 3–4; 4–6; and 6–10 cm. Crabs (approximately 1.5cm in body width) were retained in all frozen cores within the 6–10 cm segment (terminal chamber), and were wholly removed from the frozen sediment and examined to confirm the gender assigned to the burrows. Sections were dried at 60˚C for 48 hours, and then separated using a 0.5 mm sieve to remove macro-fauna and large detritus. Each section was then ground with a clay mortar and pestle to breakup any aggregates.

Organic carbon in burrow sediment

Sub-samples of each sediment section (1 g each) were acid washed with 1 M hydrochloric acid (HCl) for 24 hours to remove any inorganic compounds. HCl was washed out of the sediment with double distilled (MilliQ) water until the pH of the sediment was between 5 and 6. Samples were then dried (60˚C for 48 hours) and ground into a fine power with a clay mortar and pestle. Sub-samples (0.2 g) of each sediment section were measured using a C:N analyser (LECO TruSpec, LECO Corporation, St. Joseph, MI, USA) at the University of Technology Sydney (UTS). Rice flour and synthetic carbon standards were used to calibrate the LECO TruSpec.

Thermogravimetric analysis (TGA) of burrow sediment

To quantify the down-burrow quality of sediment organic matter (OM), sub-samples of non-acidified sediment sections (0.05 g each) were measured using a thermogravimetric analyser (SDT Q600, TA Instruments, New Castle, DE, USA), with a balance sensitivity of 0.1 µg, at UTS. This analysis works on the premise that as C_{org} becomes older (and more recalcitrant) higher temperatures are required to thermally oxidise it (Lopez-Capel et al. 2005). TGA is therefore able to quantify the contents of labile, refractory and recalcitrant organic matter (OM) and carbonates within a sample. Samples were placed inside a platinum cup, and heated under air (flow rate of 50 mL min^{-1}) at 20°C min^{-1}, until conditions reached 900°C. Sediment mass loss was identified and measured using Universal Analysis software (TA Instruments, New Castle, DE, USA), within certain temperature ranges (exotherms). Identification of these temperature ranges (exotherms) was based on the rate of change derivative (g mass loss $°C^{-1}$), which indicated clear temperature-dependant sediment mass losses (Lopez-Capel et al. 2005). The first sediment exotherm interval (labile OM) ranged from 200 to 400°C, followed by the second (recalcitrant OM) from 400 to 550°C, and third (refractory OM) from 550 to 650°C (Lopez-Capel et al. 2005). Exotherms 1–3 were recalculated as a proportion of total OM from 200 to 650 °C. One core from each treatment was analysed based on the most representative C_{org} content among the replicates. However, all samples (n = 5) from the 6–10 cm depth were analysed due to differences shown in C_{org} analysis.

Stable isotope analysis and C:N ratios of sediment

To identify the sources of C_{org} in the 6–10 cm burrow depth (identified during the burrow C_{org} analysis as having the greatest difference among treatments), a subset of both the previously acidified and un-acidified sediment samples (n = 4) from this depth were sent to the University of Hawaii Hilo analytical laboratory. Samples were analysed using an Isotope Ratio Mass Spectrometer (Thermo-Finnegan Delta V IRMS) to establish the stable isotope ratios of $^{14}N/^{15}N$ ($\delta^{15}N$) and $^{12}C/^{13}C$ ($\delta^{13}C$). Elemental C and N were again measured and used to calculate corresponding C:N ratios. Samples were compared with potential OM sources using known $\delta^{15}N$ and $\delta^{13}C$ values of epiphytes, *Z. muelleri* and filamentous algae from the sampling site (Andersen et al. 2005), and C:N values also from the site (Jarvis et al. 2014, Carter et al. 2015).

^{13}C-CPMAS solid state nuclear magnetic resonance (NMR) spectrometry

A single sample from each treatment (from the 6–10 cm burrow depth)was also analysed using solid-state ^{13}C- Cross-Polarisation Magnetic-Angle Spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Baldock et al. 2014). A 200 Avance spectrometer (Bruker Corporation, Billerica, MA, USA) fitted with a 4.7 T (wide-bore superconducting magnet; resonance frequency of 50.33 MHz) was used to obtain sample spectra. Pre-weighed sediment samples (200–400 mg) were filled into zirconia rotors (7 mm diameter) with Kel-F® end caps and spun at 5 kHz. Scans (20,000) were obtained for each sample using cross-polarisation ^{13}C-NMR (CP) analysis with a 90° pulse of 3.2 μs at 195 W, using 1 ms contact and 1s recycle time. An inversion recovery pulse sequence was used to establish the period of the recycle delay, which was determined to be five times that of sample specific T_1H values. The processing of all obtained spectra followed methods described in Baldock et al. (2014) using the Bruker TopSpin 3.1. The total signal intensity for each sample was allocated into regions of chemical shift: alkyl (45 to 0 ppm); N-alkyl/methoxy (60 to 45 ppm); O-alkyl (95 to 60 ppm); di-O-alkyl (110 to 95 ppm); aromatic (145 to110 ppm); O-aromatic (165 to145 ppm); and amide/carboxyl/ketone (215 to 165 ppm).

NMR spectral intensities for each chemical shift region were transferred into a terrestrial soils molecular mixing model (Baldock et al. 2004) in order to predict macromolecule content (carbohydrate, lignin, lipid, protein carbonyl and charcoal) of

the sediment. Data produced by the model was constrained with the elemental C and N contents of the sediment.

Statistical analysis

Sediment C_{org} was analysed using univariate (two-factor) ANOVA. Treatment (burrow gender) and sediment depth were treated as fixed independent variables. Both C:N, and $\delta^{13}C$ and $\delta^{15}N$ ratios of sediment collected from the 6– 10 cm depth were compared using separate one-way ANOVA's, with treatment as the fixed factor in both cases. Levene's test for homogeneity of variance was performed on data prior to analysis. Tukey's post-hoc test was used to establish which variables produced a significantly different interaction.

To compare OM composition (OM quality *via* TGA analysis) between the gender of crabs constructing burrows and control sediment, samples were transformed using a square root transformation, and then ordinated using multi-dimensional scaling (nMDS) in PRIMER (version 6) software, with a stress value < 0.2 considered acceptable. The Euclidean Distance Resemblance Matrices were used to develop associations between burrow type. Differences between the sediment OM compositions were examined using a one-way Analysis of Similarities (ANOSIM).

Statistical tests were not applied to the NMR mixing model results.

Results

Organic carbon in burrow sediment

Sediment C_{org} (Fig. 1.1a) in burrows differed significantly according to burrow type ($F_{2, 0.03} = 3.117$, $p = 0.048$). Female burrows contained a significantly higher amount of C_{org} compared to male burrows ($p = 0.041$). There was no significant difference between female or male burrows, and control (undisturbed) sediment ($p = 0.872$; $p = 0.128$ respectively). The 6–10 cm zone (terminal burrow chamber) in female burrows contained a significantly higher concentration of C_{org} compared to the same depth in both male burrows ($p = 0.039$) and in control (undisturbed – without bioturbators) sediment ($p = 0.009$). There was no difference between male burrows and control sediment at this depth ($p = 0.706$). Additionally, there was no difference in C_{org} concentration among burrow types or control sediment at any other sediment depth ($p > 0.050$).

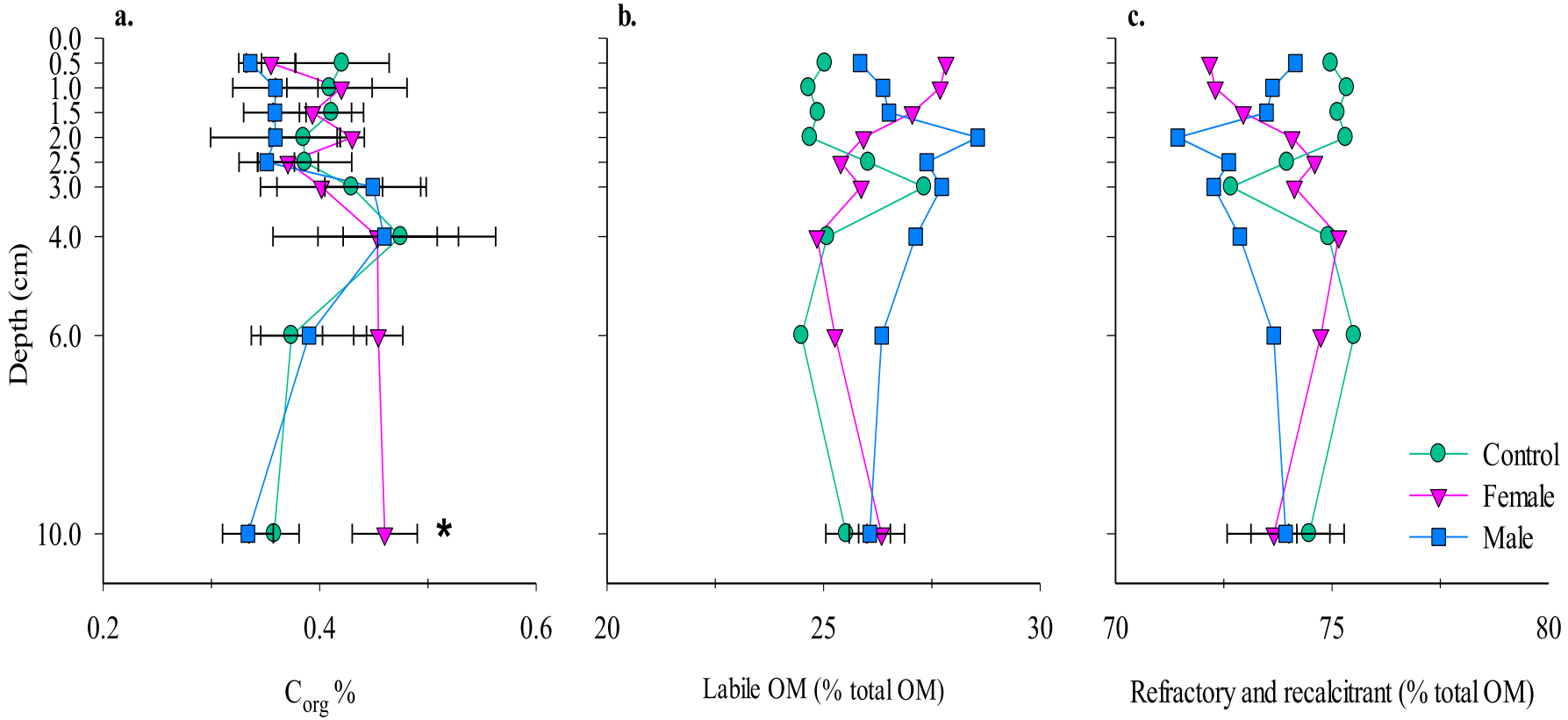

Fig. 1.1: (a) Mean down-core variations (± SE) in organic carbon (%C_{org}), comparing burrows constructed by male and female crabs, to control (undisturbed) sediment (n = 5). Down-core variation in OM content (%) of (b) labile (mass lost between 200–400°C) and (c) refractory and recalcitrant OM (mass lost between 400–550 and 550–650 °C respectively), among male and female burrows, and control (undisturbed) sediment. Depths at 6–10 cm (n = 5) are mean ± SE. An "*" indicated where significant differences lie.

Thermogravimetric analysis (TGA) of burrow sediment

The one-way analysis of similarities (ANOSIM) test on OM quality analysis (as measured *via* TGA) indicated a significant difference in OM quality among the burrow types (R = 0.266, $p = 0.003$, perm = 999). Male burrows showed a clear difference from control sediment (R = 0.478, $p = 0.001$, perm = 999) and female burrows (R = 0.319, $p = 0.005$, perm = 999). There were no difference observed between control sediment and female burrows (R = -0.012, $p = 0.463$, perm = 999). Sediment profiles displayed a greater proportion of labile OM (see Fig. 1.1b) at the surface (0–1 cm) of female burrows, compared to male burrows or control sediment. Comparatively, male burrows appeared to contain a higher proportion of refractory and recalcitrant OM (see Fig. 1.1c) in the same burrow areas. Within the 6–10 cm depth, no significant difference was observed in the amount of labile ($p = 0.401$) or refractory and recalcitrant OM ($p = 0.401$) between burrow types or sediment types. The differences observed between male and female constructed burrows, and control sediment were supported by MDS ordinations (Fig. 1.2).

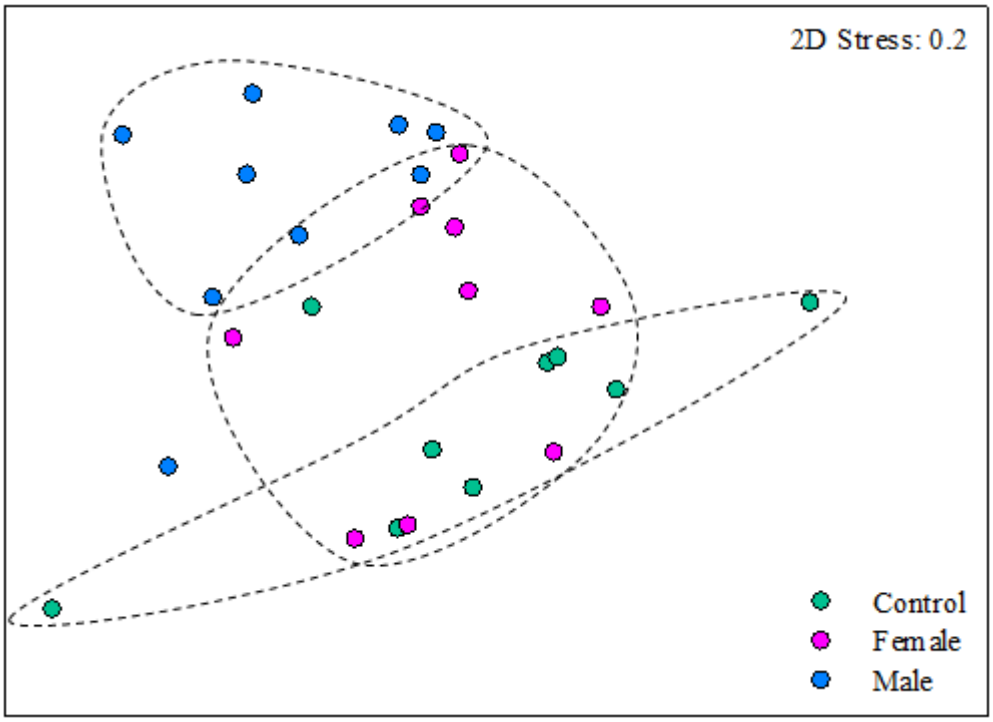

Fig. 1.2: Non-metric Multi-Dimensional Scaling (MDS) ordination plot of sediment OM composition (thermogravimetric analysis) of all depths within a subset of female and male constructed burrows, and control (undisturbed) sediment. Plot is based on a Euclidean Distance Matrix of similarity among treatments. Kruskal's 2D stress value of 0.2 indicates that the configuration is a sufficient representation of dissimilarity among the three treatments. Dashed lines indicate where treatment types are approximately clustered, and do not indicate statistical grouping.

Stable isotope analysis and C:N ratios of sediment

Stable isotope analysis (Fig. 1.3a) suggested that most of the C and nitrogen (N) in all burrow types (male and female burrows, control sediment; 6–10 cm depth) came from seagrass and filamentous algae sources. Female burrows appeared to have a high amount of seagrass-derived C, while both the undisturbed (control) sediment and male burrows appeared to have a mixture of sources. Similar results were obtained when C:N ratios and $\delta^{13}C$ data were compared (Fig. 1.3b). A similar signal (comparison of both C:N ratio and $\delta^{13}C$) for *Z. muelleri* was observed for female burrow sediment, whereas a mixed signal (i.e. filamentous algae and *Z. muelleri*) was observed for male burrow sediment and control sediments. C:N ratios of sediment showed a significant difference among burrow types ($F_{2, 12.030} = 5.837$, $p = 0.024$).Sediment from female burrow sediment showed a significantly difference in the C:N ratio compared to both control (undisturbed) sediment ($p = 0.05$), and sediment derived from male constructed burrows ($p = 0.031$). There was no differences in C:N ratios between male burrow sediment and control sediment ($p = 0.947$). Additionally there was no significant difference among burrow types in $\delta^{13}C$ ($F_{2, 4.53} = 0.384$, $p = 0.692$) or $\delta^{15}N$ ($F_{2, 0.031} = 0.096$, $p = 0.909$).

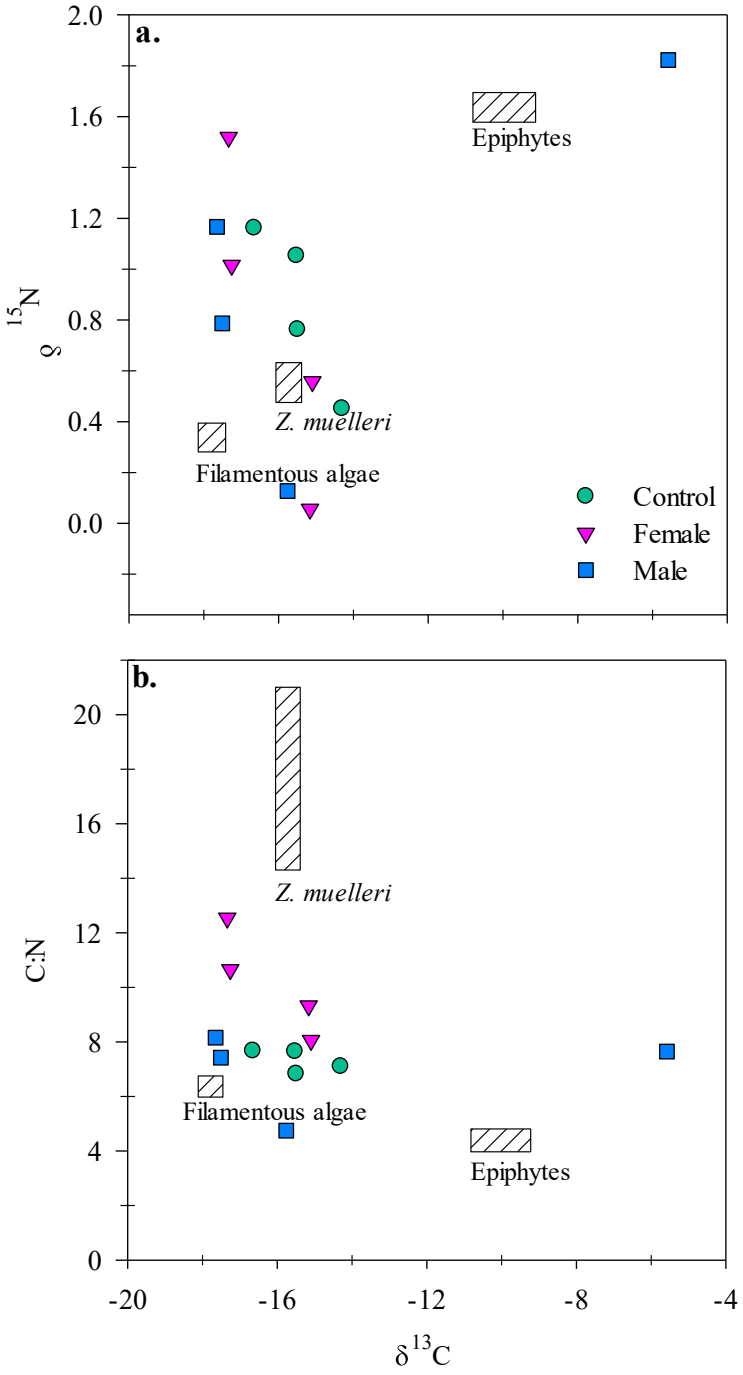

Fig. 1.3: Composition of burrow sediment (6–10 cm depth) from burrows constructed by male and female crabs, and control (undisturbed) sediment. (a) Stable isotope plot of $\delta^{15}N$ against $\delta^{13}C$ values (n = 4) of the sediments compared with reference values (hashed boxes) from potential sources (Andersen et al. 2005); and (b) sediment C:N ratios against $\delta^{13}C$ values compared (n = 4) to reference values (hashed boxes) from potential sources (Andersen et al. 2005, Carter et al. 2015). Only reference values within the range of the analysed samples are shown.

^{13}C-CPMAS solid state nuclear magnetic resonance (NMR) spectrometry

NMR analysis of sediment from the 6–10 cm depth followed by spectral interpretation with a molecular mixing model (Fig. 1.4) indicated that C_{org} components differed among burrow types. Female burrows contained a greater proportion of C in lignin (10.1% mass weight) and lipid (13.6%) and less protein (34.4%) compared to male burrows (0% lignin; 5.0% lipid; 49.5% protein) and controls (1.5% lignin; 6.7% lipid; 45.2% protein). Carbonyl content was greater in control sediments (3.8%) than in female (1.5%) and male (0%) burrows; charcoal and carbohydrate levels were fairly steady across burrow types and control sediment. Comparing the results of the raw NMR spectra, presence of a larger signal intensity at the Alkyl-C (0–45 ppm region; represented as lipids in the mixing model results) peak compared to the O-Alkyl signal (60–95 ppm region); represented as an Alkyl-C/O-Alkyl ratio, was observed in burrows constructed by females (1.34) compared to ratios observed in burrows constructed by males (1.11) or control sediment (1.26).

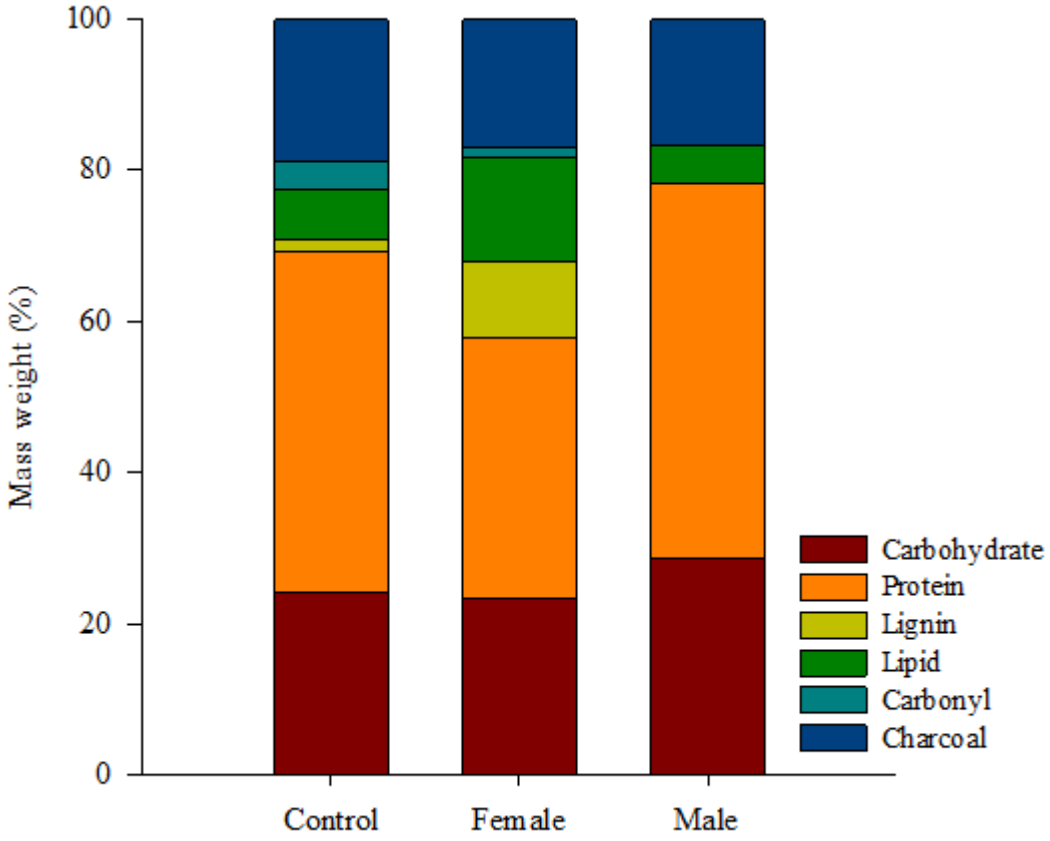

Fig. 1.4: Nuclear magnetic resonance (NMR) spectral molecular mixing model data displaying the predicted macromolecule content (carbohydrate, lignin, lipid, protein, carbonyl and charcoal) of the sediment (6–10 cm depth) of burrows constructed by male and female crabs, and control sediment (n = 1).

Discussion

Coastal seagrass sediment C_{org} comes from a mixture of sources, and can occur as more reactive and degradable OM (labile), and less reactive forms (recalcitrant and refractory) (Burdige 2007). The tougher (refractory and recalcitrant) OM is less susceptible to microbial degradation due to high structural complexity (Trevathan-Tackett et al. 2015), and is therefore more likely to remain in the sediment and contribute to long-term seagrass blue carbon (BC) stocks (Mcleod et al. 2011). Conversely, labile OM is often quickly remineralised by microbes and macro-fauna (Burdige 2007). It is therefore important to distinguish the quality of C_{org} within a system, as well as factors affecting the distribution of C_{org} within the sediment.

The terminal chamber within the fiddler crab burrow has been identified as a hot-spot of activity for females who garden microbes and eventually nest (Christy 1982). We found that there was a larger amount of C_{org} found at this depth (6–10 cm) in female burrows compared to both male burrows and undisturbed sediment, suggesting an additional input source of fresh OM. Fanjul et al. (2014) found that bioturbation by crabs altered the dispersal of OM in other vegetated coastal habitats, by homogenising the distribution of labile C within the sediment profile. NMR data indicated that the C found in female burrows within the terminal chamber had a higher proportion of both lignin and Alkyl-C (particularly the Alkyl-C/O-Alkyl ratio) than that found in male burrows or undisturbed sediment. Coupled with the seagrass stable isotope signature and with no discernible increase in the amount of refractory or recalcitrant OM in the terminal chamber (compared to all other burrow depths), these two results are consistent with both burial and decomposition, (Baldock et al. 1997) and accumulation of more biologically stable components of seagrass within the female burrows.

These results suggest that female fiddler crabs may store or passively trap seagrass detritus within their burrows. Fiddler crabs have been seen to increase benthic metabolism in other BC habitats 2- to 3-fold (Nielsen et al. 2003). Due to this increased metabolic activity associated with burrows, we predict that any labile C_{org} within detritus is likely to be lost from the system as DOC and CO_2 faster when buried or passively trapped by females burrows, than if buried under undisturbed conditions (Fanjul et al. 2014). Recalcitrant C_{org} (i.e. lignin, and lipid-associated Alkyl-C) within seagrass detritus is generally more resistant to degradation than more labile forms of

C_{org} (i.e. carbohydrates) (Trevathan-Tackett et al. 2015); concentration and retention of these recalcitrant compounds may result in some replenishment of sedimentary C stocks. We suggest that modification of the sediment by female fiddler crabs is potentially contributing to changes in the way C is stored within these seagrass meadows.

Males appeared to have no distinct effect on C distribution within their burrows. Male fiddler crabs are known to only occupy their burrows under threat of predation and during high tide (Christy 1982). Less time is therefore spent inside the burrow, and we predict that compared to burrows constructed by female fiddler crab, they have a reduced effect on redistribution of OM and C_{org} within the sediment. Any small-scale effects on sediment OM and C_{org} is likely to occur during burrow creation, resulting in increased surface area and sediment oxygenation.

There are some differences in C:N ratios and stable isotope signals between burrow sediment samples and reference samples. Both $\delta^{13}C$ and $\delta^{15}N$ of seagrass is known to be variable, with seagrass $\delta^{13}C$ in-site variability ranging ±9.6‰, and for $\delta^{15}N$, ±18.3‰ (Fourqurean et al. 2015); therefore we can expect some shift from the control samples used. We can also expect that the detritus within the female burrow's terminal chamber is fresher than that present in either the male burrow or within the undisturbed (control) sediment due to the more active maintenance of burrows, therefore differences in C:N ratios would be expected. Additionally, reduced vegetative C:N ratios, compared to fresh reference samples, is often attributed to lower light levels reaching leaves and increased microbial biomass (Abal et al. 1994, Grice et al. 1996, Holmer and Olsen 2002), both of which are conditions expected within a burrow.

Our results suggest two key effects of fiddler crab bioturbation: (1) the influence of male fiddler crabs on seagrass C stocks is negligible, and (2) in the short-term, females may contribute to changes in sediment C distribution, but these changes may result in loss of stable C stock in the long-term by both increasing benthic metabolism (Fanjul et al. 2014), and altering the natural stratification of labile and more recalcitrant forms of OM. Bioturbation by crabs has been demonstrated to impact C sequestration in coastal sediment, with densely populated mangrove systems having lower C sequestration rates than those with reduced or no crab populations (Altieri et al. 2012, Atwood et al. 2015). The C that is stored in the sediment within our study is at risk of being lost at a faster

rate due to bioturbation by female fiddler crabs. Furthermore, loss of seagrass biomass due to bioturbation is also likely to reduce the C capacity of a meadow by reducing C_{org} available to be buried (Woods and Schiel 1997).

This is the first evidence that a difference in bioturbator gender affects the distribution of C_{org} within seagrass meadows. Bioturbators are already functionally-categorised based on differences in physical morphology, behaviour and activity (Kristensen et al. 2012). We propose that behavioural and physical differences within species should also be considered in regards to bioturbation impact. In our study, differences within one species of bioturbator led to significant differences in both the distribution of OM and associated C within the sediment. Predation of fiddler crabs is heavily biased –males are significantly more vulnerable than females to predation due to greater time spent outside of burrows (Koga et al. 2001). In systems with greater predation, crab populations may therefore be temporarily skewed to be female-dominated, having an influence on C stocks. Globally, we lack sufficient impact assessments of seagrass C stocks in areas affected by bioturbation (Atwood et al. 2015), and we further hypothesise that changes in population structure and distribution may therefore have lasting impacts on seagrass meadow C_{org} stocks and stock assessment.

Chapter 2:

The impact of Callianassid bioturbation on recent versus ancient seagrass carbon stocks

Abstract

Sediment organic carbon within seagrass meadows, known as 'blue carbon', characteristically consists of young, freshly sequestered carbon (C) on the sediment surface, and ancient C deep within the sediment profile. The ability of seagrass meadows to effectively sequester and preserve this blue carbon (BC) is often limited by factors that disturb the sediment structure, oxygen supply, and microbial community. Burrowing fauna (bioturbators) which inhabit seagrass meadows, actively rework and oxygenate the sediment need to be assessed based on their ability to potentially alter the storage of BC both on the surface, and stored deeper within the sediment.

We performed a mesocosm experiment comparing the susceptibility of sediment organic C taken from surface (young) and deep (ancient) seagrass sediments to bioturbation by *Trypaea australiensis* (Callianassidae), a burrowing shrimp. Over 2 months, bioturbation reduced surface sediment C stocks by 35%, while deep stocks appeared to be unaffected by bioturbation. To explain the difference in the vulnerability of surface and deep sediments to bioturbation, we assessed changes in microbial density and sediment oxygen flux (a proxy for microbial respiration and C breakdown).

Our data suggests that bioturbation altered chemical and microbial processes within the surface sediment (420–825 years old), fuelling an increase in C remineralisation. Deep sediment C stocks (1,365 – 5,005 years old) remained constant, indicating that its recalcitrance made it relatively resistant to the biogeochemical changes induced by bioturbation. These results have implications for our understanding of seagrass C stocks, and the vulnerability of surface C stocks to ecosystem change and disturbance i.e. bioturbator populations.

Introduction

Seagrass is one of the most proficient organic carbon (C) sequestration ecosystems worldwide, with meadows storing up to 83 mega-tonnes of atmospheric CO_2 per km^2, up to 90% of which is stored as C within its sediment (Mcleod et al. 2011, Fourqurean et al. 2012). The ability of seagrasses to capture and store this C for long periods of time (centuries to millennia) is largely due to the constant supply and burial of organic matter (OM), and being maintained in anoxic sediment (Mcleod et al. 2011). The monitoring of seagrass environments and accompanying C stocks has recently become a focus for conservation and management (Fourqurean et al. 2012); and identifying drivers of seagrass loss and disturbance, including impediment of C sequestration, have been prioritised. There are however natural and persistent disturbances ubiquitously found in seagrass ecosystems that disrupt conditions associated with long-term C sequestration, and these include bioturbation by burrowing macrofauna (DeWitt 2009).

Bioturbation impacts several key components that C sequestration is dependent upon. The activity of burrowing macrofauna ("bioturbators"), namely through the creation and maintenance of burrows, increases the sediment-water interface (Koo and Koh 2013); which in turn significantly impacts benthic metabolism and OM remineralisation (Heilskov and Holmer 2001). The introduction of oxygenated water *via* bioirrigation, as well as the integration of fresh OM and disturbance of natural sediment stratification (Aller 1982), have the potential to change the long-term storage of C in seagrass ecosystems. Sediment oxygen availability is one of the strongest drivers of sediment microbial communities, being the most favourable electron acceptor in the oxidisation of C (Kristensen and Holmer 2001, Canfield et al. 2005). Mineralisation of C may occur differently when different types of C are exposed to bioturbation, with decomposition of refractory OM seen to increase up to one order of magnitude in the presence of bioturbation (Heilskov and Holmer 2001, Kristensen and Holmer 2001). Bioturbation within coastal environments is a natural process (Kristensen et al. 2012), but recently studies (e.g. Atwood et al. 2015) suggest that the intensity and population density of bioturbation and bioturbating fauna is increasingly globally due to changes in food web structures; namely loss of top-down control. Addressing bioturbation in terms of its effect on C diagenesis and its application to the preservation of seagrass C stocks is therefore imperative.

Within seagrass environments, surface-held C is relatively young (10's to 100's of years old), and is comprised of more labile compounds persisting due to recent sediment deposition and decomposition of detritus maintaining anoxia. C buried deep (cm–m) in the sediment is viewed as a more stable stock due to its resistance to remineralisation (Burdige 2007), is typically ancient (100–1000's of years old) and may be highly refractory and recalcitrant in nature (Hemminga and Mateo 1996, Burdige 2007, Duarte et al. 2013a). As many bioturbators have the capacity to build deep and complex burrow networks (Nash et al. 1984, Kneer et al. 2013), ancient stored sedimentary C may be at risk of being remineralised at a faster rate when exposed to bioturbation. It is therefore important to consider how sediment depth and its direct relationship to sediment age, C content, and OM quality, may be affected differently by bioturbation.

In addition to affecting the geochemistry of the sediment, bioturbation has the potential to vastly affect the sedimentary microbial community. Microbial densities more than 10 times the ambient sediment have been seen within the walls of bioturbator burrows (Papaspyrou et al. 2005), with the sediment oxic/anoxic interface often found to have high bacterial activity (Fenchel and Riedel, 1970; Fenchel and Finlay, 2008). Through integration of plant detritus and bioturbator-driven sediment homogenisation, bioturbation can transfer labile C from the surface to deeper regions, thereby changing organic matter (OM) availability (i.e. detritus) within the sediment (Kristensen et al. 2012). This physical activity is regularly found to be a driver of this increased microbial activity, creating micro-niches of microbial activity (Bertics and Zeibis 2010) and diversity (Laverock et al. 2010), and changing the rate of sedimentary C mineralisation (Christensen et al. 2000). Additionally, the quality of OM within the sediment is without doubt a discriminating factor in determining the composition of the sediment microbial community (Canfield et al. 2005, Bertics and Ziebis 2009).

Thalassinidean shrimp (often referred to as "ghost shrimp" or Callianassids) are a common bioturbator in seagrass environments worldwide (Papaspyrou et al. 2004, DeWitt 2009, Kneer et al. 2013), and have an enormous capacity to impact sediment metabolism and remineralisation sediment through their deep burrowing activity and a large sediment turnover rate (Dworschak 2000). They take advantage of the high-OM content of the seagrass sediment, consuming sedimentary OM and gleaning the sediment of nutrients (Abed-Navandi and Dworschak 2005). They are known to have a

large-scale effect on sediment metabolism, increasing demand for bulk sediment oxygen up to 2-fold (Webb and Eyre 2004), and in some instances include seagrass detritus within the walls and chambers of its burrow (Griffis 1991).

In this study, we aimed to determine the impact of the Thalassinidean shrimp *Trypaea australiensis* on seagrass (*Zostera muelleri*) sediment C sequestration capacity. We ascertain that in terms of their effect on sediment C stocks, the impacts of bioturbation are not only species specific (Kristensen et al. 2012), but also specific to the type of OM (age, depth, quality) that they are exposed to. Our goal was to determine whether surface (young) sediment is more susceptible to C remineralisation *via* bioturbation, compared to deeper (more ancient) sediment. Specifically, we assessed the amount of sedimentary organic C (C_{org}) present within surface and deep sediment, as well as the influence of bioturbation on oxygen consumption and microbial composition (biomass and community analysis) at different burrow depths. Based on burrow density data we then scaled up our results to reflect the potential of large-scale impacts of bioturbators on Australian seagrass C stocks.

Methods

Study sites

Fagans Bay, NSW (-33.434 S, 151.322 E), was used as an estuarine seagrass sediment collection site for this experiment. Fagans Bay is located at the top of the Brisbane Waters estuary, with freshwater inputs from Narara Creek. Fagans Bay itself has a high sediment and nutrient load, with minimal variations in water depth (Gladstone 2006). Previous surveys of the area indicate that as much as 10% of the total seagrass macroinvertebrate community is made up of crustaceans (Gladstone 2006). *Zostera muelleri* is the dominant seagrass species within the bay, with seagrass patches being surveyed for benthic macrofauna density and species composition in February, 2014. When surveyed, no *Trypaea australiensis* were present within the meadow.

An initial field trip (2013) was conducted to sample the *Z. muelleri* meadow within Fagans Bay. Seagrass density was estimated as described in McKenzie et al. (2001) at approximately 80-90% cover at the time of sampling. Sediment cores (PVC, 5 cm diameter) were collected from within the meadow, and collected sediment from each core was sliced and weighed sub-samples were dried at 60 °C for 48 hours. Selected depths were sieved (0–2, 5–7, 25–27, 50–52 and 80–82 cm), and dried sub-samples were analysed for C content (as described below), and C age using radiocarbon dating (^{14}C) using methods described by Stuiver and Polach (1977). Due to pooling of sediment in this study from 1-10 cm and 50–70 cm depths, we assume an age range similar to that estimated from depths 0–7 and 50–82 cm.

Fauna collection and survey

Specimens of *T. australiensis* (henceforth referred to as "Callianassids") were collected from Careel bay, NSW (-33.617 S, 151.326 E). Callianassids are an upwards conveyer deposit-feeding bioturbator (Butler et al. 2009, Kristensen et al. 2012), that constructs complex burrows (typically with two openings) (Katrak and Bird 2003), ranging from < 10 cm (Butler and Bird 2008), to approximately 50 cm in depth (Stapleton et al. 2001). Individuals were extracted from the sediment at low tide from areas surrounding a *Zostera muelleri* and *Halophila ovalis* meadow using commercially available steel yabby-pumps (Wilson 30" EVA Grip Bait Pump). Approximately 20 individuals (35–40 mm in length) were collected, with juveniles and egg-carrying females excluded from

collection. Individuals were transported to the laboratory in seawater, where they were held for 72 hours in separate tanks containing artificial seawater to allow for digestive purging.

Two sites were surveyed for Callianassids, and compared to corresponding seagrass density. At Careel bay, NSW and Palm Beach, NSW (-33.587 S, 151.324 E), transect lines were run from the shoreline for 50 m through seagrass meadows dominated by *Z. muelleri*. At alternating sides of the transect line, at 2 m intervals, 50 cm^2 quadrats were placed on the seagrass. Active burrow openings (mounds, with fresh sediment on the area surrounding the opening) were counted, and corresponding seagrass density was assessed as described in McKenzie et al. (2001).

Sediment collection

Estuarine seagrass sediment samples were collected from inside a *Z. muelleri* meadow located at Fagans Bay, at low tide in March 2014. Approximately 75 L of top sediment (top 10 cm) was collected with sterilised steel shovels, with the uppermost sediment layer (approximately 0.5–1 cm) removed before sampling to ensure that highly labile benthic microalgae were excluded from collection. An additional 40 cm of sediment was removed from the surface, to a depth of 50 cm. From here, a further 75 L of deep sediment (50–70 cm depth) was collected. Both sediment types (surface and deep) were sieved (2 mm sieve) into separate bins, and topped with fresh seawater. Any visible seagrass leaves and rhizomes as well as fauna were removed during sieving. An additional 75 L of seawater (salinity of 28, pH of 8.1) was collected from Fagans Bay for use in the mesocosm experiment. Sediment and additional collected seawater were held (aerated) in dark conditions.

Mesocosm conditions

The deep sediment was distributed into 24 aquaria tanks (8 L capacity, 30 cm deep, 40 x 20 cm surface area), and left to settle and compact for 3 day, forming a 10 cm “deep” layer. Surface sediment was then added on top, to a total sediment depth of 20 cm in each tank (reflecting a mid-range burrow depth; Stapleton et al. 2001, Butler and Bird 2008). Seawater collected from the study site was then added and aerated with sterilised aerators, after which the sediment was left to settle and compact for a further 48 hours. Using 60 mL syringe cores, sediment cores were taken from each tank to establish

initial sediment characteristics (total C_{org}, bulk density and quality of OM). Finally, each of the treatment aquaria were populated with one Callianassids (0.5–0.6 g w/w) and left undisturbed for 10 weeks to allow for burrow construction. Callianassids were not fed (Bertics and Ziebis 2010), with only fresh distilled water being added to the aquaria to maintain a salinity of approximately 28. Aquaria were exposed to daily light cycles (12 h) *via* sunlight, and a constant temperature of 22 ˚C (reflective of seasonal *in situ* conditions).

Potential oxygen distribution and consumption

After the 2 month incubation, syringe cores (60 mL, cut lengthways and glued back together using soft silicon glue) were used to sample the sediment and burrows. Treatment cores (+Callianassids) were taken directly over burrow openings, with defaunated (control) cores taken randomly from within the aquaria. Cores were removed and sliced along the sealed vertical cut on the syringe using a nylon string, which allowed for minimum disturbance of the sediment and vertical cross-section of each burrow (Koo and Koh 2013). One half of each core was sequentially introduced into a flow-cell with constant-flowing (2 cm s^{-1}) temperature controlled seawater at 22 ˚C. To ensure uniform flow conditions over the sediment the core was aligned with the flow direction. The sediment was then left to acclimatise within the set-up for approximately 30 minutes to allow for oxygen gradients to stabilise.

Sediment oxygen profiles were measured using an oxygen microsensor (Unisense OX50; Unisense A/S, Aarhus, Denmark) with a 50 μm tip. The microsensor was connected to a 4-channel multimeter (Unisense A/S, Aarhus, Denmark) interfaced with a PC running data acquisition software (SensorTrace PRO, Unisense A/S, Aarhus, Denmark). During operation, the microsensors were mounted on a motorized micromanipulator (Unisense A/S, Aarhus, Denmark) controlled by dedicated software (SensorTrace PRO, Unisense A/S, Aarhus, Denmark). The electrode was linearly calibrated in 100% air-saturated seawater and anoxic seawater (amended with the O_2 scavenger sodium dithionite according to manufacturer) at experimental temperature (22 ˚C) and salinity (28). The O_2 concentration of the sediment was measured by introducing the microelectrode into the sediment in 100 μm increments. Oxygen profiles were taken (where possible) in both the surface and deep portions of the sediment, at least 2 cm from the deep/surface interface. Surface and deep sediment was identified by

the clear difference in sediment colour, as well as sample location. In treatments containing fauna (+Callianassids), oxygen profiles were conducted both within the burrow wall, and within the ambient sediment surrounding the burrow (~ 2 cm from the burrow wall). In defaunated cores, profiles were taken at random from within the surface and deep portions of the sediment cores. Given the destructive nature of this sampling, all rates are referred to as "potential" rates of oxygen consumption and penetration.

Total sediment organic carbon

To determine any change in elemental organic C (total C_{org}), initial cores (taken before introduction of Callianassids), and half of the core sliced for oxygen profiling (final cores), were sliced in half, pooling the sediment according to sediment depth (surface or deep). Samples (approximately 1 g each) were prepared by drying the sediment (60 °C for 48 hours) and measuring net water loss *via* weight change to determine dry bulk density (DBD; Mg m^{-3}). The sediment was ground with a mortar and pestle and sub-samples of each sediment section (0.5 g each) were acid washed with 1M hydrochloric acid (HCl) for 24 hours to remove any inorganic compounds. HCl was washed out of the sediment with double distilled water, and samples were re-dried (60 °C for 48 hours) and ground using a clay mortar and pestle. The C_{org} content of each sample was measured using a CN analyser (LECO TruSpec, LECO Corporation, St. Joseph, MI, USA).

Organic matter quantification

To quantify the quality of sediment organic matter (OM) with depth, sub-samples of dried, non-acidified sediment (0.05 g each) were measured using a thermogravimetric analyser (SDT Q600, TA Instruments, New Castle, DE, USA), with a 0.1 µg balance sensitivity. Sediment was taken from cores taken prior to faunal population, and post-incubation. Both the surface and deep sediment was separately pooled and compared. Thermogravimetry accounts for the presence of other compounds (i.e. oxygen) in its analysis, therefore represents OM, and is intrinsically different to the elemental analysis of C_{org}.

Dried sediment samples were placed inside a platinum cup, and heated to 900 °C under air flow (50 mL min^{-1}) at 20°C min^{-1}. The first sediment range interval (labile OM) ranged from 200 to 400 °C, followed by the second (recalcitrant OM) from 400 to 550 °C, and third (refractory OM) from 550 to 650 °C (Lopez-Capel et al. 2005). For analysis, recalcitrant and refractory sediment portions were pooled. Sediment mass loss within each temperature interval was identified and quantified using Universal Analysis software (TA Instruments, New Castle, DE, USA), within specified temperature ranges (Lopez-Capel et al. 2005).

Microbial abundance

Samples for microbial abundance were taken immediately prior to the final (10 week) sediment core sampling, by inserting 1.5 mL cut-off syringes into the sediment and sampling to a depth of 0.5 cm. In faunated treatments (+Callianassid), samples were taken immediately (< 5 mm) adjacent to the mound burrow openings, while in control aquaria samples were taken at random locations on the surface. Briefly, sediment samples were transferred into 2.0 mL cryotubes (Sarstedt AG & Co.), flash frozen in liquid nitrogen and stored at–80 °C. 1 mL of filtered (0.22 mm) seawater containing glutaraldehyde (concentration of 0.1%) was added prior to nitrogen flash freezing. Determination of bacterial abundance was performed *via* flow cytometry following the methodology of Trevathan-Tackett et al. (2014), quantifying cell counts using a LSRII flow cytometer (BD Biosciences), and with SYBR Green fluorescence and light side scatter used to identify bacterial cells (Marie et al. 1997, Seymour et al. 2007). Cell abundance and nucleic acid contents of individual bacteria cells were used to discriminate the number of presumed active cells from inactive cells (Lebaron et al. 2001) using Flowing Software (ver. 2.5). This process of discrimination separates microbes with high nucleic acid (HD-DNA) content from those with low nucleic acid contents (LD-DNA).

Microbial community analysis

Samples from the surface sediment were taken for microbial community analysis prior to faunal addition, and post-incubation. Samples were taken from the same locations (see above) and frozen in the same way as for microbial abundance analysis but without glutaraldehyde addition. Cells were extracted from the sediment using a diversity assay PowerSoil DNA Isolation Kit (MoBio Technologies, CA, USA), and tested for DNA presence using a nano-drop spectrophotometer (Thermo Fisher Scientific). Amplification of the extracted DNA was performed by targeting the V4 variable region within the 16S rDNA gene, with the 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5′GGACTACVSGGGTATCTAAT-3′) Eubacteria primers (Molecular Research, Shallowater, Texas, USA). Sequencing of amplified DNA was performed using an Illumina MiSeq platform, and further processed using the QIIME (Quantitative Insights Into Microbial Ecology) package, following the manufacturer's guidelines

(www.mrdnalab.com, TX, USA). Operational taxonomic units (OTUs) were compared against the Greengenes database (DeSantis et al. 2006) which allows for comparison and alignment of thousands of available 16S rRNA genes, using BLAST to assign taxonomy.

Scaling-up of data

Soil C density was calculated using dry bulk density (DBD; mass of dried sediment divided by the total sediment volume) and sediment layer depths. A per-burrow rate of sediment C loss for the top 10 cm of sediment was calculated assuming that maximum annual sediment C loss had occurred within the 2 months allowed for in this study; therefore rates were treated as a yearly rate. Using the per-burrow rate of loss, a calculated mean, as well as low and high rates of loss were established. Burrow density (using the observed number of active mound openings) was used to extrapolate a rate of C loss per hectare of seagrass and compared against the average Australian net rate of seagrass C sequestration (Lavery et al. 2013).

Statistical analysis

Field seagrass density and bioturbation intensity (number of active burrows) was analysed by pooling both sites (Careel Bay and Palm Beach), and performing a non-linear regression analysis, using a 3-parameter sigmoidal equation which provided the best fit for the data, within SigmaPlot (Ver. 12). Spearman's rank-order correlation was then applied to determine the nature of the relationship. Sediment C_{org} and OM composition (OM quality *via* TGA analysis) were analysed using univariate (two-factor) repeated-measure ANOVA. Time and treatment (± fauna) were treated as fixed independent variables. High density DNA (HD-DNA) microbial cell counts, and sediment net respiration (measured as diffusive exchange of oxygen ; DOE) were analysed using separate one-way ANOVA's. Sediment treatment (± fauna) and sediment depth (DOE only) were used as independent factors. Levene's test for homogeneity of variance was performed on data prior to analysis. When appropriate, Tukey's post-hoc test was used to establish which variables produced a significantly different interaction. Tests were performed using IBM SPSS Statistics (Ver. 22) with a significance level of $\alpha = 0.05$.

To compare bacterial community composition (bacterial rarefied, class level) between treatments and across time, reads per sample was initially used to normalise each sample, with sample data then transformed using a square root transformation, compared using Bray-Curtis similarity between profiles, and then ordinated using multi-dimensional scaling (nMDS) in PRIMER (version 6) software. A one-way analysis of similarities (ANOSIM) was used to determine if any differences in assemblages between treatments (± fauna) were significantly different. Similarity percentage analysis (SIMPER) was used to identify the class-level ordinated taxonomical units (OTUs) controlling any composition shifts.

Results

Study site sediment C age and content

Clear trends between sediment age and Corg content were observed at the field collection site (Fagans Bay) seagrass meadow. Sediment Corg (Fig. 2.1a) content generally decreased with depth, varying from 3.3% Corg at the sediment surface (0–2 cm) to 1.4% Corg at the deepest sampled point of 80–82 cm. The age of the seagrass sediment sampled varied between 420 and 5005 years in the top 82 cm (Fig. 2.1b). Respective to sediment used in the mesocosm portion of this study, sediment from the "surface" (including the 0–2 and 5–7 cm sampled depths) varied between 420 and 825 years old. Deeper sediment (including the 50–52 cm depth, and stopping short of the 80–82 cm sampled depth) was aged between 1,365 and 5,005 years respectively.

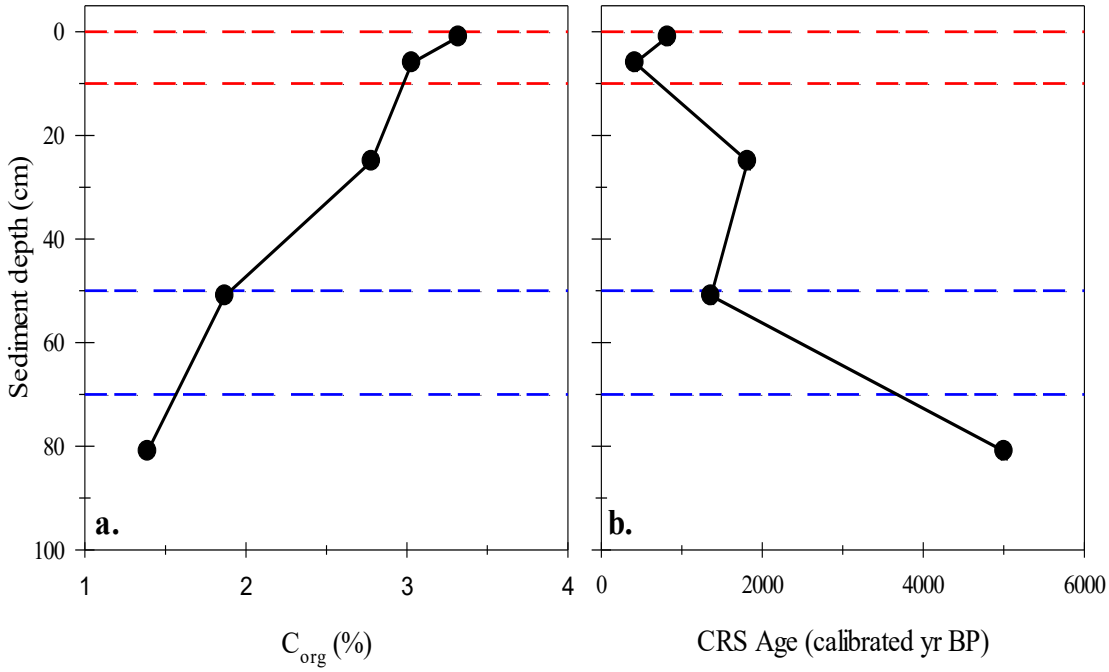

Fig. 2.1: Relative (a) organic carbon (Corg %) content and (b) age (constant rate of supply; CRS Age) of sediment at Fagan's Bay, respective to depth from sediment surface. Dashed lines indicate the surface (red) and deep (blue) sediment depths that sediment was collected from. Due to the pooling of collected sediment in this study from 1–10 cm and 50–70 cm depths, we assume an age range similar to that estimated from depths 0–7 and 50–82 cm. Symbols represent means ± 1 SE (n = 3). In some places, error bars are too small to be visible.

Faunal field survey

Based on the number of active burrows observed (Fig. 2.2), there was no significant correlation between bioturbation burrows (m^{-2}) and seagrass cover (%) ($r_{s\ 78}$ = -0.195, p = 0.087). In areas with seagrass density > 40%, there was an average of 36 active

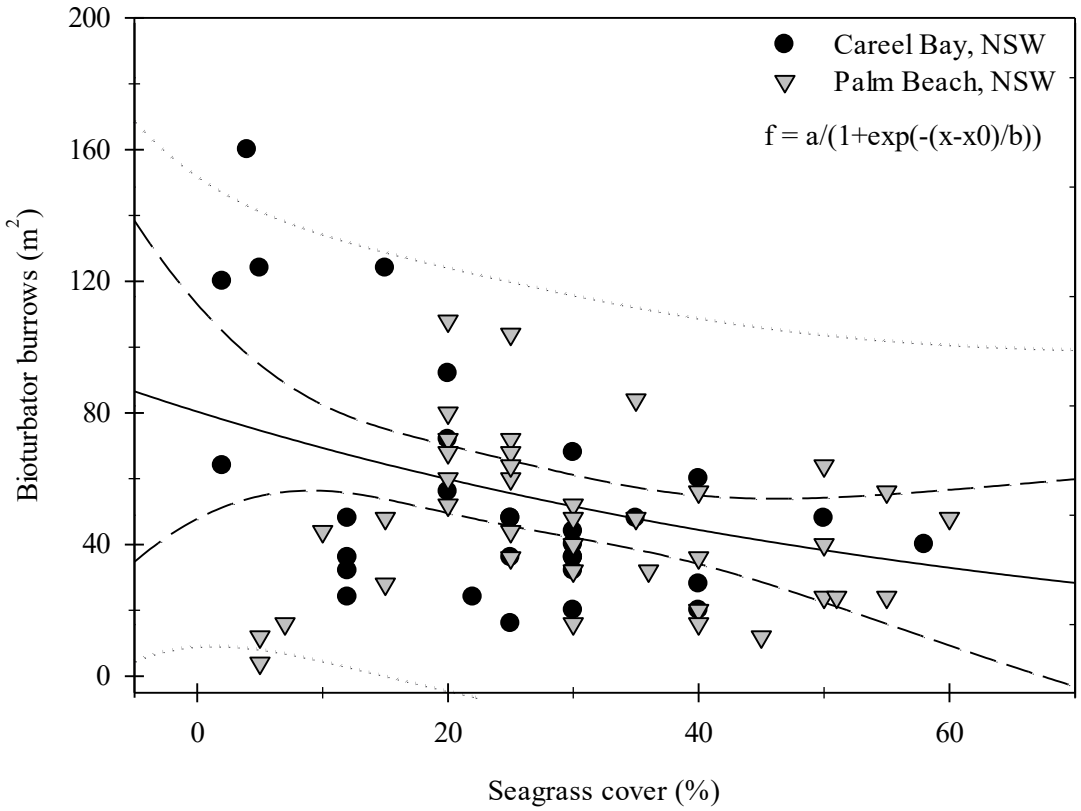

Fig. 2.2: Non-linear regression of seagrass density to density of active Callianassid burrow openings, at two sites (proximal to Sydney, AUS), showing an approximate 95-percent confidence envelope (dashed line) and predictor variable envelope (dotted line). The 3-factor equation of the regression line is given.

Total sediment organic carbon

The presence of *Trypaea australiensis* decreased surface sediment C_{org} (Fig. 2.3a) over time by > 35% ($F_{1, 1.556} = 11.197$, $p = 0.010$), with the decrease also being significantly greater than control (defaunated) treatments ($F_{2, 1.032} = 4.485$, $p = 0.035$). There were no significant changes over time in the defaunated treatments. Additionally, there were no significant changes in C_{org} within the "deep" sediment (Fig. 2.3b) over time, or among treatments. Overall, the deep sediment layer had significantly less C_{org} initially than the surface sediment layer ($F_{1, 3.199} = 4.823$, $p = 0.031$).

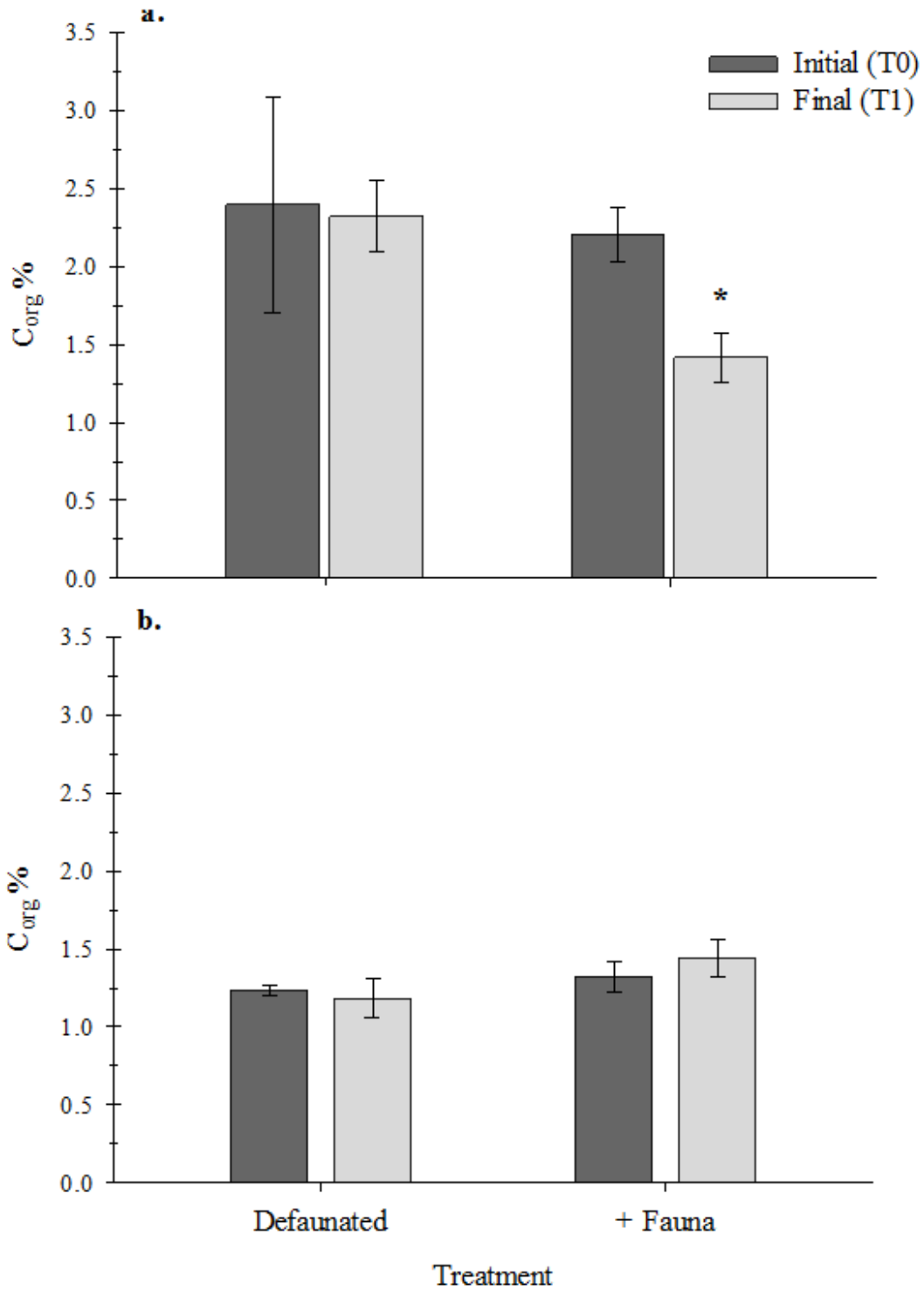

Fig. 2.3: Mean changes ±SE in (a) "surface" sediment; and (b) "deep" sediment C_{org} in faunated (+Callianassid) and defaunated sediment treatments between initial (T0) and final (T1) sampling. An "*" indicates where significant differences among treatments lie. Bars represent means ± 1 SE (n = 5).

Organic matter quantification

Encompassing the quantification of all organic compounds within the sediment (i.e. cellulose, lignin, proteins, etc.), deep sediment contained less overall OM (Fig. 2.4) compared to surface sediment, and therefore overall less labile ($F_{1, 8.562} = 40.796$, $p < 0.001$), refractory and recalcitrant OM ($F_{1, 8.562} = 40.796$, $p = 0.002$). The amount of labile OM in surface sediment (Fig. 2.4a) declined over time ($F_{1, 2.213} = 10.413$, $p = 0.012$). Surface sediment populated with Callianassids lost 35% OM over time, compared to a loss of 16% total OM in defaunated surface sediment. Similarly, the amount of pooled refractory and recalcitrant OM in surface sediment declined over time ($F_{1, 2.027} = 9.487$, $p = 0.034$), with surface sediment populated with Callianassids losing 34% of its refractory and recalcitrant portion, and control surface sediment losing 15% of its labile OM portion over time. There was no significant change in labile or refractory and recalcitrant OM in deep sediment (Fig. 2.4b) over time or with bioturbation.

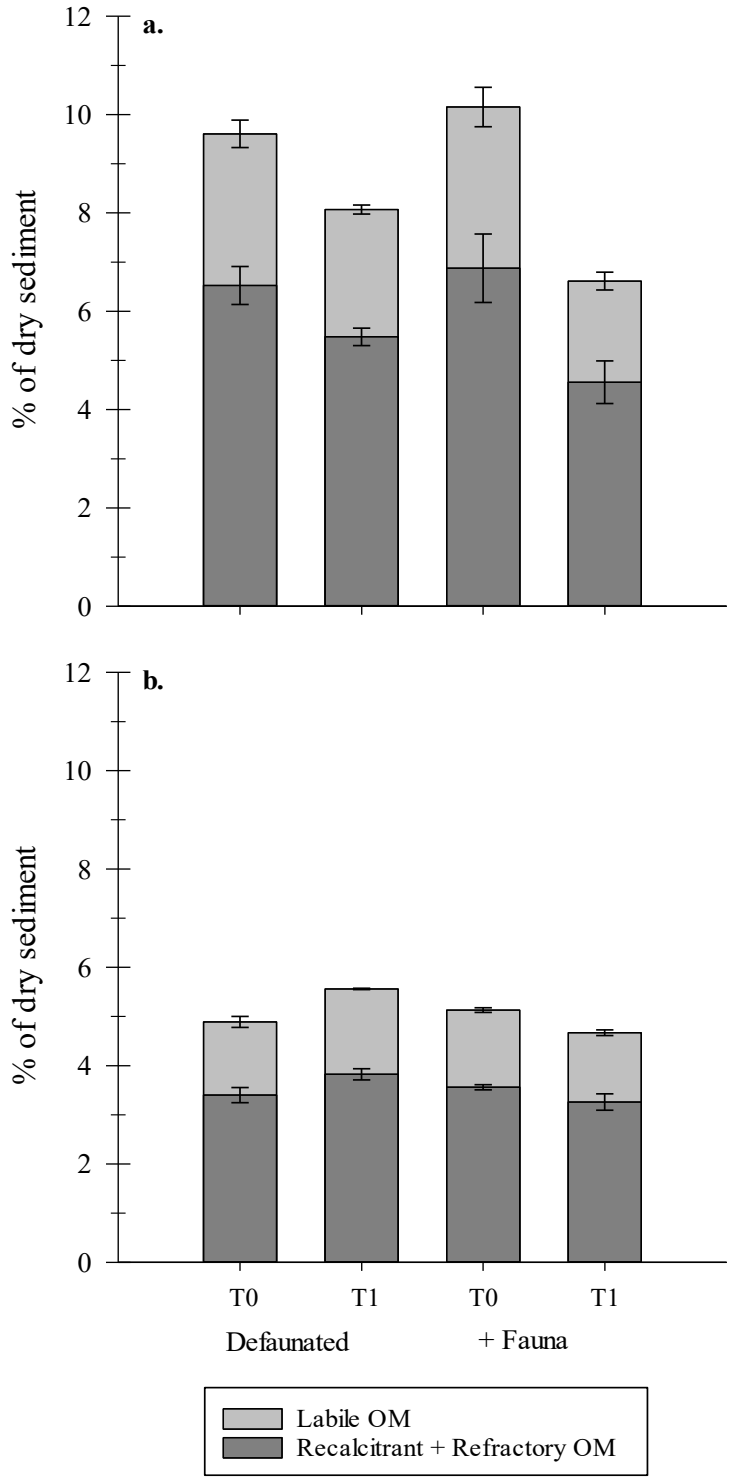

Fig 2.4: Mean changes ±SE in (a) "surface" sediment; and (b) "deep" sediment organic matter (OM) composition in faunated (+Callianassid) and defaunated sediment treatments between initial (T0) and final (T1) sampling. Lighter portion of graph indicates sediment composed of labile OM, while the darker portion indicates sediment composed of both recalcitrant and refractory OM. Bars represent means ± 1 SE (n = 5).

Microbial Abundance and Community Analysis

Bioturbation resulted in a doubling of the number of HD-DNA cells in the sediment (Fig. 2.5), compared to that of defaunated sediment ($F_{2, 1.906} = 8.466$, $p = 0.02$).

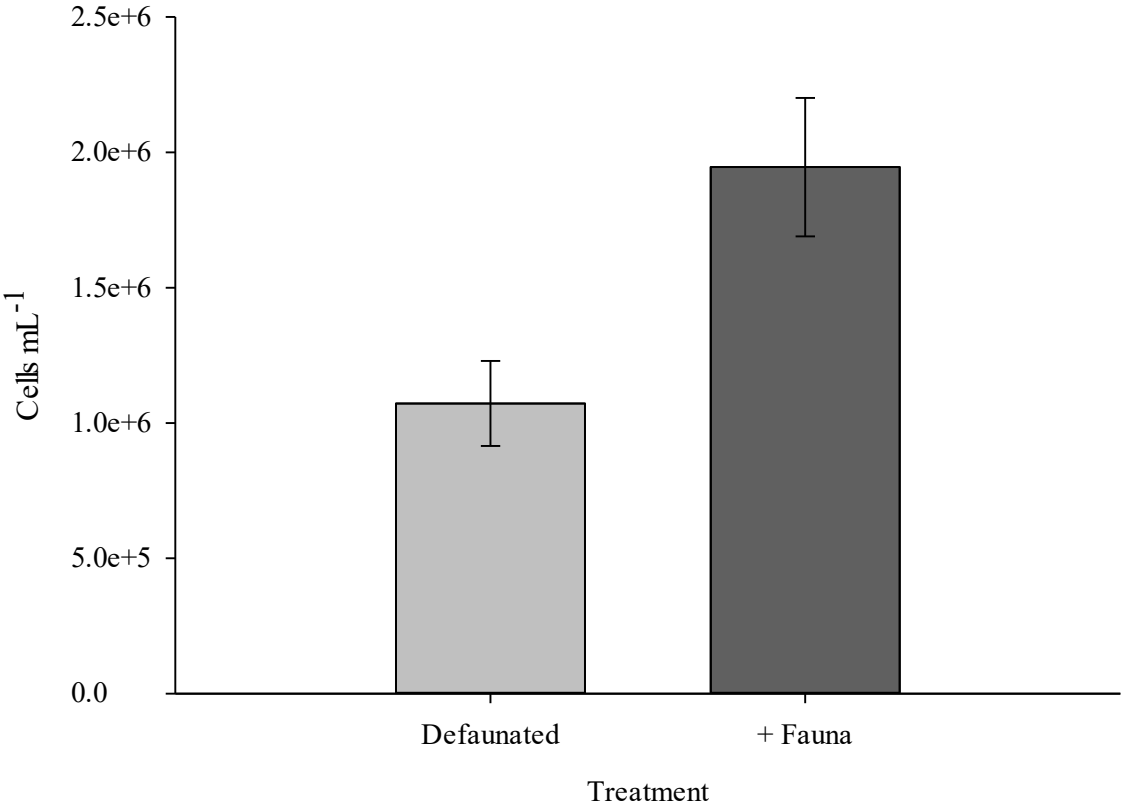

Fig. 2.5: Mean high-density DNA (HD-DNA) bacterial cell counts ±SE in "surface" sediment of faunated (+Callianassid) and defaunated treatments. Bars represent means ± 1 SE (n = 5).

Analysis of the sediment bacterial communities through 16S amplicon sequencing (Fig. 2.6) showed no overall significant differences at the class level over time, or between treatments. However, SIMPER analyses revealed that time had a significant impact on the bacterial community composition in defaunated sediment (R = 0.144, p = 0.008). In those treatments where changes in community composition were observed, there was a trend of increasing importance of *Anaerolineae* and *Epsilonproteobacteria* over time.

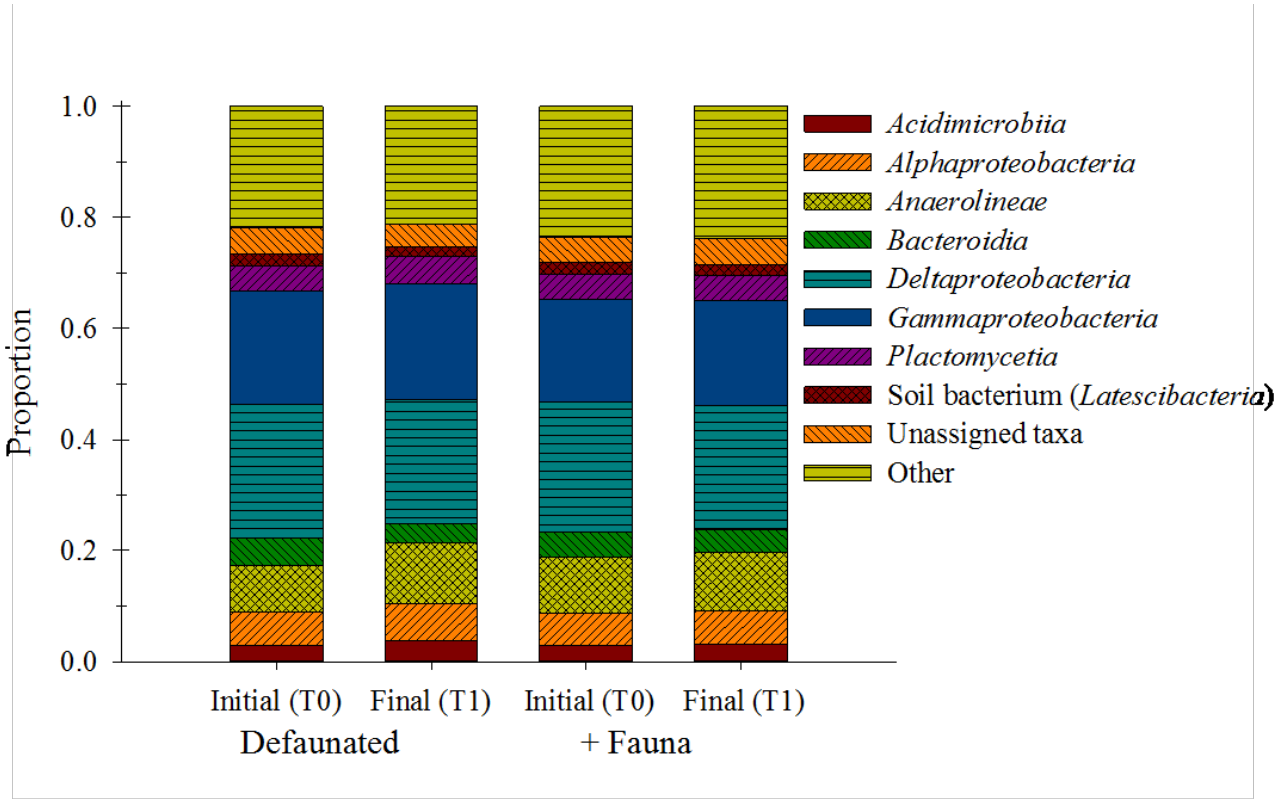

Fig. 2.6: Identities of bacterial communities across time and sediment treatment. Community assemblages are represented as average (n = 5) relative abundance of the top 10 most dominant bacterial classes in faunated (+Callianassid) and control (defaunated) sediments. The category designated 'other' cumulatively represents less abundant taxa (i.e. less than 2%). Bars represent means ± 1 SE (n = 5).

Potential sediment oxygen distribution and consumption

Oxygen penetration (Fig. 2.7) within both the surface (Fig. 2.7b) and deep (Fig. 2.7c) sediment increased with the introduction of Callianassids, with oxygen penetration in burrow walls increasing approximately 25% in both surface and deep sediment.

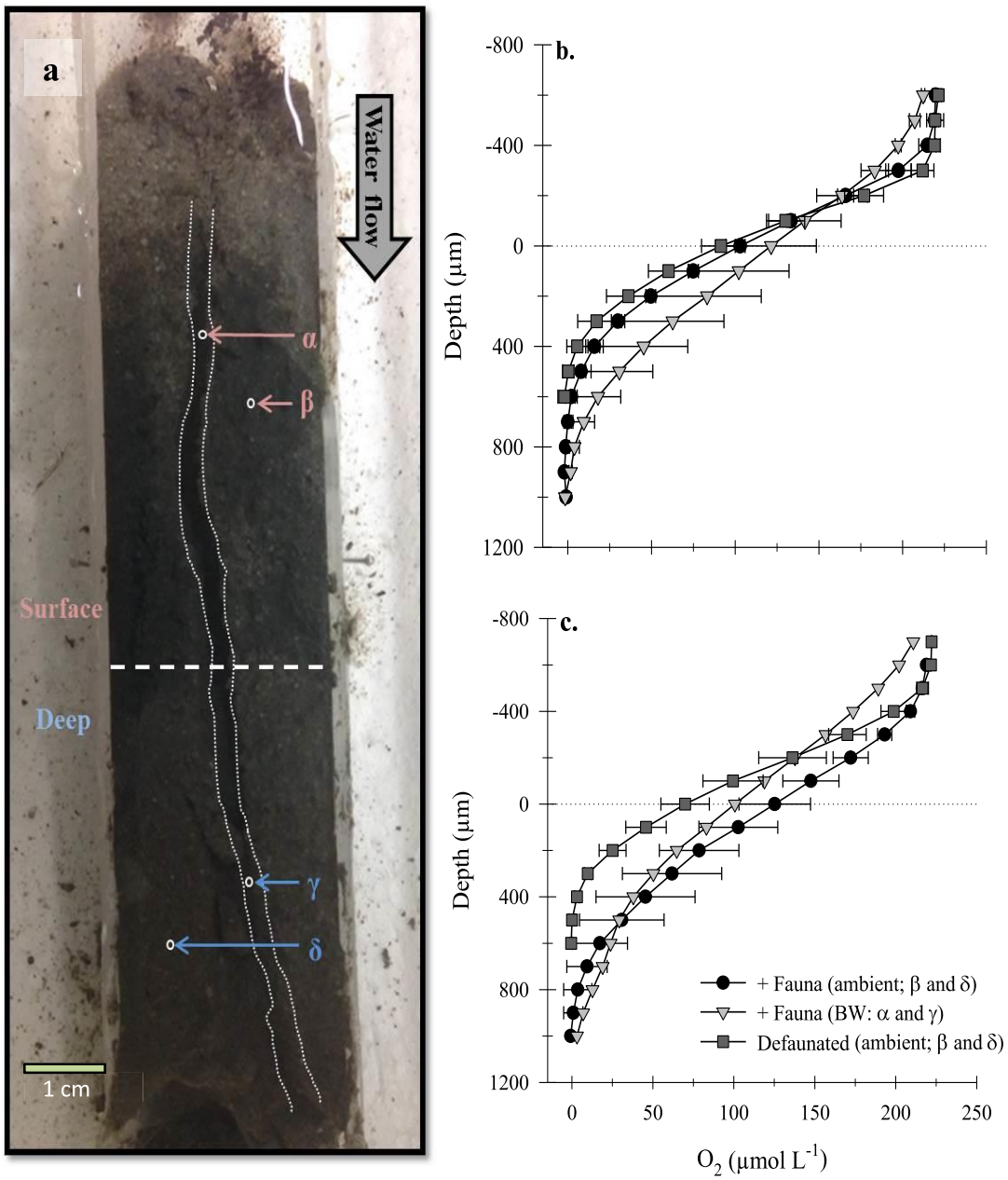

Figure 2.7: (a) Vertical display of a Callianassids burrow (outlined by dotted line) in a sediment core, displaying (red) surface and (blue) deep sediment. Approximate microprofile locations in burrow walls (α and γ) and ambient sediment (β and δ) are shown. The dashed line indicates the approximate interface of surface and deep sediment. Corresponding oxygen microprofiles (n = 2–5) of (b) surface and (c) deep sediment in sediment cores, with ±SD (where applicable) in +Fauna (+Callianassid) and defaunated sediments. Measurements within faunated treatments were taken from within the burrow wall (BW), and from the surrounding ambient sediment approximately 2 cm from the burrow wall ambient). The dotted line (through Y-axis at 0) designates the sediment surface

Net sediment respiration (diffusive oxygen exchange, DOE, Fig. 2.8) decreased significantly ($F_{4, 2624.949}$= 12.926, $p < 0.001$) in both surface and deep sediment populated with Callianassids. Bioturbation resulted in a net decrease in sediment respiration by ~32 and ~35% in the surface and deep sediment, respectively.

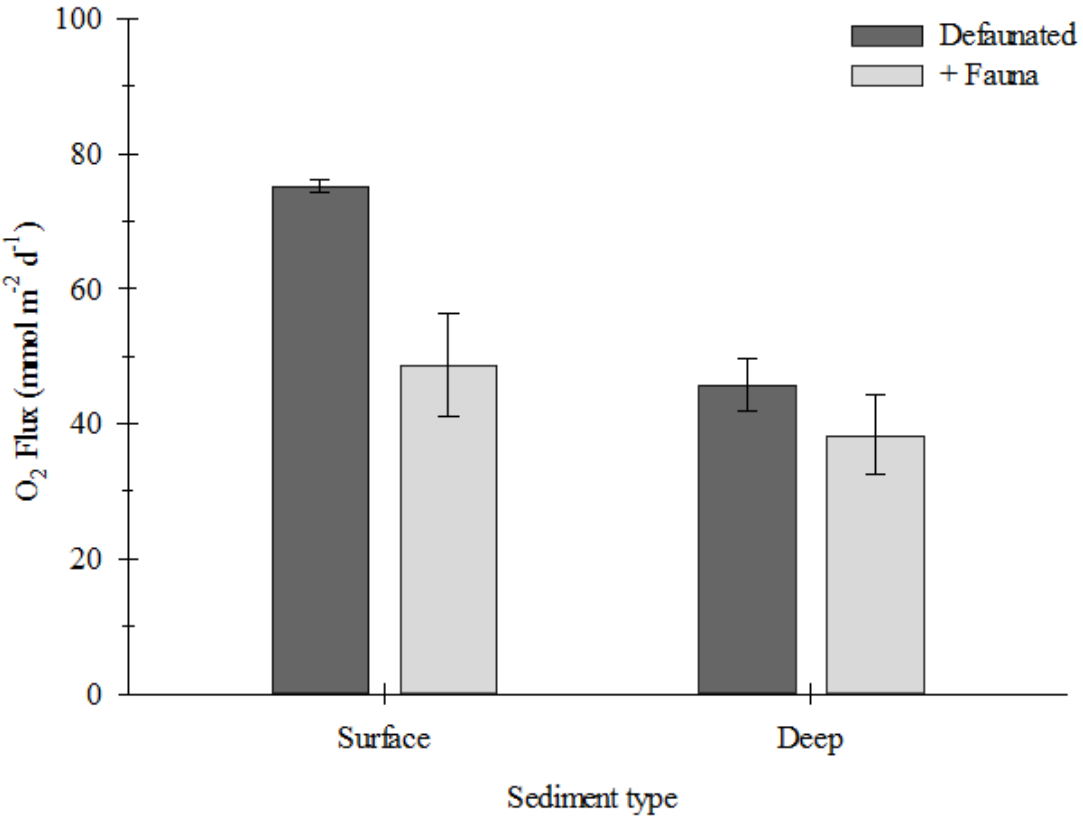

Fig. 2.8: Mean net sediment respiration (calculated as diffusive oxygen exchange) ±SE of surface and deep sediment, in defaunated and faunated (+Callianassids) treatments.

Scaling up of data

Scaled-up the results of this study (Fig. 2.9) reflected high sediment C loss. According to this model, and considering average densities of Callianassids observed in the field (30–35 burrows per m^2), Callianassids bioturbation can potentially result in a loss of 0.060–0.876 Mg C ha^{-1} y^{-1} (depending on faunal biomass and activity), with a probable loss of 0.293 Mg C ha^{-1} y^{-1} for average densities of Callianassids around Sydney, Australia.

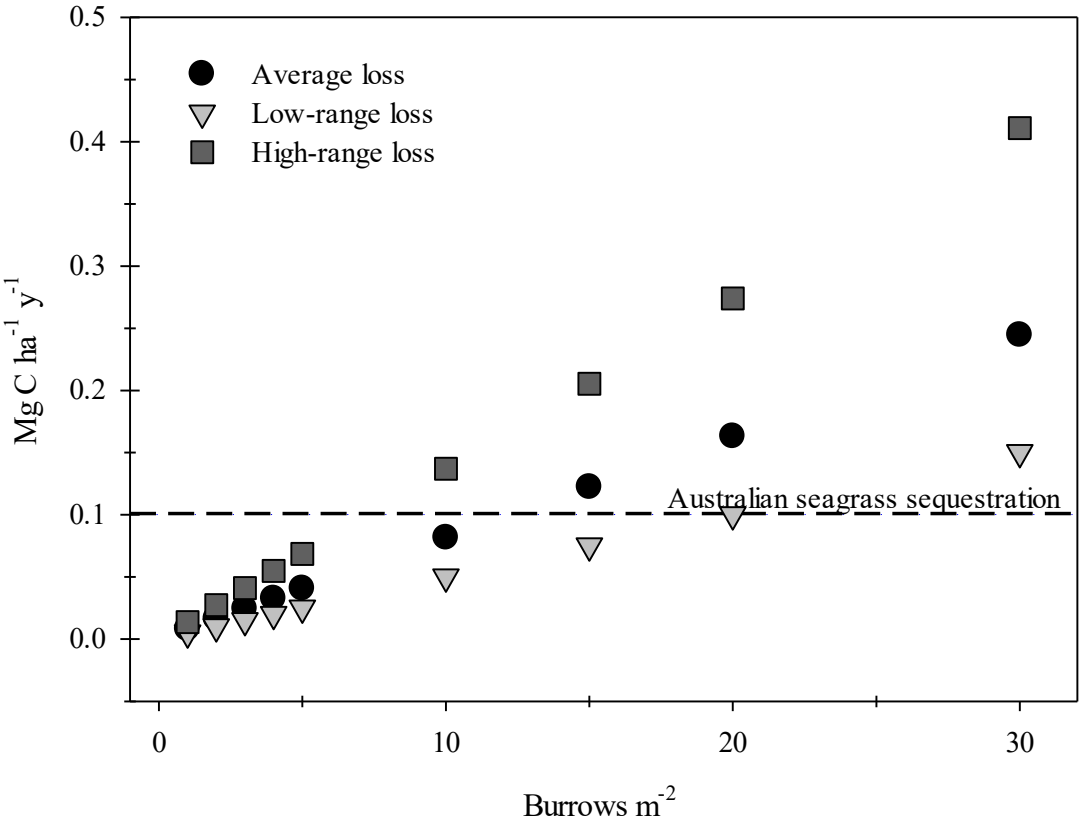

Fig. 2.9: Predicted loss of surface sedimentary C_{org} due to Callianassids bioturbation, at different burrow densities, compared to the Australian seagrass annual rates of C sequestration. Australian seagrass sequestration calculated from published data (Lavery et al. 2013). Average loss reflects the average calculated loss of C_{org} that 1 burrow would lose annually, as seen in this study. Low-range and high-range loss values are calculated based on the lowest and highest (respectively) loss per burrow of C_{org} seen in this study. All loss values are scaled up according to densities per m^2.

Discussion

Macrofauna living within coastal environments, especially within 'blue carbon' habitats, hold global significance given their distribution within seagrass meadows (DeWitt 2009). Within this study, we found that due to the effects of bioturbation by Callianassids, large portions of surface sediment C_{org} were lost, while deep sediment C_{org} remained relatively conserved. We therefore propose that the loss of surface C_{org} in this study indicates that sediment surface stocks in seagrass meadows is particularly vulnerable to bioturbation.

Typically, C found within deep (ancient) marine sediment is more resistant to microbial degradation due to its recalcitrance (Burdige 2007), and in this study, deep sediment (1,365 – 5,005 years old) appeared to be stable against the impact of bioturbation. Conversely, C stocks in surface sediment (420–825 years old) reduced significantly when exposed to bioturbation. Increased rates of benthic metabolism are common in areas populated by Thalassinidean shrimp (Papaspyrou et al. 2004, Webb and Eyre 2004), and it appears that in this study, increased sediment metabolism (displayed *via* changes in microbial density and oxygen consumption), is likely to be the driving factors in the reduction of C_{org} in surface sediment. We can elicit that the observed stability of deep sediment C stocks against the effects of bioturbation is a result of this deep C being more recalcitrant, being exposed to both reduced oxygenation and reduced microbial activity in comparison to surface sediment, and therefore being relatively resistant to remineralisation.

Although sediment containing Callianassids had a greater microbial biomass after 2 months, we suggest that these microbes were comparatively less active at the time of sampling. An increase in sediment oxygenation caused by bioturbation is the likely the driver behind increased microbial density in faunated sediment (Papaspyrou et al. 2005); however, increased oxygen penetration, like that observed within the burrow walls, is typically connected with a slower influx of oxygen to that sediment due to lower microbial activity (Kristensen 2000). Increases in microbial activity and microbial cell density within burrows immediately after introduction of Callianassids (and thus oxygenation), would have likely resulted in rapid remineralisation of available C (Aller and Aller 1998). A significant reduction in labile OM within sediment containing Callianassids was observed, indicating that remaining C would likely be more resistant

to degradation (Burdige 2007), resulting in a (comparative) reduction in microbial activity and therefore oxygen consumption at the time of sampling. The observed higher microbial density and oxygen penetration in sediment affected by Callianassids are likely to be artefacts of this rapid mineralisation process.

Surprisingly, we did not see any shift in the microbial community structure with the introduction of Callianassids. Typically, introduction of active bioturbators, such as Thalassinidean shrimp, leads to significant shifts in sediment bacterial communities (Laverock et al. 2010). The minimal effect on the microbial community may have been caused by: (1) samples were taken from the surface of the burrow, an area of highly-oxidised sediment, and therefore not that different to defaunated surface sediment (Bertics and Ziebis 2009); and/or (2) surface sediment homogenisation reduced surface microbial biofilms (Pillay et al 2007); and/or (3) microbial communities within sediment exposed to bioturbation most reflect communities from the sediment surface (Laverock et al. 2010), and are therefore no different to control communities. Comparatively, in defaunated sediment, the surface sediment remained undisturbed, and a natural evolution or progression (albeit small) of the bacterial communities was therefore allowed (i.e. increases in *Epsilonproteobacteria*, and *Anaerolineaea*). In order to detect any changes in microbial community with certainty, a more intensive sampling regime, including sampling along the burrow and within burrow chambers is required.

Given the densities of fauna seen in the field, and the low rate of Australian seagrass sequestration (Lavery et al. 2013), bioturbation within coastal C ecosystems may have significant impacts on C storage. In the instance of dense burrowing fauna, stored atmospheric CO_2 (as sedimentary C) which may have been preserved for several hundred years, is at risk of being re-released to the atmosphere as CO_2 *via* remineralisation of surface sediment stocks. In terms of coastal C preservation, the conservation of deep ancient C stocks against bioturbation is beneficial; however, the rapid loss of C from surface sediment may have detrimental effects on the persistence of coastal C stocks. If the quality and quantity of C_{org} preserved within C stocks decreases, this may result in the overall reduction in C stock quality over time

Assessments for bioturbation, and indeed their distribution, only exist for a limited number of species, settings and areas (Teal et al. 2013, Atwood et al. 2015). Bioturbating macrofauna provide many essential ecosystem services to our coastal

environments (Schaffner et al. 1997, Hansen and Kristensen 1998, Kristensen 2000); however, if bioturbator, and indeed specifically Callianassid populations drastically increase, coastal C stocks may be negatively affected (Atwood et al. 2015). The monitoring of seagrass ecosystems and associated C stocks has in recent time become more of a focus for conservation (Fourqurean et al. 2012). Reflecting on the results of this study, we suggest an inclusive approach to ecosystem management; monitoring not only the health and persistence of the meadow and its C stocks, but also the fauna utilising and living within the meadow. This way, changes in faunal populations may be compared and monitored against seagrass health and C stocks.

Chapter 3:

Stability of estuarine carbon sinks: How does bioturbation affect the degradation of seagrass meadow carbon sources?

Abstract

Seagrass meadows are one of the most effective carbon (C) sequestering ecosystems globally. They are able to store C for long periods of time (millennia) largely due to the constant supply and burial of slowly degrading seagrass detritus that is rich in structural polymers (i.e. lignin). However, other forms of organic matter, including macroalgae, are common in seagrass meadows. Bioturbating macrofauna commonly alter the distribution of organic matter within the sediment; however, the impact this bioturbation has on organic matter degradation and overall C preservation is poorly understood. We performed a mesocosm experiment to compare the degradation of relatively labile macroalgae (*Fucus vesiculosus*) detritus with more recalcitrant seagrass (*Zostera marina*) detritus, in sediment with and without bioturbation by the common polychaete *Arenicola marina*. Additionally, we investigated if there was any effect on degradation when macroalgae and seagrass detritus were buried simultaneously.

After 4 weeks, a significant proportion of seagrass (41%) and macroalgae (56%) detritus were degraded in sediment without bioturbation. Surprisingly, bioturbation impacted both the burial and degradation of seagrass and macroalgae detritus differently. Seagrass material was rapidly buried deeper into the sediment as a discrete layer (45% degraded). Conversely, bioturbation macerated macroalgae detritus into fine particulate organic matter (81% degraded). In sediment containing detritus from both macroalgae and seagrass, an additional 13–15% of seagrass material recovered, indicating preferential preservation of seagrass material.

We conclude that bioturbation will impact C stocks differently depending on the dominant macrophyte within a marine ecosystem. In ecosystems with both seagrasses and macroalgae, the total C-preservation from seagrasses will be higher due to preferential degradation of the most labile organic matter (i.e. macroalgae). Ultimately, bioturbation will enhance C-preservation in seagrass ecosystems, but lower C-preservation in macroalgal-dominated systems. This altered rate of detrital degradation

and associated CO_2 release has implications for the loss of seagrass C stocks through time, and strongly points to the potential impact of bioturbating infauna on C storage in sediments.

Introduction

Preservation of coastal carbon stocks is considered of high priority for the effective mitigation of atmospheric greenhouse gases and climate change (Mcleod et al. 2011, Duarte et al. 2013a). Coastal and estuarine wetland ecosystems (including seagrass beds, saltmarshes marshes, and mangrove forests) are highly productive and sequester large amounts of carbon (C) in sediment (Duarte et al. 2005, Fourqurean et al. 2012). Preservation of coastal C stocks therefore, is considered of high priority to effectively mitigate atmospheric greenhouse gases and climate change (Fourqurean et al. 2012).

Carbon captured by coastal and estuarine wetland ecosystems persist for long periods of time within the sediment (1000s of years), due to the preservation of structurally complex organic matter (OM) under anoxic conditions (Mateo et al. 2006, Valdemarsen et al. 2014) in the sediment. It is assumed that within these environments C sequestration and stocks are static, and that dramatic events such as storms or dredging, alters C stocks. The contribution of natural or chronic disturbances (i.e. variability or invasions of benthic fauna and bioturbation) to C stocks is typically not considered.

Within seagrass ecosystems, OM burial is one of the key factors that determines the storage of coastal C (Duarte et al. 2013a). Plant detritus, rich in refractory structural polymers (i.e. lignocellulose), enables C to persist in anoxic sediments (Trevathan-Tackett et al. 2015). Accumulation of C within seagrass systems relies on input from both autochthonous and allochthonous sources, with 50–75% of the stored OM in some seagrass systems derived from allochthonous sources, which can include a portion of algal material (Gacia et al. 2002, Kennedy et al. 2010). Thus, macroalgae (i.e. Fucales, Laminariales, and Plocamiales) have recently been proposed as significant contributors to the C inventory in seagrass sediments (Hill et al. 2015, Trevathan-Tackett et al. 2015), because they are high in biomass and are readily available sources of OM within coastal seagrass ecosystems. However, macroalgae lack the structural polymers that seagrass tissues possess, and they are degraded rapidly once deposited on the sediment. The slower degradation of seagrass detritus thus makes it a better source for C burial (Trevathan-Tackett et al. 2015). How bioturbation impacts the persistence of macroalgal-C compared to seagrass-C while undergoing degradation in sediment, is largely unknown

Large conveyer-belt feeding bioturbators such as those belonging to the polychaete *Arenicola marina* (hereby referred to as "*Arenicola*") often occupy sediment adjacent to seagrass meadows in temperate zones (Valdemarsen et al. 2011, Govers et al. 2014). The capacity of *Arenicola* for particle-reworking and burrow ventilation results in altered sediment texture, biogeochemistry and benthic metabolism, and therefore dispersal and burial of OM within the sediment (Banta et al. 1999, Kristensen 2001, Papaspyrou et al. 2007, Kristensen et al. 2012, Wendelboe et al. 2013). This reworking can stimulate sediment microbial metabolism up to 123% (Banta et al. 1999). Furthermore, *Arenicola* burrow ventilation increases oxygen availability and aerobic conditions for the microbial degraders that are located in the deeper sediment layers, which in turn results in increased rates (141–270%) of C mineralisation (Banta et al. 1999, Papaspyrou et al. 2007). Consequently in bioturbated sediment regions, high levels of microbial activity and detritus degradation occurs in sediment layers that were previously protected from high rates of remineralisation (Kristensen 2001). The metabolic activity may be further promoted by the presence of labile macroalgae detritus, leading to enhanced degradation of buried seagrass detritus i.e. 'priming' (Kuzyakov et al. 2000, Guenet et al. 2010).

We investigated the effects of *Arenicola* bioturbation on C burial and retention within coastal sediment. Our objectives were to: (1) determine how bioturbation affects seagrass C preservation by altering decomposition of buried seagrass C; (2) discern whether deposited macroalgae would induce a priming effect on seagrass C remineralisation; and (3) determine whether this degradation would be further stimulated in the presence of bioturbation. We hypothesised that degradation of both macroalgae (*Fucus vesiculosus*) and seagrass (*Zostera marina*) is enhanced by *Arenicola* bioturbation, and that degradation of refractory C sources (seagrass) is enhanced when microbes simultaneously degrade labile C sources (macroalgae), as well as seagrass.

Methods

Sediment and animal collection

Sandy sediment with a median grain size of 210 µm and organic content of 0.4%, and individuals of juvenile *Arenicola marina* (Polychaeta) were collected on separate days from Bregnør Bay in Odense Fjord, Denmark. The top-most 0.5–1 cm of sediment) was removed before sediment sampling to ensure that highly labile benthic microalgae were excluded. Sediment was wet-sieved through a 0.5 mm mesh and homogenised on site. *A. marina* (henceforth referred to as "*Arenicola"*) were recovered through careful sieving (0.5 mm mesh), and transferred to buckets for transportation to the laboratory. Once in the laboratory, *Arenicola* were transferred to petri dishes containing seawater (salinity of 20), left to acclimatise at 15°C for 36 hours and weighed thereafter. Only healthy juvenile (0.30 ± 0.08 g) *Arenicola* (still active after the 36 hour acclimation) were used in experiments to compensate for the mesocosm size employed.

Macrophyte collection

Zostera marina (henceforth referred to as "seagrass") was collected at Enebærodde in Odense Fjord, Denmark. Seagrass leaves were gathered from a seagrass bed approximately 10 m from shore, and kept in seawater during transport back to the laboratory. *Fucus vesiculosus* (henceforth referred to as "macroalgae") was harvested near Odense River in the upper reaches of Odense Fjord. The thalli of macroalgae, which were attached to boulders, were harvested, and transported back to the laboratory while submerged in seawater. Leaves (seagrass) and fronds (macroalgae) were gently cleaned for epiphytes, washed with distilled water, and chopped into uniform detritus pieces (< 15 mm). Subsequently, known weights of the detritus were separated into portions (5 g w/w), each representing an experimental core (described below). Mixed macrophyte enrichments consisted of 2.5 g w/w of each macrophyte type. Additional macrophyte material (both seagrass and macroalgae) was prepared for chemical analysis.

Mesocosm experimental set-up

The effect of *Arenicola* bioturbation on macrophyte degradation within the sediment was assessed over 28 days. A total of 8 experimental treatments were established (n = 3), consisting of faunated and defaunated sandy sediment cores, without enrichment (Control) or enriched with seagrass detritus (+SG), macroalgae detritus (+MA) or a mix of seagrass and macroalgae detritus (+MIX). Sediment cores were prepared by adding sediment into 24 acrylic (30 cm long, 8 cm diameter) core tubes, to a depth of 18 cm. Cores were left to compact overnight (approximately 16 hours at 15˚C). The following day, 130 g of clean sediment, corresponding to a 2 cm layer, was added to control cores (n = 6). Macrophyte detritus (5 g w/w) was mixed with clean sediment (60 g w/w) and added to the remaining cores in a 1 cm thick layer on top of the sediment cores. An additional 1 cm layer of clean sediment was then added. All cores were left to settle for 4 hours, so that the final sediment depth was 20 cm. Each core was then topped up with seawater, and all 24 sediment cores were transferred to four 90 L seawater tanks (salinity of 20). Two tanks received cores containing bioturbators (*Arenicola*) and two tanks received cores that were defaunated. Cores were separated in different tanks to prevent the migration of bioturbators from one core to another. Water was circulated using stirring magnets (1.5 cm long) fitted to the core tubes, and driven by a rotating external magnet (~ 60 rpm). Each tank was fitted with two air stones to aerate and mix the water, and was kept in the dark for the entire experimental period to restrict the growth of benthic microalgae.

Characterisation of macrophyte detritus used for sediment enrichment

Initial samples of macroalgae and seagrass detritus (n = 5) were analysed for C and nitrogen (N) content, and wet weight to dry weight conversion (ww/dw). Approximately 2 g (wet weight; w/w) of each macrophyte was washed in distilled water, dried with a paper tissue and weighed. Samples were then dried at 60°C for 48 hours, and re-weighed to establish ww/dw. Dried samples were ground using a clay mortar and pestle, and analysed using a CN analyser (LECO TruSpec, LECO Corporation, St. Joseph, MI, USA).

Gas flux measurements

Exchange of total CO_2 (TCO_2) and O_2 between the sediment and overlying water was determined by conducting flux incubations before, and 1, 6, 13, 20, and 27 days after *Arenicola* was added to the sediment cores. Cores were left to compact for approximately 48 hours before the first flux measurement was performed. On day 2 of the experiment, two *Arenicola* worms were added to each faunated core (12 cores in total, total 0.6–0.7 g w/w in each core). *Arenicola* were allowed to construct burrows for 24 hours before the second flux measurement was initiated on day 3. Cores were sealed with gas-tight rubber bungs and water was mixed with continuous stirring as described above during the incubations. All cores were incubated in darkness at a constant temperature (15°C). Measurement incubations were carried out for 2–3 hours, and water O_2 concentration at the beginning and end of each incubation was determined for individual cores using a fiberoptic O_2 dipping probe connected to a Microx 4 transmitter (PreSens, Germany). Water samples were taken from each core using a 60 mL syringe before and after each incubation to determine rates of TCO_2 production. Each water sample was transferred to 3 mL gas tight glass vials and preserved with saturated $HgCl_2$ (50 µL). Water samples were stored in darkness at 5°C for < 7 days and analysed by flow injection (Hall and Aller 1992). The exchange of O_2 and total CO_2 between sediment and water was calculated from changes in core water concentration before and after incubations, using the following equation:

$$\text{Gas Flux} = \frac{[\text{Conc } T_1(mmol\ L^{-1}) - \text{ Conc } T_0\ (mmol\ L^{-1})] \text{ x Water volume } (L^{-1})}{\text{Sediment surface area } (m^{-2}) \text{ x Time } (d^{-1})}$$

Slicing of the core sediment

Each core was sliced on day 29 of the mesocosm experiment. A sample to establish background CO_2 and DOC concentrations in the overlying water in each tank was collected before core slicing. Faecal casts on the sediment surface were gently flattened to ensure a consistent sediment surface, before cores containing *Arenicola* were sliced. This accumulated sediment in faunated cores was sliced into 1 cm intervals. All sediment cores from the original surface sectioned into 1 cm intervals to 6 cm depth, and 2 cm intervals to 22 cm depth. Each section was homogenised, and subsamples of

every section were taken for porewater analysis (described below), and sediment bulk density and organic carbon (C_{org}) analysis.

Sediment porewater analysis

Porewater was extracted by centrifuging sediment subsamples in double centrifuge tubes containing GF/C filters for 10 min at 1500 rpm. Subsamples of extracted porewater were fixed with saturated $HgCl_2$ (1:100 v/v) and stored for CO_2 analysis as described above. Other porewater subsamples were stored in glass vials at -20°C for DOC and SO_4^{2-} analyses. Porewater SO_4^{2-} concentrations were analysed using ion chromatography and standardised against chloride concentrations, using methods described by Martin and Banta (1992). DOC concentrations were measured on a TOC Analyser (Shimadzu TOC-5000).

Recovery of macrophyte material (coarse POC > 0.5 mm) from sediment cores

To determine the quantity and vertical distribution of macrophyte material (coarse particulate organic C; POC) remaining in the sediment at the end of the 28 day mesocosm experiment, all large pieces of particulate detritus > 0.5 mm were recovered from each sediment slice from each core. Sediments not used for other purposes after slicing were sieved (0.5 mm mesh) slice by slice and all visible coarse POC was retained. The collected coarse POC was washed in distilled water, and dried at 60°C for 24 hours.

Characterisation of sediment

The sediment was characterised in homogenised sediments taken from each core slice during final core sectioning. Sediment density was analysed gravimetrically with a 4 mL cut-off syringe. Samples were transferred to aluminium trays, and the wet weight of each sample was recorded. Sediment was dried at 60°C for 24 hours to establish both wet and dry bulk density (mg cm^{-3}), and each sediment sample was then ground with a mortar and pestle. Subsamples of each section (0.5 g each) were acidified by fumigation and re-dried according to Komada et al. (2008). The C_{org} content of each sample was measured using a CN analyser (LECO TruSpec, LECO Corporation, St. Joseph, MI, USA).

Measurement of *Arenicola* metabolism

The contribution of *Arenicola* respiration to CO_2 and O_2 flux was determined in a separate experiment. *Arenicola* were individually weighed, and transferred to 50 ml Winkler vials filled with O_2 saturated seawater. Initial and final seawater samples for TO_2 and CO_2 were taken (as described above; gas flux measurements), before and after a 2 hour incubation. Incubation bottles were held in darkness at a constant temperature (15°C) and salinity (20) throughout the experiment. Samples were analysed for TCO_2 and O_2 flux as described above. *Arenicola* growth for the experimental period was estimated using a 25% increase in biomass, based on observations of faunal biomass in juvenile *Arenicola* by Beukema and De Vlas (1979).

Statistical analysis

The impact of *Arenicola* on the degradation of seagrass and macroalgae degradation was tested with separate two-way ANOVAs based on total detritus recovery and flux data (time integrated release). The addition of *Arenicola* (defaunated and faunated sediment), and macrophyte enrichment (+SG, +MA, +MIX, and Control) were used as fixed independent factors.

Levene's test for homogeneity of variance was performed on data prior to analysis. When appropriate, Tukey's post-hoc test was used to establish which variables produced a significantly different interaction. All tests were performed with a significance level of $\alpha = 0.05$, using IBM SPSS Statistics (Ver. 22).

Results

Visual observations of the sediment conditions

Upward movement of sediment as a result of *Arenicola* defecation caused an average additional 1.2 cm of sediment on the surface of faunated cores. There was a clear appearance of oxidised burrow walls in all *Arenicola* cores, and the fecal material on the sediment surface displayed extensive sediment oxidisation. In defaunated cores, the light coloured oxidised sediment layer extended approximately 2–3 mm from the surface, and was met by a dark coloured sediment band of iron sulfide. Defaunated cores and especially cores containing macrophyte detritus, developed small white patches, indicative of sulfide oxidising bacteria, on the sediment surface within the first week. Sulfide oxidising bacteria were largely absent in all cores containing *Arenicola*. By the end of the 28 day mesocosm experiment, 100% of the added *Arenicola* were recovered in the final core sectioning.

Characteristics of macrophyte detritus used for sediment enrichment

C_{org} analysis of the fresh macroalgae and seagrass material revealed the original C_{org} content of macrophyte enrichment within each respective treatment. Macroalgae enriched (+MA) core sediments originally received approximately 63.5 g C m^{-2} of macroalgal-C, while seagrass enriched (+SG) core sediments received 65.0 g C m^{-2} of seagrass-C. Mixed (+MIX) enrichment core sediments received approximately 31.7 g C m^{-2} of macroalgae and 32.5 g C m^{-2} of seagrass-C material. Carbon to nitrogen (C:N) ratios of the two macrophytes varied considerably, with the original seagrass (leaf) material displaying a C:N ratio of 30.6 ± 1.3, and the original macroalgae (blade) material exhibiting a ratio of 21.6 ± 1.5.

Recovery of macrophyte material (coarse POC > 0.5 mm) from sediment cores

All of the macrophyte detritus recovered from defaunated sediment cores was retrieved close to the initial burial depth (3–4 cm); however, sediment reworking by *Arenicola* radically altered the distribution of detritus in all faunated cores (Fig. 3.1). Seagrass detritus was buried a further 4–8 cm into the sediment (8–4 cm depth), compared to macroalgae detritus, which was spread evenly throughout the sediment down to 14 cm. This same pattern was observed in faunated sediment when macrophyte detritus was added individually or as a mixture. The appearance of seagrass detritus was almost unaltered compared to the initial state. Large pieces of macroalgae (> 5 mm) were only found in defaunated cores, while macroalgae was predominantly recovered as small organic particles (< 5 mm) in faunated cores, suggesting maceration or degradation of macroalgae detritus.

Overall, more seagrass detritus (macro-particles) was recovered from the sediment than macroalgae ($p < 0.001$). Recovery of macroalgae was significantly reduced by both the presence of buried seagrass ($p = 0.002$) and *Arenicola* ($p < 0.001$). Recovery of seagrass material, however, was not significantly affected by the presence of either *Arenicola* ($p = 0.380$) or buried macroalgae ($p = 0.672$). Based on the difference between the mass of added material, and the mass of material recovered from the sediment, 58.2% of seagrass and 43.8% of macroalgae detritus in defaunated treatments was recovered (Fig. 3.1). Degradation of seagrass within the mesocosm experiment was not significantly affected by burial of detritus by *Arenicola* into the deep and anoxic sediment (54.6% was recovered). Conversely, increased degradation of macroalgae was observed in faunated sediment (only 19.5% was recovered). In treatments with mixed detritus (+MIX), 13–15% more seagrass was recovered than in sediment containing only seagrass, with 66.7% of seagrass, and 27.3% of macroalgae detritus recovered in defaunated cores. The addition of *Arenicola* increased the degradation of macroalgae by an additional 60% and seagrass by nearly 10%, with 61.5% of seagrass and 16.6% of macroalgae detritus recovered.

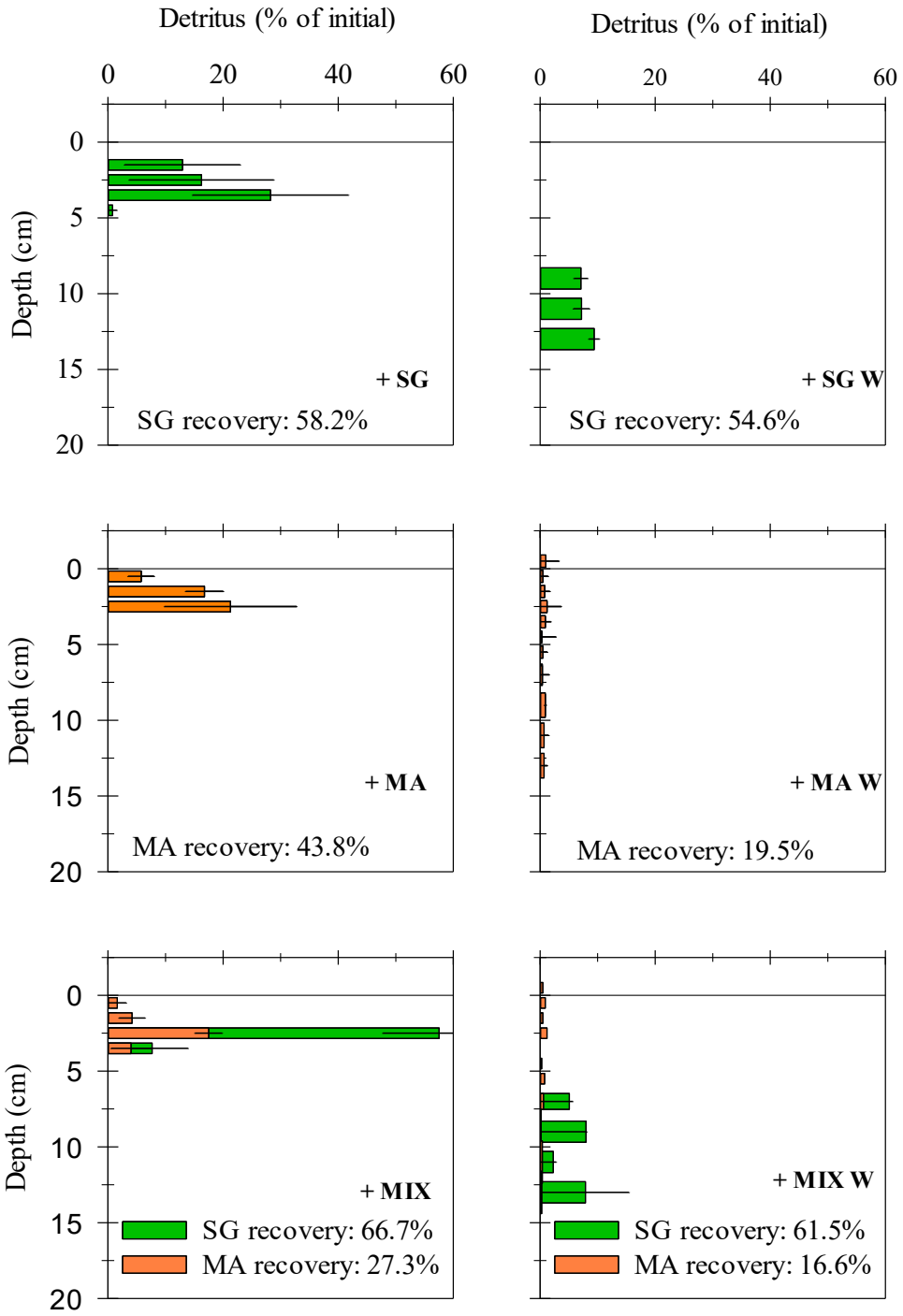

Fig. 3.1: Vertical profiles of macrophyte recovery of coarse POC (> 0.5 mm) in sediment cores enriched with seagrass (+SG), macroalgae (+MA), and mixed (+MIX) macrophyte material. Left panels: defaunated cores. Right panels: faunated cores (+W). Percentages indicate the portion (%) of the original material that was recovered post-experiment. The straight reference line (0 cm depth) indicates the original sediment surface depth. Original burial depth of macrophyte material was 3–4 cm. Error bars: SE (n = 3).

Gas flux and exchange rates

O_2 was consumed and CO_2 released by the sediment in all cores (Fig. 3.2). O_2 uptake was almost identical at about 4.6–5.8 mmol m^{-2} d^{-1} in the control and +SG (seagrass) treatments initially, while the +MA (macroalgae) treatment showed 2.5–3 times higher initial rates. *Arenicola* increased the O_2 uptake of all treatments instantly after addition. The increase was about 2–10 times higher than initially at Day 2; highest in the +MA treatment and lowest in the control treatment. The O_2 uptake in defaunated sediments was constant over time in control sediments, with a slightly increasing rate over time in all macrophyte enriched treatments.

Defaunated treatments with +MA initially produced significantly more CO_2 (71.1 mmol m^{-2} d^{-1}) than all other sediments; twice the amount of CO_2 compared to control sediment ($p < 0.001$), 1.5 times more than the +SG treatments ($p < 0.001$), and 1.2 times more than the mixed macrophyte enrichment (+MIX) ($p = 0.018$) (Fig. 3.2). *Arenicola* bioturbation resulted in CO_2 release immediately after introduction of *Arenicola* (day 2) by a factor of 2–20 in all cores and subsequently it decreased in an exponential fashion throughout the experimental period. CO_2 release for faunated sediment was always highest in treatments with macroalgae (+ MA, +MIX), with values 1.6 times greater than for both seagrass and control treatments. The time-integrated release of CO_2 over the entire experimental period in all *Arenicola* treatments was more than double that of defaunated treatments ($p < 0.001$). The enrichment type also had a significant impact on CO_2 release – macroalgae significantly increased CO_2 production ($p < 0.001$).

DOC flux was variable throughout the experimental period, fluctuating in almost all treatments between production and consumption (Fig. 3.2). DOC flux within defaunated control sediment and defaunated sediment enriched with macroalgae (+MA) remained relatively consistent. +MA sediments displayed DOC consumption rates of between 6.6 and 23.2 mmol m^{-2} d^{-1}. Conversely, defaunated control sediments displayed production of DOC throughout the experimental period, with production rates between 6.6 and 25.1mmol m^{-2} d^{-1}. There were no other clear trends in DOC flux over time in either faunated or defaunated cores, or for type of macrophyte enrichment.

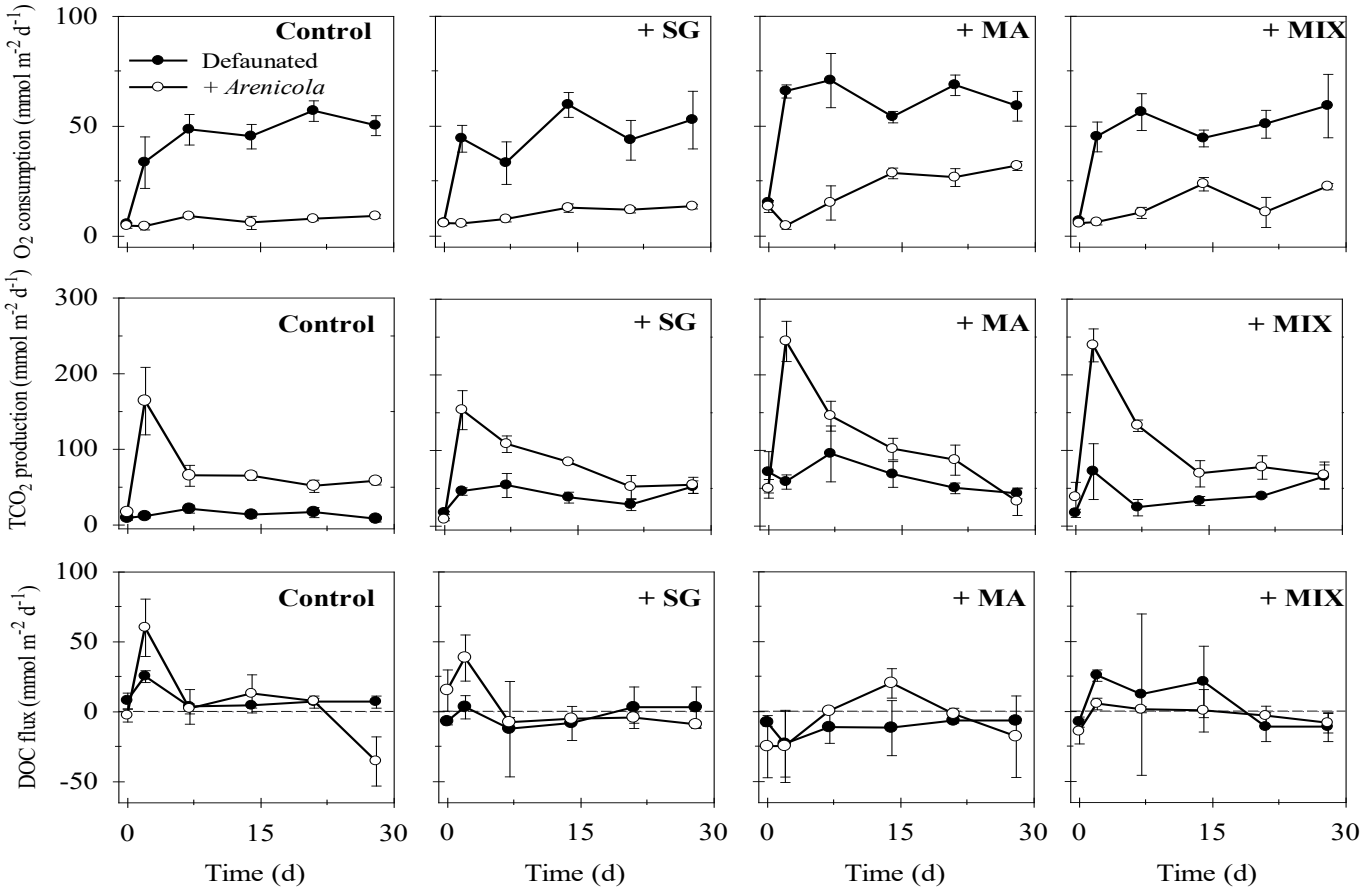

Fig. 3.2: Total dissolved organic C (DOC), oxygen (O_2) and carbon dioxide (TCO_2) effluxes over time in sediment cores enriched with seagrass (+SG), macroalgae (+ MA), and mixed macrophyte material (+MIX); and without (control) enrichment. The two curves in each graph show efflux profiles in sediment cores with (+ *Arenicola*; white symbols) and without (Defaunated; black symbols) fauna. Error bars: SE (n = 3).

Sediment organic carbon content and composition

Defaunated sediment containing macroalgae had higher C_{org} (0.22–0.25 mmol C cm^{-3}) in the top 4 cm (original detritus burial depth) of sediment, compared to all the layers below this depth (0.15–0.18 mmol C cm^{-3} C_{org})) (Fig. 3.3). C_{org} within all other defaunated treatments was relatively constant (~0.17 mmol C cm^{-3}) at all sediment depths. Downcore profiles of C_{org} were similar in all cores containing *Arenicola* (Fig. 3.3). C_{org} concentrations were relatively variable (0.14–0.24 mmol C cm^{-3}) in the top 15 cm of all faunated cores, but less (0.08–0.12 mmol C cm^{-3}) in the bottom quarter (15–20 cm) of the sediment.

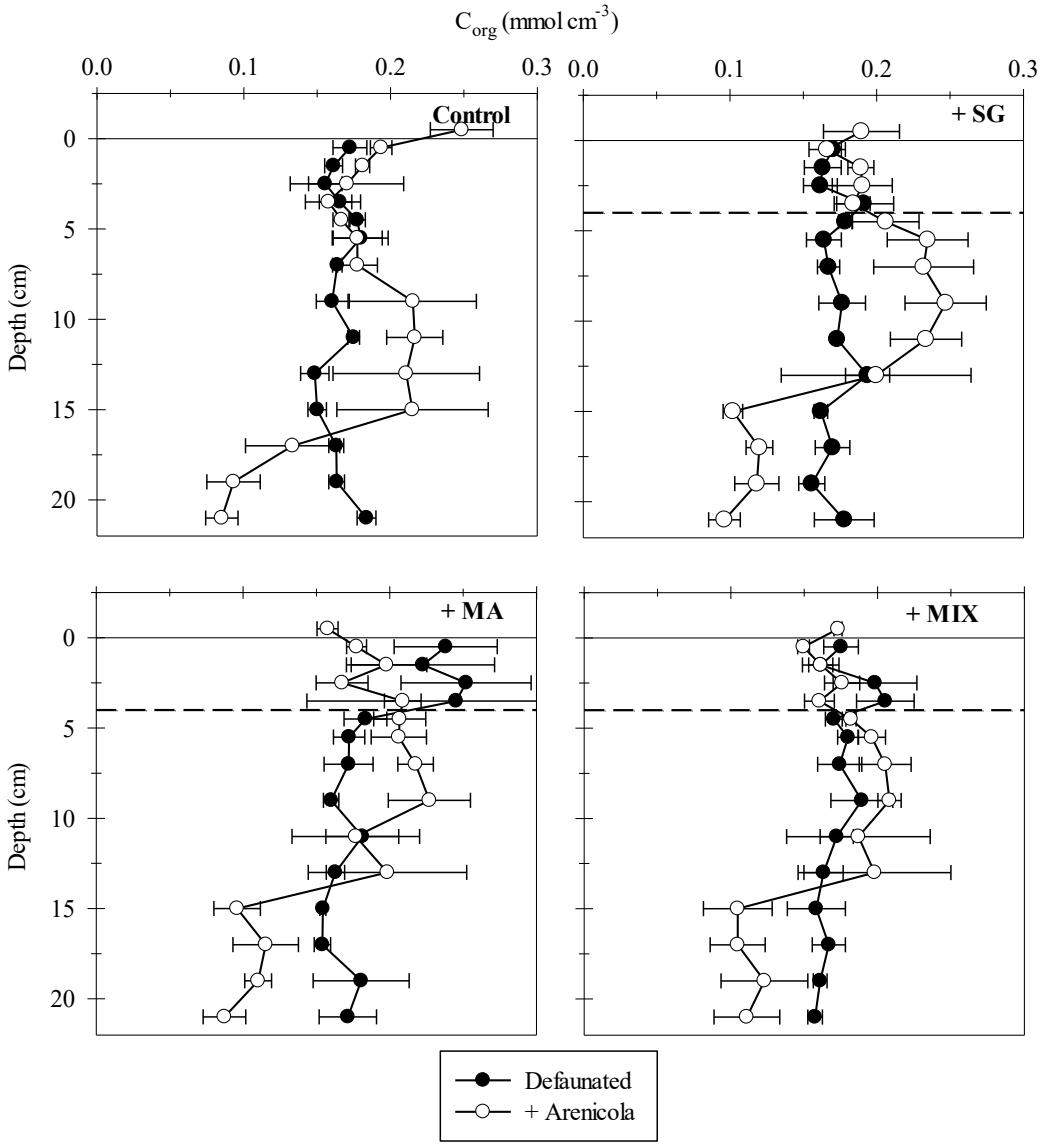

Fig. 3.3: Content of total organic carbon (C_{org}) in core sediments with seagrass (+SG), macroalgae (+MA), and mixed macrophyte material (+MIX); and without (control) enrichment. The two curves in each graph show sediment cores with (+*Arenicola*; white symbols) and without (Defaunated; black symbols) fauna. The dashed line (4 cm depth) indicates the initial depth of macrophyte enrichment. The straight reference line (0 cm depth) indicates the original sediment surface. Error bars: SE (n = 3).

Distribution and concentration of sediment porewater solutes

The distribution of porewater solutes was strongly influenced by the ventilation and bioirrigation of *Arenicola* bioturbation (Fig. 3.3). Defaunated cores displayed solute concentration gradients in the upper 4 cm of the sediment (from the surface to a 4 cm depth respectively, SO_4^{2-} decreased, from 15.4–10.4 to 9.3–0.7 mmol; CO_2 increased, from 3.3–9.1 mmol to 9.9–39.8 mmol,; and DOC increased, from 0.7–1.9 to 1.6–6.7 mm), particularly in sediment enriched with macrophyte material (+ SG, +MA, +MIX). While CO_2 and DOC accumulated to high levels in this zone of enriched cores (CO_2; 30–45 mmol, and DOC; 0–10 mmol), SO_4^{2-} was strongly depleted (1–10 mmol). The only solute to accumulate with depth in defaunated sediment without macrophyte enrichment was CO_2. The flushing of porewater solutes due to *Arenicola* bioirrigation in faunated sediment evened out the profiles markedly to such an extent that concentrations of all solutes were not significantly different to that of the overlying water.

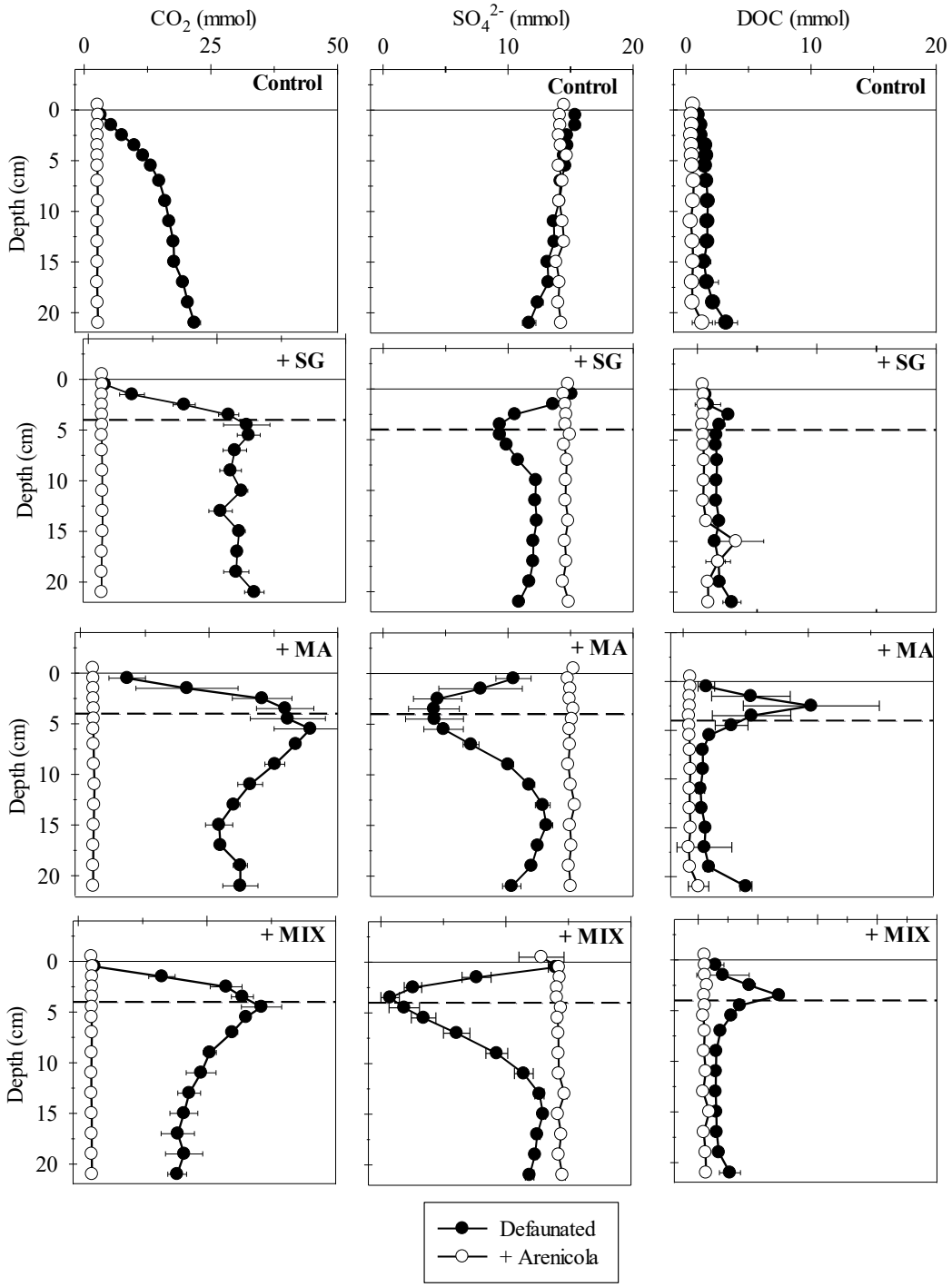

Fig. 3.4: Vertical porewater profiles of total carbon dioxide (TCO_2), sulfate (SO_4^{2-}), and dissolved organic C (DOC) in sediment cores with seagrass (+SG), macroalgae (+MA), and mixed macrophyte material (+MIX); and without (control) enrichment. The two curves in each graph show profiles in sediment cores with (+*Arenicola*; white symbols) and without fauna (Defaunated; black symbols). The dashed line (4 cm depth) represents the initial burial depth of macrophyte material. The straight reference line (0 cm depth) represents the original sediment surface. Error bars: SE (n = 3).

Metabolism of *Arenicola* worms; contribution to TCO_2 and O_2 flux

The respiratory consumption of O_2 and production of TCO_2 by *Arenicola* tested in a separate experiment were 60.6 ± 7.7 µmol g^{-1} d^{-1} and 90.0 ± 11.9 µmol g^{-1} d^{-1} respectively (Table 3.1). When extrapolated to the biomass in each core, *Arenicola* contributed an overall average of 10–14% of the total TCO_2 production and 13–18% of the total O_2 consumption by bioturbated sediments. These values range from a relatively low contributions to the total CO_2 production and O_2 consumption in the initial stages of the experiment (4.8–7.2% of the total CO_2 production and 12.0–17.0% of the total O_2 consumed) to a large contributions (21.3–45.7% of the total CO_2 produced and 16.2–19.6% of the O_2 consumed) at the final stages of the mesocosm study (day 28). These estimates included a 25% predicted growth in *Arenicola* biomass (Beukema and De Vlas 1979) and no observed *Arenicola* mortality.

Table 3.1: Details of *Arenicola marina* biomass added to each treatment, predicted end biomass, and calculated metabolism contributions to TCO_2 and O_2 fluxes.

	+ SG	**+ MA**	**+ MIX**	**Control**
Initial *Arenicola* biomass (g core $^{-1}$)	0.62	0.66	0.64	0.66
Estimated final *Arenicola* biomass (g core $^{-1}$)	0.78	0.82	0.80	0.83
TO_2 (µmol g^{-1} d^{-1})	37.77	41.02	37.43	40.17
TCO_2 (µmol g^{-1} d^{-1})	56.14	60.97	55.63	59.71
RQ (TCO_2 / TO_2)	1.49	1.49	1.49	1.49

**Arenicola* added to the experiment for a total of 26 days.

Discussion

Although seagrass meadows are well recognised coastal carbon (C) sinks (Mcleod et al. 2011, Fourqurean et al. 2012), there are limited studies that investigate the effects of bioturbation on the fate of C within seagrass sediments. We found that bioturbation by *Arenicola* affects the degradation and burial of various C sources differently. Considering that allochthonous sources of C make up a large proportion of seagrass sediment C (Gacia et al. 2002, Kennedy et al. 2010, Greiner et al. 2016), the observed impact of bioturbation on both seagrass and macroalgae burial and degradation has significant implications for the persistence of coastal C stocks.

In coastal sediments, *Arenicola* worms are known to completely homogenise the top 15 cm of the sediment profile (Riisgaard and Banta 1998). Surface sediment containing a smaller grain size than undisturbed sediment is generally attributed to areas surrounding *Arenicola* burrows (Volkenborn et al. 2007, Wendelboe et al. 2013), as *Arenicola* show strong preference for feeding on particles < 200 µm (Cadée 1976); however, larger particles (up to 2 mm) may also be consumed (Krüger 1971).Particles not ingested by *Arenicola* are passively buried (Valdemarsen et al. 2011). It is likely, that this physical reworking and active grain selection by *Arenicola* impacts on the burial of macrophyte detritus.

Our data showed, that unlike macroalgae, seagrass detritus was transported as a discrete layer deeper into the sediment. Furthermore, we observed that *Arenicola* had no effect on the overall degradation of seagrass. We may expect that given this burial of seagrass material, the lignocellulose within the seagrass tissue will be preserved (Godshalk and Wetzel 1978) as a result of it being buried in the most anoxic (deepest) depth of burrowed sediment (Kristensen et al. 1995). Unlike macroalgae, it is conceivable that bioturbated seagrass will contribute positively to sediment C stocks in areas with ample supply of seagrass detritus

While *Arenicola* bioturbation increased the overall degradation of macroalgae by 20%, the physical reworking of the sediment by *Arenicola* also resulted in the spread of macroalgae tissue throughout the entire sediment profile. It is evident from the presence of macroalgae particles in *Arenicola* faecal casts (-2–0 cm) that macroalgae was being ingested by *Arenicola.* This activity would have resulted in the mixing of material

within the sediment and subsequently increased the macroalgae surface area. In field studies investigating the decay of macroalgae on the sediment surface, little to no macroalgae tissue remained after 60 days (Lopes et al. 2011). Given the short time span of this study (4 weeks), it is highly unlikely that the macroalgae biomass would remain within the sediment after an extended period of time (an additional 1-2 months; Lopes et al. 2011). It is therefore not likely that, macroalgae such as *Fucus* would serve as a suitable donor to seagrass C stocks in areas occupied by *Arenicola*. Long-term preservation of buried macroalgal-C may be significant when *Arenicola* or similar bioturbators are absent. However, burial in anoxic sediment is required and must therefore occur by other physical processes, such as deposition by waves and currents.

In sediments containing mixed detritus (both macroalgae and seagrass), significantly more macroalgae was lost than in sediment containing only macroalgae, while more seagrass was retained. These results suggested that the addition of a labile OM source (macroalgae) diverted microbial metabolism away from refractory or recalcitrant OM (seagrass) (Canfield 1994, Zonneveld et al. 2010). Oxygenation and macroalgae maceration by *Arenicola* bioturbation appeared to have further stimulated sediment metabolism (see Fig. 3.1). We suggest therefore, that such stimulated degradation is indicative of 'selective' or 'preferential' degradation (Canfield 1994). We can therefore anticipate that when seagrass detritus is buried together with macroalgae detritus, i.e. when trapped among seagrass leaves and buried *via* bioturbation or other physical means, macroalgae will be preferentially degraded, while seagrass C will be conserved.

Compared to the comparatively persistent distribution of C_{org} in defaunated sediment, the introduction of *Arenicola* to the sediment may result in particle sorting (Longbottom 1970, Baumfalk 1979) and therefore changes to sediment C_{org} stratification. *Arenicola* characteristically consumes organic particles small enough (<500 µm) to digest (Andresen and Kristensen 2002), which due to its feeding behaviour, are transported to the surface. Larger particles (i.e. sand) that are generally low in C, remain deep within the sediment. This grain size stratification may explain the lower C_{org} content observed in deep sediment (>15 cm) in cores containing *Arenicola.*

Concentrations of porewater solutes (TCO_2, SO_4^{2-}, and DOC) were drastically changed with the introduction of *Arenicola* displaying a strong irrigation effect (Banta et al. 1999). This effect was distinct even in sediment immediately surrounding the original

macrophyte enrichment layer (2–6 cm), where accumulation of solutes was substantial in defaunated sediment. In fact, porewater flushing caused by *Arenicola* irrigation was so strong that porewater profiles in faunated sediments were completely uniform. We suggest that any mineralisation products (TCO_2 and DOC) accumulated within the sediment would therefore be quickly flushed to the overlying water *Arenicola*, and at the same time, electron acceptors would be replenished within the sediment (Quintana et al. 2013). Persistence of coastal C stocks also relies on microbial activity in the environment where C is stored. It is evident that microbial activity was stimulated substantially, corresponding to the original macrophyte enrichment layer in defaunated sediment.

Fluxes of TCO_2 and O_2 observed within our laboratory experiment were comparable to published data (Kristensen 2001, Papaspyrou et al. 2007). Within all sediment cores, there was a 2- to 5-fold increase in benthic flux in the presence of *Arenicola*. Although *Arenicola* respiration contributed a considerable portion of both TCO_2 production and O_2 consumption, stimulation of sediment flux was still significantly larger (1.7–4.8 times) compared to defaunated sediment. The respiratory quotient (RQ; CO_2 efflux to O_2 consumption) ranged from 1.2–3.1 in all treatments. Sediment containing *Arenicola* had lower RQ values (1.2–1.3) than defaunated sediment (1.8–3.1). Defaunated treatments containing seagrass (including mixed macrophyte enrichments) had the highest RQ values (3.1 and 2.0 respectively) of any cores. Aerobic sediment respiration typically results in relatively balanced respiratory quotients of 1.0 (Banta et al. 1999), while unbalanced values favouring CO_2 release usually indicate considerable changes to metabolic processes, such as increased rates of anaerobic decomposition (Hargrave and Phillips 1981). We suggest that flushing of the sediment and detritus burial by *Arenicola* resulted in a comparatively more balanced system, while discrete burial of C-rich relatively un-degraded seagrass detritus stimulated anaerobic microbial activity (Kristensen 2000).

Total C mineralisation over the entire experiment was estimated as the sum of the total CO_2 and DOC flux, and total CO_2 and DOC porewater accumulation (Table 3.2). Total C mineralisation of all enriched sediments (+SG, +MA and +MIX) was relatively substantial throughout the 28 day experiment (47–68%), with the largest loss seen in cores enriched with macroalgae (+MA; 66–68%). However, C budgets in faunated and

defaunated sediments imply that *Arenicola* did not significantly stimulate net C mineralisation in seagrass or macroalgae enriched sediments. In most cases, the addition of *Arenicola* in macrophyte enriched cores did not have a huge effect on C mineralisation (increased by 3% in +SG and decreased by 3% in +MA treatments). While bioturbation by *Arenicola* led to a large efflux of TCO_2 from the sediment, a comparable amount of porewater mineralisation products (TCO_2 and DOC) accumulated within defaunated sediments. However, in mixed treatments (containing both seagrass and macroalgae), *Arenicola* stimulated overall C mineralisation by nearly 30%. This was the only treatment with a distinct effect of *Arenicola* on C mineralisation. Recovery of coarse POC (detritus) indicated that a larger portion of macroalgae was degraded in these treatments compared to their defaunated counterparts. While this may be indicative of 'negative' or 'synergistic priming'(Kuzyakov et al. 2000, Zonneveld et al. 2010), coupled with the preferential degradation in detritus observed in mixed treatments, this process ultimately led to relatively higher persistence of seagrass biomass and therefore C. This finding has significant implications for both the persistence of seagrass C, as well as the status of sedimentary C stocks as "sinks". The increased preservation of seagrass C ultimately may lead to larger C burial.

Table 3.2: Calculated budget of carbon (C) mineralisation in faunated (+*Arenicola*) and defaunated core sediments, with seagrass (+SG), macroalgae (+MA), mixed (+MIX) macrophyte material, and without (control) enrichment. Porewater accumulation (± SE) was calculated using depth integrated TCO_2 and TDOC concentrations at the beginning and at the end of the experiment. Time integrated TCO_2 flux rates (± SE) were calculated using average fluxes from day 7 onwards (post initial sediment flushing), and all data is presented for the total experimental period of 28 days.

	Defaunated								**+ Arenicola**							
(mmol m^{-2})	**+SG**		**+MA**		**+MIX**		**Control**		**+SG**		**+MA**		**+MIX**		**Control**	
Added C_{org} (enrichment)	5411	±9	5293	±20	5326	±7	-		5425	±7	5293	±20	5338	±3	-	
Recovered coarse POC (detritus)	3162	±33	2076	±242	2507	±171	-		2974	±624	1122	±83	1726	±335	-	
TCO^2 Efflux	1142	±98	1823	±133	1147	±73	408	±60	2347	28±0	3210	30±7	2931	17±3	1992	±162
TCO^2 Δ PW	1337	±36	1687	±100	1164	±68	663	±18	-131	±2	-138	±3	-132	±6	-127	±2
Total DOC Production	-51	±92	384	±239	-17	19±7	-81	12±0	252	±89	287	±52	151	±83	-175	18±4
TDOC Δ PW	38	±4	107	±32	69	±6	37	±7	-15	±14	-39	±8	-38	±4	-38	±4
Net C - degradation	2516.2		3616.9		2380		1827.8		2467.7		3320		2913		1108.3	
Net C - degradation (% of initial)	47%		68%		45%		-		48%		66%		58%		-	

Our results indicated that macroalgae detritus does not remain in the sediment long enough for it to be considered a viable source for permanent C burial. The overall degradation and remineralisation of seagrass appeared to be independent of *Arenicola* bioturbation, indicating that bioturbation may promote storage of C in seagrass environments by burying seagrass detritus into deep, anoxic sediment. Buried seagrass will over time degrade slower than it would when exposed to oxygen (Kristensen et al. 1995). Furthermore, in coastal and estuarine ecosystems with both seagrasses and macroalgae available as a source of C, the total C preservation from seagrass will be higher than in systems without macroalgal burial, due to preferential degradation of the more labile organic matter.

In areas within and surrounding seagrass meadows occupied by an active bioturbator community such as *Arenicola*, C transported in the form of macroalgae detritus (i.e. macroalgae) is likely to be lost, while seagrass C (i.e. seagrass) is likely to contribute to long-term stocks. Thus, the burial of seagrass by *Arenicola* benefits the marine ecosystem in terms of C sequestration. If these detrital sources are present and mixed together in a seagrass meadow, a much larger and targeted degradation of macroalgae and subsequent release of C is likely to occur. Ultimately, this study highlights that the source of C determines its fate in regards to C stock, and that consideration of a whole ecosystem, including its fauna, must be taken when assessing C stock persistence.

Chapter 4:

The effect of estuarine lugworm *Arenicola marina* on sediment reworking and anaerobic microbial degradation of carbon

Abstract

Common bioturbators, such as the Northern European estuarine lugworm *Arenicola marina*, have the ability to alter sediment and benthic processes through their burrowing and ventilation activities. Preservation of seagrass carbon (C) stocks, named "blue carbon", relies on sediment anoxia and relatively dormant microbial activity. However, the ability of *A. marina* to transport both particles and water throughout the sediment increases C degradation within the sediment.

We performed two laboratory incubation experiments to observe the effects of bioturbation by *A. marina* on 1) changes in anaerobic microbial activity and 2) sediment reworking and bioirrigation (burrow irrigation), and how this impacted the degradation of macroalgal (e.g. *Fucus vesiculosus*) and seagrass (*Zostera marina*) detritus buried within the sediment. We observed that the rapid and continuous reworking of the sediment by *A. marina* caused burial of macrophyte detrital material, resulting in high rates of sediment bioirrigation. *A. marina* bioturbation of the sediment resulted in a consistently larger grain size in sediment deeper than 15 cm. Furthermore, a stable isotope analysis of *A. marina* tissue revealed a preferential ingestion of macroalgae material, which resulted in increased rates of macroalgal C degradation.

Sediment enriched with seagrass material displayed further increases of anaerobic microbial reaction rates (production of carbon dioxide, sulfate, and dissolved organic C within the porewater) compared to both control sediment and sediment enriched with macroalgae. Subsequently, anaerobic rates of C degradation were almost doubled in bioturbated sediment containing seagrass. We concluded that bioturbation and bioirrigation have clear effects on the sediment anaerobic degradation of C, and that coastal seagrass sediments inhabited by *A. marina* may be vulnerable to loss of C stock. Given that seagrass ecosystems rely on the burial and subsequent storage of seagrass detritus to refresh and retain C stocks, extensive bioturbation in seagrass sediments could significantly reduce the proportion of detrital C maintained in the sediments.

Introduction

Benthic macro-fauna such as the lugworm, *Arenicola marina*, inhabit estuarine and marine sediments, and are sustained by the constant supply of organic matter and oxygen (Gutiérrez et al. 2000). *A. marina* is a prevalent and dominant large burrower along Northern European coastlines. *A. marina* is a non-selective "conveyer-belt" feeder (Kristensen et al. 2012), that is known for its extensive sediment reworking and ventilation activities (Riisgaard et al. 1996). The activities of these "bioturbators" (Kristensen et al. 2012) result in sediment particle sorting and displacement, as well as bioirrigation, shifts in microbial community composition, biogeochemical reactions, and remineralisation of buried organic matter (Papaspyrou et al. 2005, Kristensen et al. 2014a). This physical reworking also changes the biogeochemical environment of the sediment substantially (Kristensen et al. 2014a). In coastal ecosystems such as seagrasses meadows, bioturbators play an essential role in the processing of organic matter (OM) and nutrient cycling at the interface of the benthos and the water column (Aller 1982, 1994, Hansen and Kristensen 1998), but their net impact on coastal and estuarine carbon (C) sequestration is however, poorly understood.

Seagrass meadows are one of the most productive ecosystems globally. They produce and sequester vast amounts of C, and by doing so, mitigate climate change (Mcleod et al. 2011). Accretion of C in seagrass ecosystems relies on the burial of both allochthonous (macroalgae, diatoms, seston, mangrove, and foreign seagrass material, as well as terrestrial biomass) and autochthonous (seagrass biomass) sources of OM (Kennedy et al. 2010). In some cases, as much as 41% of the contributed C is derived from seagrass biomass, with a portion of the allochthonous C (<5%) being attributed to macroalgae (Greiner et al. 2016). Much of C sequestered within seagrass meadows is transferred deep into the sediment, where it is stored for hundreds or thousands of years (Fourqurean et al. 2012). The persistence of this sedimentary C relies on constant burial of OM (Burdige 2007), which restricts oxygen to the upper few millimetres resulting in sustained sediment anoxia, and therefore sediment aerobic microbial activity is limited in most of the sediment profile. Several studies have focussed on the impact of oxygen on sediment C remineralisation (Canfield 1994, Kristensen et al. 1995), but the knowledge of how bioturbation affects subsurface sediment process, and indeed C mineralisation, is limited (Kristensen and Holmer 2001)

The ability of bioturbators to influence both detrital and sediment derived-C remineralisation is strongly dependent on the mode of bioturbation (reworking or ventilation; Kristensen et al. 2012). For example, deep-burrowing and active bioirrigation by *A. marina* enhance C mineralisation substantially more (4-10 fold) than observed for the less active polychaete *Nereis* (*Hediste*) *diversicolor* (Banta et al. 1999). Sediment biogeochemistry and microbial communities are strongly influenced by the extent of bioirrigation (Meysman et al. 2006a, Volkenborn et al. 2007, DeWitt 2009). Thus, (Kristensen and Holmer 2001) showed that oxygenation *via* bioirrigation may stimulate C remineralisation of buried OM in vicinity of burrows by a factor of up to 10, causing a doubling in total sediment metabolism.

The persistence of seagrass blue carbon (BC) stocks in the sediment is enhanced by anoxic conditions, as a result of the slower rates of anaerobic metabolism compared to aerobic metabolism (Kristensen 2001, Burdige 2007, Mcleod et al. 2011). *Arenicola* burrowing increases the abundance of oxygen in deeper recalcitrant sediment, thereby leading to higher levels of microbial activity, detrital degradation, and remineralisation (Kristensen 2001). Furthermore, the renewal of burrow water through ventilation serves important transport function, such as the supply of oxygen and other oxidised compounds (electron acceptors) and removal of metabolites (e.g. sulfide and ammonium) deeper into the sediment. The associated bioirrigation is an important factor for the control of microbial abundance in the sediment (Kristensen, 1988; Aller & Aller, 1998).This leads to a shift from stable sediment consisting of relatively 'dormant' microbial communities, to one that contains high-density heterotrophic anaerobic bacteria and meiobenthic bacteriovores.

Reichardt (1988) found that the oxidised wall lining of the polychaete *A. marina* burrows contained a higher abundance of bacteria and bacterial production, compared to surface sediment, ambient anoxic sediment and faecal casts. Additionally, microheterotrophic activity and concentrations of hydrolytic enzymes have also been shown to be higher in the wall lining of burrows (Kristensen 2000, Penha-Lopes et al. 2010). Altered rates of burial, degradation and anaerobic C remineralisation of OM stimulated by bioturbation and bioirrigation therefore, have the potential to release coastal and estuarine C. The direct assimilation of detritus components by animals

however, can competitively remove substrates that are otherwise available to microbial decomposers (Tenore et al. 1982).

In this study, we further the results obtained in Chapter 3, and explore the mechanisms behind *A. marina* stimulated detrital degradation. We hypothesise that *A. marina* bioturbation alters the degradation rates of buried macrophyte detritus, and consequently, affects the sediment C retention capacity. We aim to quantify how bioturbation by our model lugworm species, *A. marina*, affects: (1) the physical structure of sediment; (2) anaerobic microbial processes within the sediment; and (3) C storage. Our hypotheses were two-fold; (1) Bioturbation driven changes in anaerobic microbial activity hamper the long-term storage of C within the sediment; and (2) The degradation of macroalgal (e.g. *Fucus vesiculosus*) and seagrass (*Zostera marina*) detritus is enhanced by both direct (sediment re-working and ventilation) and indirect (bioirrigation driven changes in microbial activity) effects of *A. marina* bioturbation.

Methods

Sediment and *Arenicola marina* lugworm collection

Well mixed sandy sediment with low organic content (0.4 %) and median grain size of 210 µm was, collected together with individuals of juvenile *Arenicola marina* (Polychaeta) on separate days from Bregnør Bay in Odense Fjord, Denmark. *A. marina* (henceforth referred to as "*Arenicola"*) is native to Danish estuaries and appears in abundances ranging from 3 to 80 individuals m^{-2} (Valdemarsen et al. 2011), commonly occupying seagrass ecosystems. The top-most sediment layer (0.5–1 cm) was removed before sediment sampling to avoid excessive amounts of highly degradable benthic microalgae in the experimental sediment matrix. Sediment was wet-sieved through a 0.5 mm mesh and homogenised on site. Juvenile individuals of *Arenicola* were recovered through careful sieving, and transported to the laboratory in buckets. Individuals were then transferred to petri dishes containing seawater (salinity of 20), left to acclimatise at 15°C and to defecate for 36 h. Subsequently, only individuals that appeared healthy and were within a biomass range of 0.2–0.5 g fresh weight were used in the experiments.

Collection of macrophyte material

Fucus vesiculosus (henceforth referred to as "macroalgae") thalli were gathered from Odense River, within the upper reaches of the Odense Fjord. The thalli of macroalgae attached to the substrate were collected, as individuals from this site had also been previously analysed for their distinct $\delta^{13}C$ signature. *Zostera marina* (henceforth referred to as "seagrass") leaves were collected from a seagrass bed formerly analysed for their distinct $\delta^{13}C$ signature, at Enebærodde in Odense Fjord.

All macrophyte material was transported submerged in seawater back to the laboratory. Leaves (seagrass) and fronds (macroalgae) were gently cleaned for epiphytes, washed with distilled water, and chopped into uniform detritus pieces (<15 mm). Known weights of the detritus were separated into portions (5 g w/w), each representing an experimental core. Mixed detritus enrichments consisted of 2.5 g w/w of each macrophyte. Additional macrophyte material (both seagrass and macroalgae; n = 5) was prepared for stable isotope and C_{org} content analysis.

Experimental conditions

To observe the effect of *Arenicola* bioturbation on macrophyte degradation and the extent of sediment re-working and bioirrigation, an initial 28-day laboratory experiment was carried out. See methods described in Chapter 3 for full description of the initial incubation conditions.

Throughout the study, 8 experimental treatments (n = 3) were used, containing sediment enriched with macrophyte material. Sediments were enriched with seagrass (+SG), macroalgae (+MA) and mixed macrophyte material (equal parts seagrass and macroalgae (+MIX). Fresh macrophyte material was added to sediment cores and buried 3–4 cm from the sediment surface in a discrete layer. Non-enriched treatments consisted entirely of sandy sediment (control), with layer of additional clean sediment added to the surface after initial compaction. Cores were also either faunated (+ *Arenicola*) or defaunated (no added fauna), with 2 pre-weighed *Arenicola* individuals introduced to each faunated core (n = 10) on day 2 of the study. After the sediment was exposed to bioturbation for 4 weeks, sediment recovered from experimental cores was sliced, and portions were taken for an anoxic incubation for a further 4 weeks to determine the rates of microbial C remineralisation *via* production of porewater solutes.

Slicing of core sediment

After 28 days, sediment cores were removed and sliced. A water sample was collected from the overlying water measure CO_2 and dissolved organic C (DOC) concentrations. In cores containing *Arenicola*, accumulated sediment from *Arenicola* faecal casts on the core surface were gently flattened to ensure a consistent sediment surface. This accrued sediment in faunated cores was sliced in 1 cm sections. From the original surface in all cores, sediment was sectioned into 1 cm slices to a depth of 6 cm, at 2 cm intervals to a depth of 22 cm. Each section was homogenised, and subsamples of every section were taken for the analysis of porewater, sediment bulk density and organic carbon (C_{org}).

Measurement bromide profiles and bioirrigation rates

Bioirrigation was assessed as changes to porewater transport associated with *Arenicola* bioturbation and was measured in faunated (+*Arenicola*) cores at the end of the 28-day mesocosm experiment. A sodium bromide (NaBr) solution was added to the overlying

water in each core to a final concentration of 12 mmol. NaBr has no impact on either the fauna or the sediment microbial community. Porewater profiles of bromide (Br^-) were measured during the final core slicing approximately 24 hours after NaBr addition. Sediment subsamples from each sectioned depth were placed inside double centrifuge tubes containing GF/C filters, and porewater was extracted by centrifuging at 1500 rpm for 10 minutes. Porewater Br^- concentrations were analysed using ion chromatography and standardised against chloride concentrations, using methods described by Martin and Banta (1992). The extent of sediment bioirrigation by *Arenicola* was calculated from the inventory of Br^- in faunated cores (integrated concentration), and compared to defaunated cores of the same enrichment type. The volume of overlying water advected into the sediment was estimated from the excess inventory of porewater Br^-, the overlying water concentration and incubation time (Quintana et al. 2011).

Recovery of macrophyte detritus (> 0.5 mm)

C was quantified from each core to determine how much of the original C pool (macrophyte-derived C) remained in the sediment after the 28-day experiment. Additionally, homogenised sediment from each core slice was sampled to determine the organic C content (C_{org}) of each corresponding core depth. After sediment subsamples were taken, the remaining sediment from each sediment slice was sieved (0.5 mm sieve), and all remaining visible detritus (coarse POC) was identified and collected. Collected detritus was washed with distilled water, and dried at 60° C for 24 hours. The dried detritus was then weighed and compared to the calculated original macrophyte enrichment dry weights. See methods described in Chapter 3 for a full description of the recovery and quantification of macrophyte material and sediment C_{org} content. Results of macrophyte recovery and sediment C_{org} content in this chapter have been re-calculated to reflect the total available pool of C_{org} in each respective core.

Sediment water content and grain size analysis

Homogenised sediment from each core slice was sampled with a 4 mL cut-off syringe. Samples were transferred to pre-weighed aluminium trays, and the wet weight of each sample was recorded. Sediment was then dried at 60° C for 24 hours and re-weighed to establish water content.

Homogenised sediment (un-dried) from control cores, corresponding to the depths used for jar incubations (see below), were taken for grain size analysis. Sediment from both faunated and defaunated cores (0–2, 3–6, 8–10 and 18–20 cm; including sediment accumulated on the original surface -2–0 cm from faunated cores) were analysed for median grain size using a Particle Size Analyser (Malvern Mastersizer 3000).

Anoxic (jar) incubations – microbial reactions

The microbial reactions within four sediment depths in defaunated sediment, and five depths in faunated (+*Arenicola*) sediment were assessed from accumulation and consumption of porewater solutes during anoxic sediment incubations (Valdemarsen et al. 2012, Quintana et al. 2013). Production of total carbon dioxide (CO_2), sulfate (SO_4^{2-}), and dissolved organic C (DOC) were used as proxies for microbial response (Hansen and Kristensen 1998, Valdemarsen et al. 2012, Quintana et al. 2013). Sediment from depth intervals of 0–2 cm, 3–6 cm, 8–10 cm and 18–20 cm were pooled from replicates of each enrichment type after cores were sectioned. Faunated treatments contained an additional depth interval, -2–0 cm, from sediment added to the original surface *via Arenicola* reworking and defecation. The pooled sediment from each depth was homogenised, packed into 20 mL glass vials (hereby referred to as "jars"), and then sealed with air-tight screw caps. Ten jars were prepared for each depth interval, with all jars being buried in buckets containing sandy sediment to ensure exposure to constant temperature (15°C), darkness and anoxia.

Porewater was extracted by centrifugation of 2 jars from every jar series every 5–6 days. The jars were centrifuged upside down at 1500 rpm for 10 minutes in a centrifuge tube after replacing the screw-top lids with punctured caps containing GF/C filters. The collected porewater was analysed for SO_4^{2-} by ion chromatography and standardised against chloride using methods described by Martin and Banta (1992). DOC concentrations were measured on a Shimadzu TOC-5000 Analyser, and TCO_2 concentrations were analysed using the flow injection method (Hall and Aller 1992). Total production of C_{org} degradation by-products (DOC and TCO_2) produced within the anoxic incubation were calculated and used to produce a C budget to estimate the rates of C_{org} degradation within the sediment.

Stable isotope analysis of *Arenicola* tissue

Arenicola individuals collected initially and recovered from each faunated treatment by the end of the experiment were collected for stable isotope analysis. The worms were left to defecate overnight, rinsed with distilled water and then frozen. Each worm had their tail-end removed (to ensure digestive tract contents did not contaminate the sample), with the remaining tissue (mouth and thoracic region) of each worm dried at 60°C for 48 hours. Fresh samples of seagrass leaves and macroalgal thallus were cleaned of epiphytes, washed with distilled water, and dried at 60°C for 24 hours. All dried fauna and macrophyte samples were ground into a fine powder using a clay mortar and pestle before stable isotope analysis. Sediment samples were also ground, and a subsample was treated with 10% HCl to remove inorganic carbonates. The re-dried samples were used for $\delta^{13}C$ analysis, while subsamples for $\delta^{15}N$ were not acidified.

Corg and nitrogen (N) content as well as stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of macrophyte, fauna and sediment samples were determined with a analytical elemental analyser, (Thermo Flash EA 1112 Series) coupled *via* a isotope ratio mass spectrometer (ConFlo IV interface to a Thermo Delta V Plus). Stable isotope ratios are expressed as δ values (‰) relative to conventional standards (VPDB limestone for C and atmospheric N_2 for N) according to;

$$\delta Y = \left[\frac{R_{sample} - R_{standard}}{R_{standard}}\right] \times 10^3$$

Where "Y" represents ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ in the case of C and $^{15}N/^{14}N$ in the case of N.

Statistical analysis

The impact of *Arenicola* on the degradation of seagrass and macroalgal-derived C was tested with separate two-way ANOVAs on macrophyte detritus recovery and sediment C_{org} content. The addition of *Arenicola* (defaunated and faunated sediment) and type of macrophyte detritus used for enrichment (+SG, +MA, +MIX or control), were used as fixed independent factors in these analyses. Rates of bioirrigation among faunated sediment were compared using a one-way ANOVA, with type of macrophyte enrichment used as a fixed factor. Levene's test for homogeneity of variance was carried out prior to analysis. Where suitable, Tukey's post-hoc test was used to establish

which variables produced significantly different results. IBM SPSS Statistics (Ver. 22) was used to perform all tests, with a significance level of $\alpha = 0.05$.

Results

Bromide profiles and bioirrigation rates

Profiles of Br^- clearly revealed the extent of *Arenicola* bioirrigation in the sediment (Fig. 4.1). In control cores Br^- only penetrated to 3-4cm depth and Br^- profiles indicated transport by passive diffusion, while Br^- was actively transported deep down into the sediment in all faunated treatments. In treatments containing macrophyte enrichment, Br^- peaked at intermediate depths (10–13 cm) into the sediment, and declined below. No such decline was observed in sediment with no enrichment. Instead a fairly constant increase in Br^- concentrations occurred until the maximum sediment depth. The area-specific rate of bioirrigation, which was corrected based on *Arenicola* density, showed clear trends among treatment types. Cores enriched with both macroalgae (+MA), and mixed macrophyte detritus (+MIX) had a significantly higher rate of bioirrigation, compared to both control sediment and sediment containing only seagrass ($p = 0.032$ and $p = 0.033$ respectively). There was no difference in bioirrigation rate between sediment cores containing macroalgae (+MA) and control sediment cores ($p = 0.160$). Sediment cores enriched with seagrass (+SG) had a similar rate of bioirrigation to control sediment cores ($p = 0.908$), and significantly lower bioirrigation rates than sediment cores with mixed macrophyte detritus ($p = 0.033$).

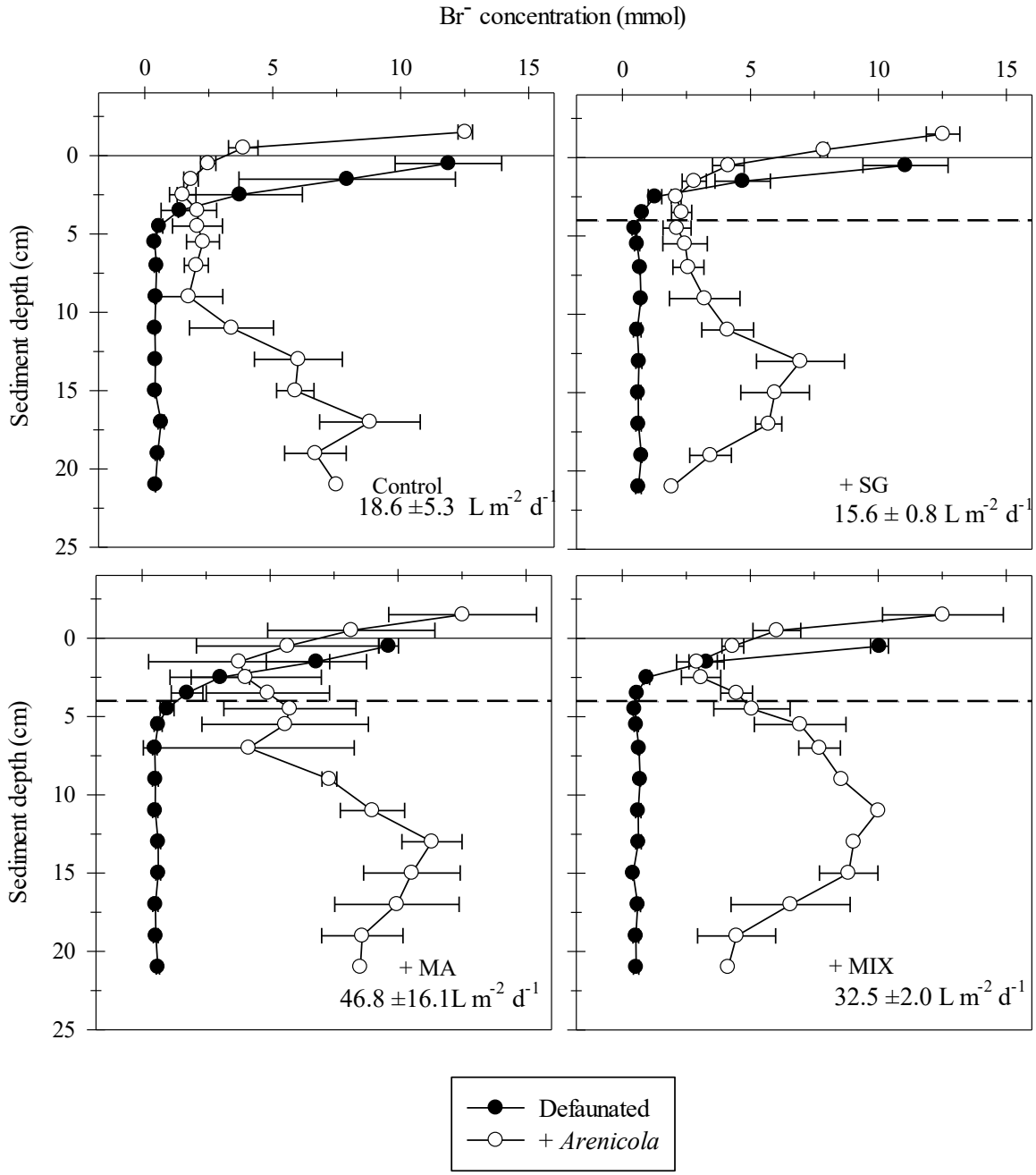

Fig. 4.1: Vertical porewater profiles of bromide (Br^-; mmol) in faunated (+*Arenicola*) and defaunated sediment cores with +SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae) and without (Control) enrichment. The dashed line (4 cm depth) indicates the initial depth of macrophyte enrichment. The straight reference line (0 cm depth) indicates the original sediment surface. Numerical values in each graph represent calculated bioirrigation rates ±SE within faunated sediments (L m^{-2} d^{-1}). Error bars: SE (n = 3).

Sediment water content

A marked increase in sediment water content was observed with the introduction of *Arenicola* (Fig. 4.2). In defaunated sediments regardless of enrichment type, water content at the surface of the cores began at around 17–18%, and decreased steadily until a depth of 4 cm (depth of macrophyte enrichment), where it remained uniform in the sediment until the bottom of the core (15–16.5%). Cores with *Arenicola* contained more water, 19–21 % to 15 cm depth, below which the water content rapidly declined to that of defaunated sediment (~ 15%).

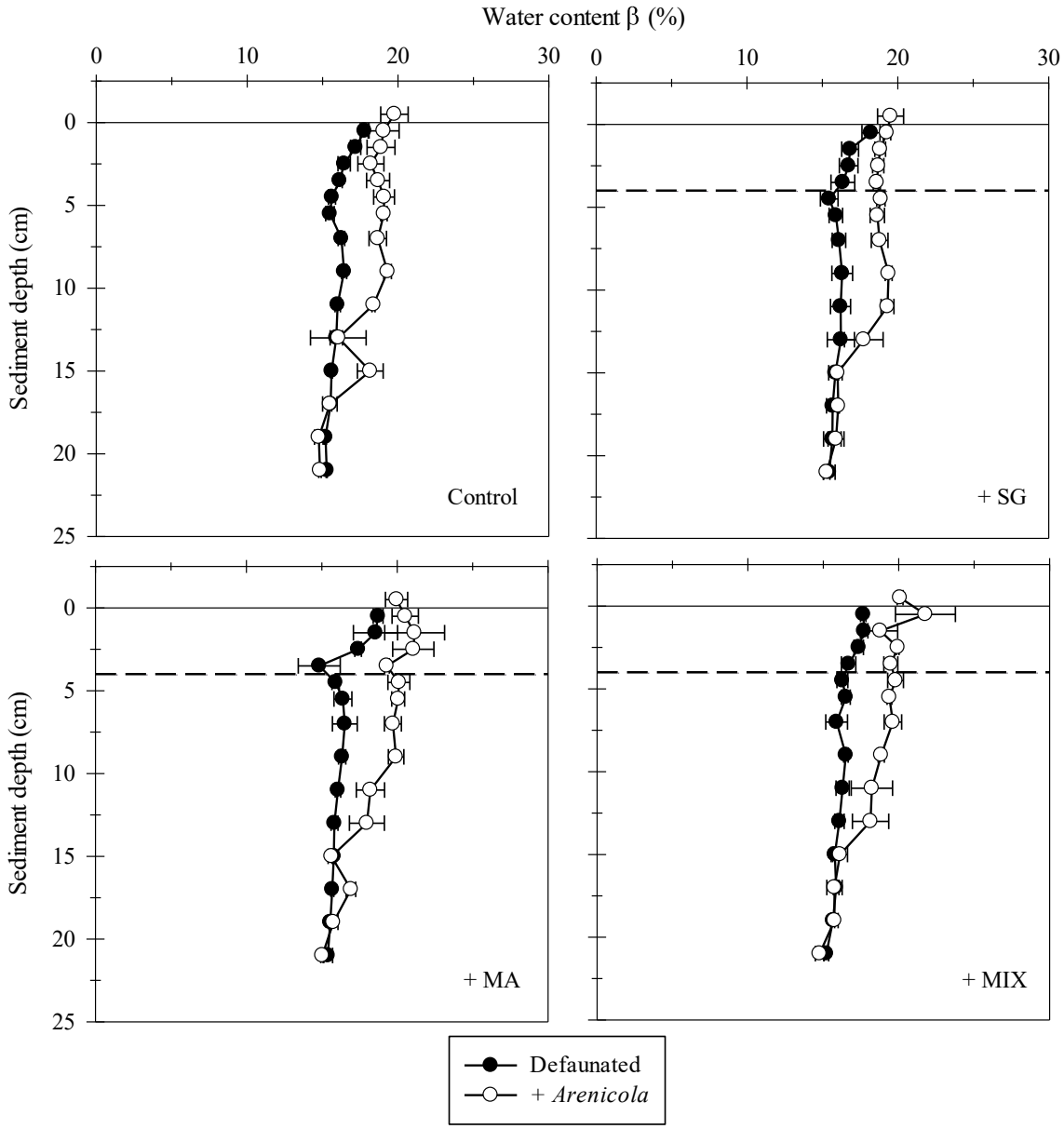

Fig. 4.2: Core profiles of sediment water content, in faunated (+ W) and defaunated sediment. Sediment was amended with +SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae) and without (Control) enrichment. The straight reference line (0 cm depth) indicates the original sediment surface, while the dashed line (4 cm) indicates the initial depth of macrophyte enrichment. Bar width is indicative of the sediment portion sampled. Symbols represent means ± SE (n = 3).

Total sediment C_{org} pool–Recovery of macrophyte detritus and quantification of sediment C_{org} content

After 28 days, the impact of *Arenicola* bioturbation on the available pool of C (detrital-derived C) was evident (Fig. 4.3). Overall, more seagrass-derived C was retained in sediment cores than macroalgal C ($p < 0.001$). Retention of macroalgal C was significantly reduced in the presence of both seagrass ($p = 0.002$) and *Arenicola* ($p < 0.001$). Seagrass C retention however, was not significantly affected by the presence of either *Arenicola* ($p = 0.380$) or buried macroalgae ($p = 0.672$). Based on the difference between added and recovered macrophyte material, 41.8% of seagrass and 56.2% of macroalgae detritus in defaunated treatments was degraded (Fig. 4.3). This pattern was significantly altered by *Arenicola* bioturbation, in which seagrass detritus was preserved after rapid burial of detritus into the deep (>15 cm) and anoxic sediment (45.4% degraded), and comparably, twice as much macroalgae was degraded in the presence of *Arenicola* (80.5% was degraded). In treatments with mixed macrophyte material (+MIX), 23.3% of seagrass and 72.7% of macroalgae detritus was degraded in non-bioturbated cores, while the addition of *Arenicola* increased the degradation of macroalgae by an additional 60% and seagrass by approximately 10% over a 28 day period.

In defaunated sediment, the majority of detritus was retrieved close to the original burial depth (3–4 cm). Physical re-working by *Arenicola* buried seagrass detritus an additional 6 cm deeper into the sediment (8–14 cm depth), with retrieved detritus appearing similar in size and condition to detritus recovered in defaunated sediment. Macroalgal detritus recovered from faunated cores was visibly macerated and spread through the sediment to a depth of 14 cm. This pattern was similar in treatments containing *Arenicola* with macroalgae (both +MA and +MIX). Neither bioturbation ($p = 0.991$) nor macrophyte enrichment ($p = 0.316$) made a significant difference to total sediment C_{org}.

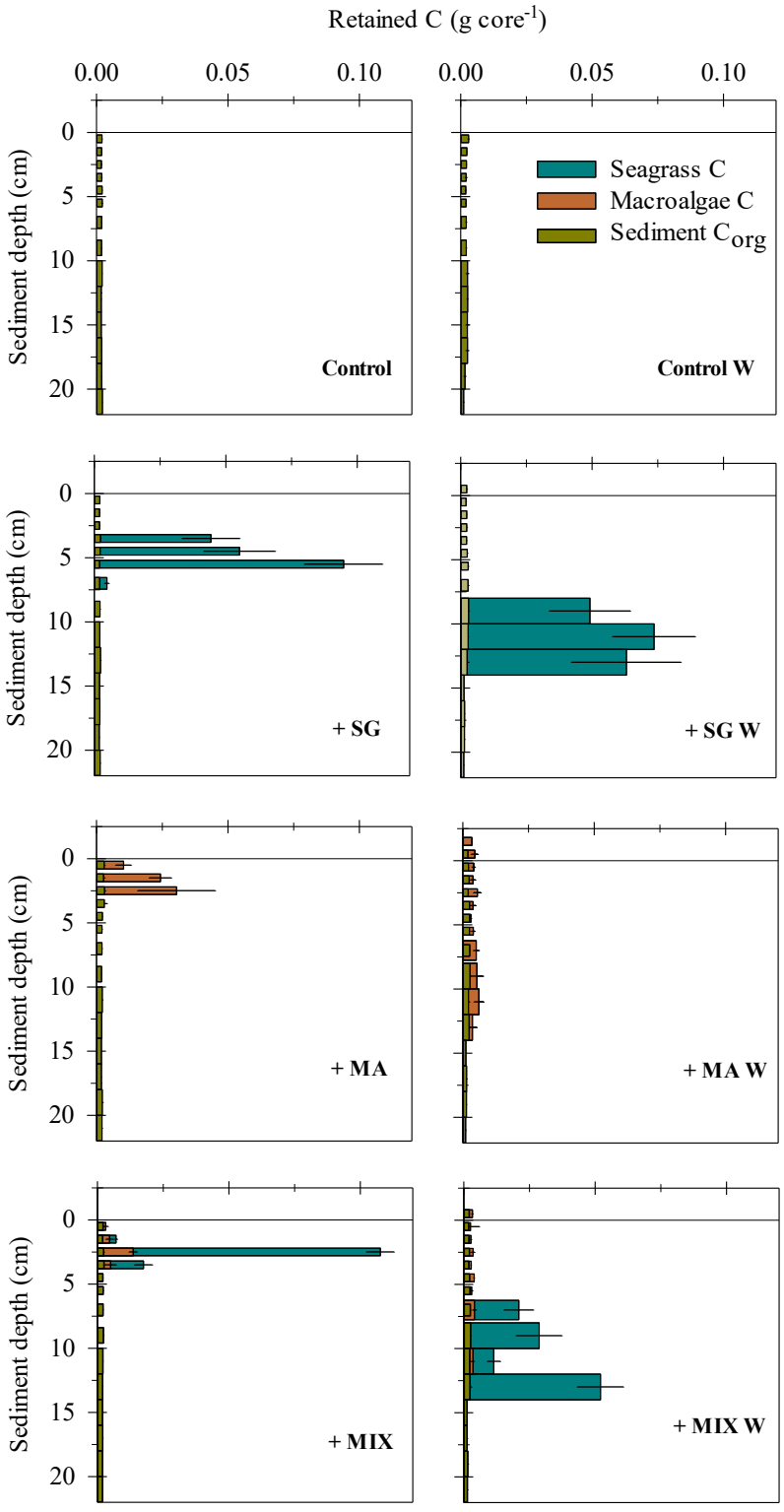

Fig. 4.3: Calculated recovery of detritus derived C, and sediment C_{org}, in sediment cores with +SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae) and without (Control) enrichment. Original enrichment depth was 3–4 cm. The straight reference line (0 cm depth) indicates the original sediment surface depth. Bar width is indicative of the sediment portion sampled. Error bars: SE (n = 3).

Sediment grain size, and silt and clay content

Selected sediment samples showed a distinct effect of *Arenicola* on both grain size distribution, and silt and clay content (Fig. 4.4). Although defaunated treatments remained relatively homogenous in both grain size distribution (median grain size 0.23–0.25 mm) and silt and clay content (4.7–5.5%) throughout the sediment column, treatments populated with *Arenicola* were considerably altered. A trend for increased grain size below the macrophytes burial depth was observed in faunated sediment, with considerably smaller grains (0.20 mm) observed in the mixed layer (0-10 cm), and larger grains (0.43 mm) present in the 18–20 cm sediment depth. Additionally, a higher content (4-7%) of silt and clay was evident in the mixed layer of these sediments compared to that in deeper sediment (1.5 %).

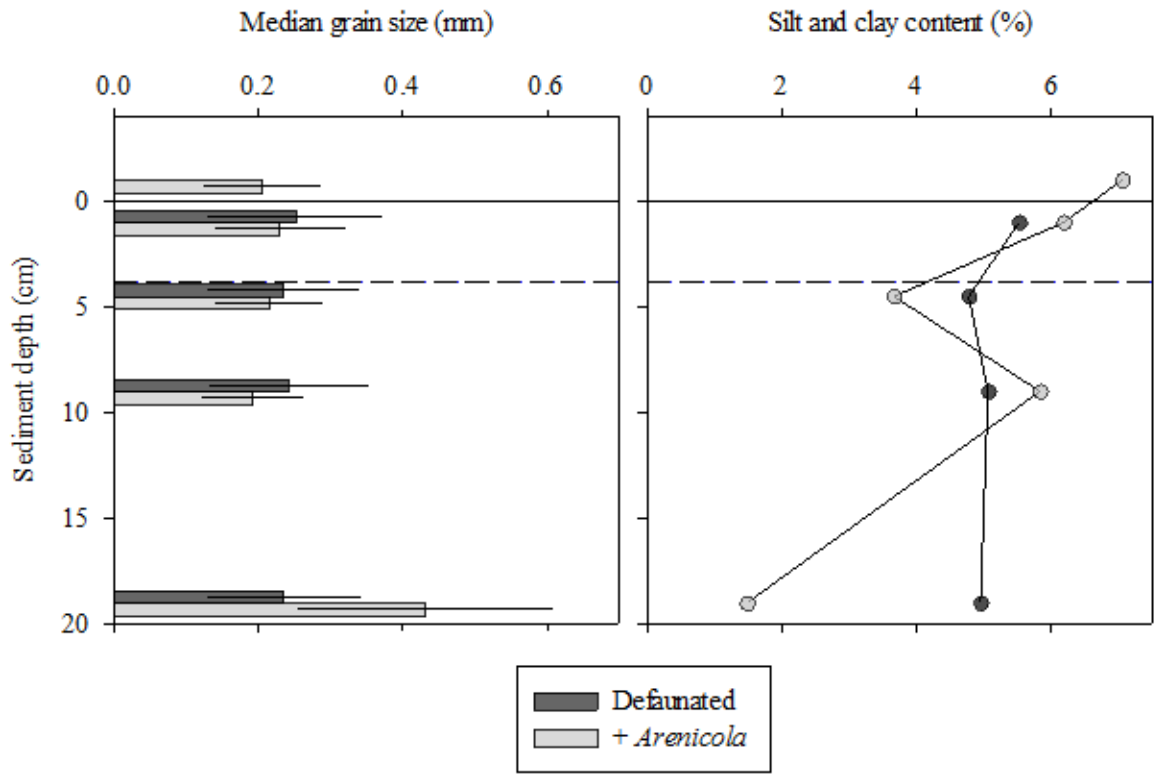

Fig. 4.4: Vertical profiles of median grain size (mm) distribution and silt and clay content (%) of sediment collected from faunated (+ *Arenicola*) and defaunated sediment. Samples were taken from sediment within incubated jars from -2–0, 0–2, 3–6, 8–10 and 18–20 cm depths. Bar width is indicative of sediment portion sampled. The dashed line (4 cm depth) indicates the initial depth of macrophyte enrichment. The straight reference line (0 cm depth) indicates the original sediment surface.

Anoxic jar incubations–microbial reactions

Production of TCO_2, SO_4^{2-} and DOC in anoxic sediment (jar) incubations revealed a clear effect of *Arenicola* on the microbial response (Fig. 4.5). Notably, there was an accumulation of C remineralisation byproducts (both TCO_2 and DOC) in faunated (+ *Arenicola*) sediment enriched with seagrass (180 and 412 nmol cm^{-3} d^{-1} respectively). DOC production in all other faunated treatments was consistently low throughout the sediment (0–33 nmol cm^{-3} d^{-1}). In defaunated sediment containing macrophyte detritus enrichment, a peak in DOC production appeared in the upper depths (3–5 cm) of the sediment containing seagrass detritus (244–320 nmol cm^{-3} d^{-1}) in macroalgae and mixed detritus (+MIX) treatments. Similarly, SO_4^{2-} consumption was consistently low throughout the sediment in all treatments (28–62 nmol cm^{-3} d^{-1}), except for sediment enriched with seagrass. In defaunated sediment, a peak in consumption appeared at a sediment depth of 3–5cm (140 nmol cm^{-3} d^{-1}). TCO_2 production in defaunated sediment was relatively uniform, with slight peaks seen in the upper sediment depths (97–197 nmol cm^{-3} d^{-1}). Although peaks of TCO_2 production were observed in seagrass enriched faunated sediment, a peak in TCO_2 accumulation at 8–10 cm was also observed in faunated sediment enriched with macroalgae (166 nmol cm^{-3} d^{-1}).

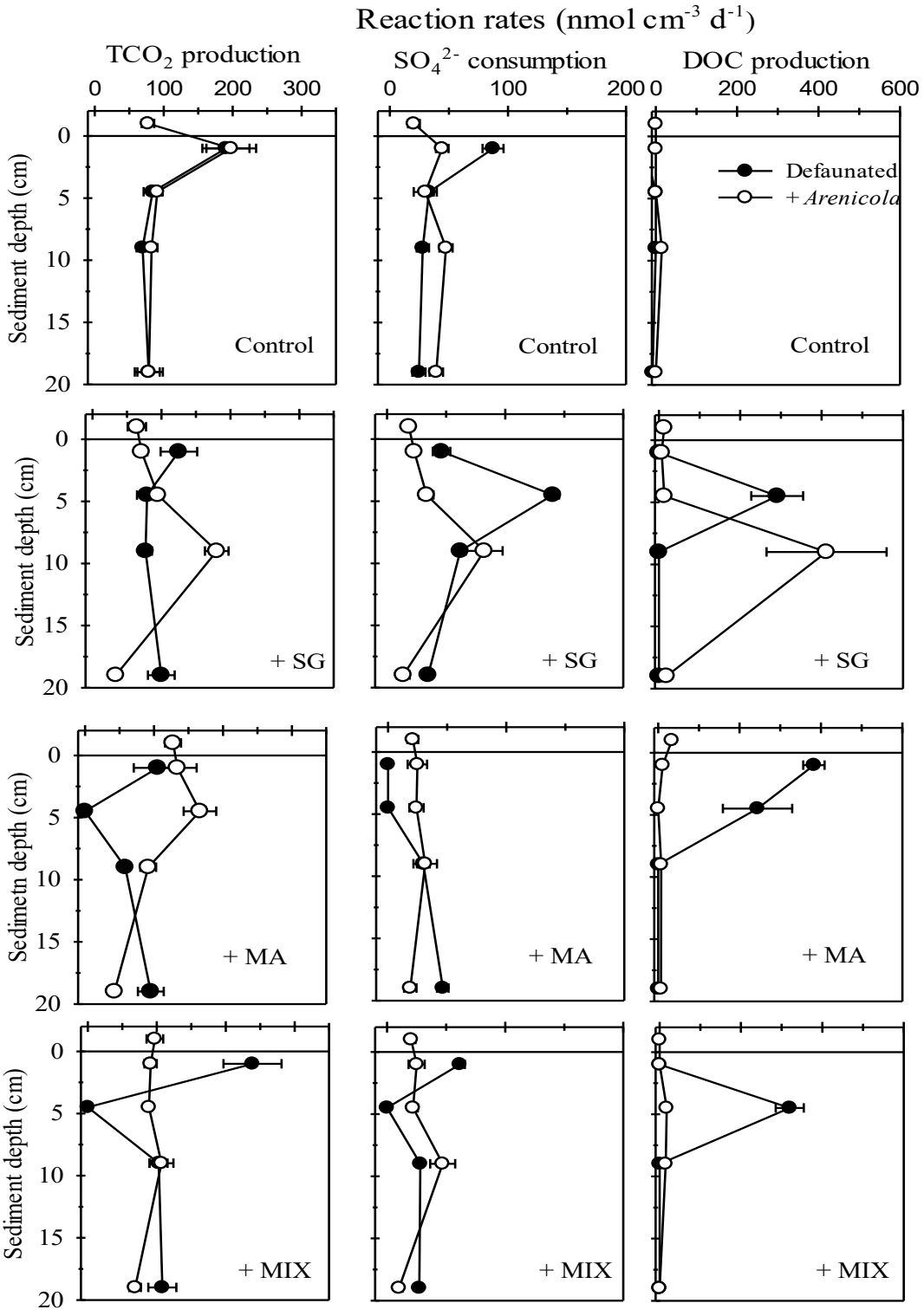

Fig. 4.5: Vertical porewater profiles of total carbon dioxide (TCO_2), sulfate (SO_4^{2-}), and dissolved organic carbon (DOC) microbial reaction rates (nmol cm^{-3} d^{-1}) in faunated (+*Arenicola*) and defaunated sediment cores with +SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae) and without (Control) enrichment. Error bars: SE (n = 10).

Anoxic (jar) carbon budget

Rates of C_{org} degradation (Table 4.1), calculated from the accumulation of total depth-integrated porewater degradation by-products (DOC and TCO_2) in anaerobic incubations, displayed clear differences among treatment type (Table 4.1). Sediment containing macroalgae (both macroalgae and mixed macrophyte treatments) previously populated with *Arenicola* showed a decrease (54% and 43% respectively) in C degradation. A small increase (17%) in C_{org} degradation remineralisation was observed in control sediment previously populated with *Arenicola*, compared to defaunated sediment. However, in sediment containing only seagrass, a marked increase (30%) in C remineralisation was observed in sediment previously containing *Arenicola*, compared to defaunated sediment. Faunated seagrass sediment had a C_{org} degradation rate almost double that of any other faunated treatment (1,035 mmol m^{-2} compared to 489–540 mmol m^{-2} over 25 days). Defaunated sediment containing macrophyte material had similar C remineralisation rates (790–854 mmol m^{-2}), all of which were greater than the C_{org} degradation observed in control sediment (423 mmol m^{-2}).

Table 4.1: Total production of porewater solutes (mmol m^{-2}), based on calculated anaerobic microbial reaction rates in incubated jars with sediment collected from -2–0, 0–2, 3–6, 8–10 and 18–20 cm depths, with calculated values representing depth-integrated reaction rates. Sediment was collected from faunated (+ *Arenicola*) and defaunated cores, with +SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae); and without (Control) macrophyte enrichment.

Microbial Reaction Rates (mmol m^{-2})	Defaunated				+*Arenicola*			
	+ SG	+ MA	+ MIX	Control	+ SG	+ MA	+ MIX	Control
TCO_2	463	329	494	456	460	503	452	505
DOC	327	514	360	-33	575	38	38	19
Total C_{org} degradation	790	843	854	423	1035	541	490	523
C_{org} degradation of initial (%)	1.9	2.0	2.1	1.2	2.5	1.3	1.2	1.4

* All values are represented as mmol m^{-2} for the 25 day anoxic incubation

Stable isotope analysis of *Arenicola* tissue

Stable isotope signatures of *Arenicola* retrieved from the experimental cores were compared with those of the various organic materials initially present in the sediment (i.e. *Arenicola*, eelgrass, macroalgae and C_{org} in sediment). Initial seagrass leaves had $\delta^{13}C$ ranging from -10.7 to -11.9, and $\delta^{15}N$ ranging from 5.1 to 6.2; while macroalgae had markedly different signatures with $\delta^{13}C$ ranging from -15.9 to -19.8 and $\delta^{15}N$ ranging from 14.9 to 16.4. Sediment samples from the sample site had $\delta^{13}C$ of -15.9 to -16.4, and $\delta^{15}N$ of 7.3 to 9.0; while initial *Arenicola* tissue had a $\delta^{13}C$ of -13.3 to -14.7, and $\delta^{15}N$ of 8.1–11.8 (Fig. 4.6). *Arenicola* tissue retrieved from experimental cores at the end had stable isotope signatures that were displaced compared with the initial situation. *Arenicola* retrieved from both seagrass and bare sediment treatments displayed signatures ($\delta^{13}C$ of -14.6 to -16.0 and $\delta^{15}N$ of 10.8 to 12.9) similar to that of initial *Arenicola* tissue. *Arenicola* tissue retrieved from cores containing macroalgae (both those containing strictly macroalgae, and mixed macrophyte treatments), on the other hand, had stable isotopes values ($\delta^{13}C$ of -14.6 to -18.7 and $\delta^{15}N$ of 11.0 to 13.8) approaching initial macroalgae values, with a stronger trend for tissue retrieved from strictly macroalgae enriched treatments than mixed macrophyte enrichment treatments.

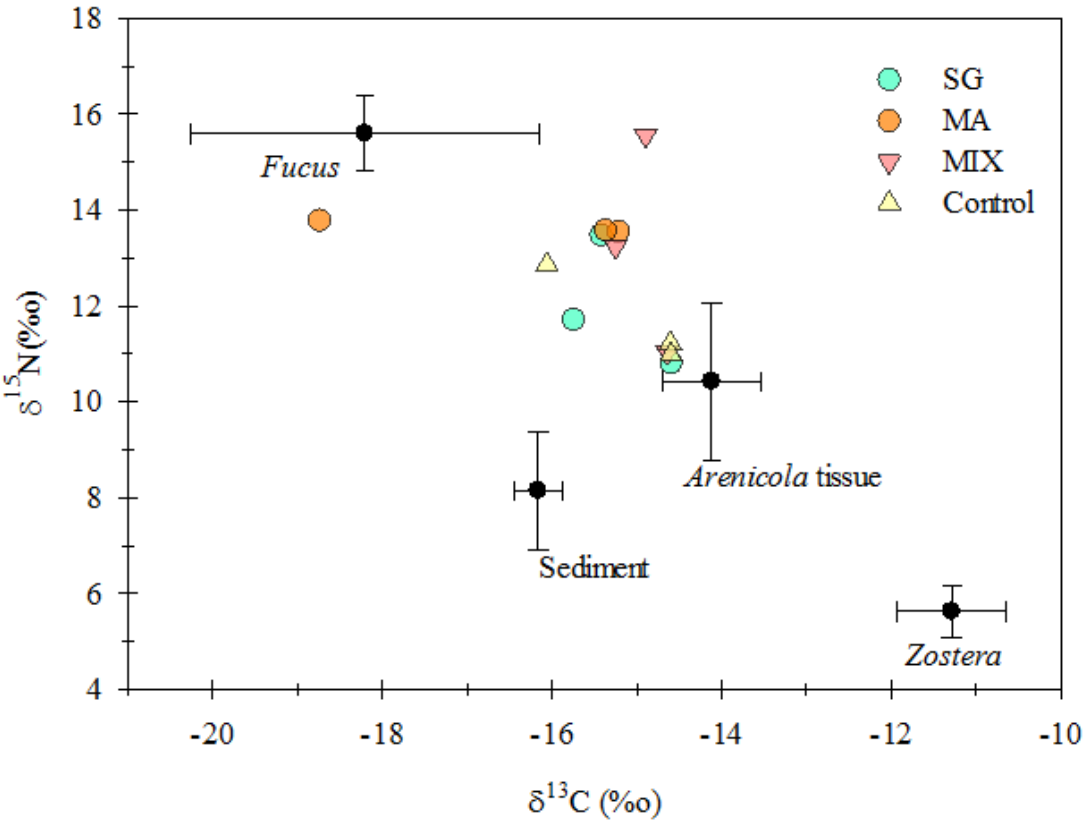

Fig. 4.6: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values for *Arenicola* tissue, taken from animals removed from macrophyte-enriched (+ SG (seagrass), +MA (macroalgae), and +MIX (seagrass and macroalgae)) and non-enriched (Control) sediment. The labelled symbols ± SD bars indicate reference samples taken from *Fucus* and *Zostera* tissue, sediment and non-exposed *Arenicola* tissue. For reference and treatment samples, n = 3.

Discussion

Worldwide, seagrass sediments are important reservoirs for organic carbon (C). Possibly the most notable finding in this study is that bioturbation by *Arenicola* promotes the anaerobic degradation of seagrass-derived C. Considering that seagrass detritus contributes on average half of the sediment C in seagrass ecosystems (Kennedy et al. 2010), the finding that *Arenicola* bioturbation stimulates microbial activity to degrade this C has significant implications for our knowledge of the preservation of seagrass blue carbon (BC) stocks.

The rates of sediment bioirrigation in faunated sediment were similar, albeit smaller, with rates previously found for this species (Kristensen 2001), which may reflect the smaller juvenile individuals used in this study. In sediments enriched with seagrass, bioirrigation rates were lower than that of the control sediment, and less than half of that observed in macroalgae enriched sediment. Bioturbation and physical reworking of the sediment displaced the buried seagrass detritus as a discrete layer deeper into the sediment (10–15 cm), corresponding to a decline in sediment bioirrigation (peak at 12 cm, then declining thereafter).

In this study, the clogging of interstices with particles, such as detritus, had clear effects on sediment permeability in *Arenicola* burrows (Volkenborn et al. 2007). As the seagrass detritus was recovered from faunated sediment in a discrete layer, it is plausible that restricted irrigation also occurred. Sediment particle re-working had clear effects on the physical structure of the sediment, with faunated sediment containing higher water content than defaunated sediment. The J-shape of *Arenicola* burrows enables substantial advective porewater transport into the surrounding sediment (Kristensen 2001, Meysman et al. 2006a), increased rates of which have been linked to organic C degradation (Banta et al. 1999, Papaspyrou et al. 2007), and stimulation of both biogeochemical processes and microbial activity *via* supply of oxygenated water (Mermillod-Blondin et al. 2004, Volkenborn et al. 2007). Adult *Arenicola* can pump up to 1.5 mL min^{-1} of oxygenated water through their burrows (Riisgaard et al. 1996), thereby replacing important electron donors important for C remineralisation within the sediment. In this study, the intensity of bioturbation clearly enhanced the initial rate of organic matter (OM) degradation.

In sediment cores containing *Arenicola,* physical re-working of the sediment was substantial, and resulted in significant restructuring of the sediment profile. *Arenicola* are known to be extremely active in their grain size stratification of sediment (Baumfalk 1979), whereby particles (> ca. 3 mm) not ingested by *Arenicola* are buried (Valdemarsen et al. 2011). The resulting "biosorting" of sediment grains has implications for the export of fine particle DOC and POM. *Arenicola* fecal casts can deposit as much as 10 cm of high POC sediment on the surface per year (Cadée 1976, Gutiérrez et al. 2000). These deposited fecal casts are quickly washed away by tidal movements, and fine particles are removed from the sediment *via* resuspension (Volkenborn et al. 2007, Wendelboe et al. 2013). While tidal export of C could not be measured within this study, deposition of fine grains and particles of macroalgae detritus on the sediment surface was noted. Additional transfer of C *via* tidal export, and accumulation of C due to particle trapping (Ward et al. 1984) is therefore likely within *in situ* conditions.

Together with Chapter 3, the results from this study show that after one month, the more easily degradable macroalgae were mostly (~81–83 %) consumed, while the more recalcitrant seagrass C remained preserved in the sediment (~45–48% degraded). Stable isotope analysis of worm tissue recovered from sediment cores indicated that those worms recovered from cores amended with macroalgae had consumed some portion of the macroalgae-derived material. Since macroalgae lack structural (i.e. lignin) polymers (Hill et al. 2015, Trevathan-Tackett et al. 2015) and are relatively soft, it is likely that they were macerated during intensive physical reworking by *Arenicola.* Although initially large (~15 mm), the labile macroalgae would have become smaller during particle re-working and maceration by Arenicola, and degradation in the sediment (Greiner et al. 2016). These processes would have led to the macroalgae increasing in surface area to volume ratio, and subsequently making it easier for Arenicola consumption. This is further supported by the presence of fine macroalgal particles recovered from the fecal cast layer in sediment cores. Additionally, we suggest that deposition of macroalgae on the sediment surface *via Arenicola* defecation increased the bioavailability of the macroalgae tissue to microbial degradation. Increased microbial biomass and activity, introduced through faunal consumption (Grossmann and Reichardt 1991), would have likely aided the degradation of the macroalgal tissue (Kristensen and Mikkelsen 2003, Papaspyrou et al. 2007).

Arenicola can stimulate C oxidation up to 3-times more than other fauna, i.e. *Nereis diversicolor* (Banta et al. 1999), and while bioturbation prevented the loss of seagrass C within mesocosm conditions to an extent, we suggest that during exposure to bioturbation, the seagrass detritus in faunated sediment would have likely been exposed to a mixed consortia of microbes and oxygenated water. In the anoxic incubation, sources of DOC for sulfate-reducing bacteria, were supplied by the degrading macrophyte material (both macroalgae and seagrass) of which, seagrass was in greater supply. The enhanced remineralisation of seagrass C (double that of any other faunated treatment) in the anoxic incubations is therefore, likely to be attributed to a wider range of organic substrates provided by the detritus that was buried deeper into the sediment in the initial (relatively) aerobic incubation, and likewise supported greater diversity of microbial groups and enzymes to mineralise the detritus (Guenet et al. 2012).

Microbial degradation of seagrass C is supported by peaks of DOC and TCO_2 production, as well as SO_4^{2-} consumption in faunated sediment, at depths where the bulk of seagrass detritus was recovered (8–10 cm). A small increase in the ratio of depth-integrated TCO_2 production and SO_4^{2-} consumption in faunated treatments (C:S = 2.5–4.0) compared to defaunated sediment (C:S = 1.4–3.8) indicates slight differences in sediment C remineralisation pathways. While the stoichiometry of sediment sulfate-reducing C remineralisation is ~2:1, signifying electron transfer directly from C to SO_4^{2-}, the higher values observed in our data may be associated with alternative anaerobic pathways (i.e. fermentation) (Canfield et al. 2005, Valdemarsen and Kristensen 2010). It is therefore evident that heterotrophic sediment processes were stimulated by the presence of seagrass detritus at these depths. Interestingly, only in faunated sediment was CO_2 production significantly stimulated, indicating that *Arenicola* bioturbation potentially altered both the microbial community composition and the availability of electron acceptors (Kristensen 2001).

It is clear that the effects of *Arenicola* bioturbation have impacts on the sediment biogeochemistry, microbial community and C remineralisation. In this instance, it appears as though the below-ground microbial and chemical environment was altered so that degradation of seagrass C doubled (compared to other treatments). Further to this, bioturbation-stimulated processes (i.e. changes to microbial activity) may have led to instances of greater C degradation. In seagrass sediments containing C as buried

detritus, bioturbators such as *Arenicola* may significantly increase the vulnerability of C to alternate conditions, resulting in enhanced rates of C remineralisation and CO_2 release. While more labile sources of C (i.e. macroalgae) are likely to be degraded through sediment masceration and integration, recalcitrant sources of C (i.e. seagrass) may be initially preserved, but are vulnerable to bioturbator-stimulated microbial degradation. The effect of bioturbation on the degradation and remineralisation of more recalcitrant macrophyte tissue (i.e. seagrass) deserves further investigation namely to address long-term impacts, as it has on-going effects for the preservation of seagrass BC.

Chapter 5:

Bioturbator-stimulated loss of seagrass carbon stocks *via* microbial priming

Abstract

Seagrass ecosystems are highly productive, and are sites of significant carbon sequestration. Sediment-held carbon stocks can be many thousands of years old, and persist largely due to sediment anoxia and a relatively "dormant" microbial community. However, the carbon sequestered in seagrass ecosystems may be susceptible to remineralization *via* the activity of bioturbating fauna. Primarily, the hypothesis that bioturbation could stimulate sediment microbial priming whereby remineralisation of sediment carbon (recalcitrant organic matter) was stimulated bioturbation-mediated burial of a labile source of organic matte (seagrass) was investigated.

We carried out a 2-month mesocosm study to compare the remineralisation (measured as release of CO_2) from buried seagrass (*Zostera muelleri*) to the total rate of sediment organic matter remineralisation in sediment populated with and without a common Australian bioturbator *Trypaea australiensis* (Decapoda: Thalassinidae). Our findings revealed that bioturbation led to clear changes in benthic metabolism and resulted in the burial of seagrass detritus to a depth of 23 cm. Additionally, a clear bioturbator-stimulated priming effect was discovered. Bioturbation led to a 2 to 5 fold increase in total CO_2 release, and the calculated bioturbator-stimulated priming effect was equivalent to 15% of the total daily sediment-derived CO_2 releases. In control sediment containing seagrass, we observed a negative priming effect where seagrass remineralisation was favoured over sediment remineralisation, re-affirming that bioturbation causes seagrass sediments to become carbon sources rather than carbon sinks.

The results of this study suggested that the impact of bioturbator-stimulated microbial priming could have significant impacts on our understanding of seagrass carbon stocks. We conclude that bioturbation-mediated microbial priming effectively "leaks" sediment-held carbon as CO_2, and hypothesise that significant change to seagrass faunal communities may have negative repercussions on seagrass carbon stocks.

Introduction

Estuarine and coastal ecosystems, including seagrass, saltmarsh and mangroves (known as "blue carbon" ecosystems) are global hotspots for carbon (C) sequestration and storage (Mcleod et al. 2011, Fourqurean et al. 2012). Investigating how marine ecosystems sequester and release C has been proposed as a scientific priority (Guenet et al. 2010), however, there is paucity of data on the factors affecting C cycling in coastal seagrass sediments, due in part to a lack of knowledge about microbial processes within these environments. Within seagrass ecosystems, labile organic C, that is released directly from the rhizosphere or through microbial hydrolysis of particulate organic C (POC), is rapidly degraded by microbial activity within the sediment (Guy 2010). The remaining POC can stay buried for centuries to millennia due to relatively dormant microbes and anoxic sediment conditions (Burdige 2007, Mcleod et al. 2011). This burial represents a large pool of relatively "stable" C, however, in terrestrial systems it has been shown that inputs of labile organic matter (OM) can lead to a disproportionate remineralisation, or release, of this "stable" C *via* the microbial priming effect (Kuzyakov et al. 2000, Fontaine et al. 2003, Guenet et al. 2010).

Burrowing macrofauna are a common feature in coastal benthic ecosystems (Kristensen et al. 2012). Understanding the impact of these bioturbators on the persistence of coastal C stocks has been suggested as a research priority (Macreadie et al. 2014). The physical activity of macrofauna can have a major effect on coastal C cycling and sequestration, given their influence on the sediment, and their relationship with sediment microbes (Kristensen 2001, Papaspyrou et al. 2004, Papaspyrou et al. 2005, Papaspyrou et al. 2010, Maher and Eyre 2011, Kristensen et al. 2012). Direct impacts include mechanical re-working of sediment to disperse C-rich deep sediment onto the sediment surface in oxygenated water, and indirect impacts include feeding processes that alter the microbial community within the deeper parts of the burrow (Kristensen 2008).

Bioturbators can act as a physical catalyst for sediment metabolism, by incorporating organic matter (OM) e.g. seagrass detritus (Aller 1983, Kristensen et al. 1985) into burrow walls and introducing oxygen into an otherwise anoxic environment. Indeed, Thalassinidean shrimp burrows are often seen lined with seagrass detritus, which is actively integrated into the sediment by reworking and faunal 'gardening' (Dworschak 2001). Bioturbator burrows also support extensive microbial populations, with up to 11

times higher microbial biomass found within the walls of burrows than in surrounding sediment (Papaspyrou et al. 2005). This coupling of high microbial biomass, and increased supply of labile organic matter can stimulate CO_2 release; with the magnitude of this sediment stimulatory effect depending on bioturbator activity, density of fauna, and most critically, the quality and quantity of OM (Hansen and Kristensen 1998, Banta et al. 1999, Papaspyrou et al. 2007).

Although seagrass ecosystems are ideal for rapid, permanent C storage, preservation of these stocks is threatened by anthropogenic activity, including habitat loss (Marbà et al. 2006, Fourqurean et al. 2012, Macreadie et al. 2015), and loss of top-down predator control affecting food webs and trophic cascades (Atwood et al. 2015). Microbial priming occurs when moderate changes in environmental conditions (e.g. physical disruptions or fresh OM inputs) 'prime' or activate microbes into causing leakage (efflux) of stored 'stable' C from the sediment (Kuzyakov et al. 2000). Terrestrial studies have shown that inputs of labile OM can lead to significant increases of recalcitrant OM remineralisation (Kuzyakov et al. 2000, Fontaine et al. 2003, Fontaine et al. 2007), in some cases increasing soil respiration rates up to 11-fold (Blagodatskaya and Kuzyakov 2008).

The effect of priming on C cycling has only recently garnered attention in marine systems (Guenet et al. 2010, Gontikaki et al. 2015, Steen et al. 2015). Indeed, the stimulation of sediment metabolism by bioturbating macrofauna could be a trigger for remineralisation of recalcitrant C in deep sediment layers *via* microbial priming. However, how bioturbation affects the remineralisation (CO_2 release) of different sediment C sources (i.e. detritus) and buried recalcitrant OM stocks in seagrass ecosystems has yet to be investigated.

The aims of this study were two-fold: (1) to determine whether the burial of a labile organic matter (LOM) source (i.e. seagrass detritus) into deeper sediment by bioturbating fauna would stimulate microbial priming; and (2) to characterise the source of CO_2 released from the system (i.e. seagrass or sediment organic matter) by using isotopically amended seagrass detritus and tracing the evolution of ^{13}C-CO_2.We hypothesised that incorporation of seagrass (labile organic matter; LOM) detritus into the sediment *via* bioturbation would lead to increased levels of CO_2 released into the

water column, and that a microbial priming effect (PE) would increase metabolism of sediment recalcitrant organic matter (ROM).

Methods

Sediment and seagrass collection

High organic carbon (C_{org}) sediment (1.5–3%) was collected from Fagans Bay, Brisbane Waters NSW in August 2015. Collection occurred along the edges and within an existing *Zostera muelleri* meadow. The top 1-2 cm of sediment was removed prior to collection to ensure microalgae were excluded. Sediment was collected to a depth of 30–40 cm, wet sieved and homogenised on site using a 2 mm sieve to remove any plant material and macrofauna. Collected sediment was covered in fresh seawater to limit oxygen exposure.

Whole *Z. muelleri* (henceforth referred to as "seagrass") plants were collected from Fagans Bay, NSW. Plants were gathered from a meadow approximately 10 m from shore and kept in seawater during transport back to the laboratory. Only plants that appeared visually healthy were collected, with those showing signs of heavy epiphyte colonisation or senescence, excluded from collection.

Collection of Callianassids

Trypaea australiensis (a burrowing Callianassid shrimp, henceforth referred to as "Callianassid") were collected from within a shallow mixed *Z. muelleri* and *Halophila decipiens* meadow located within the Narrabeen Lagoon, NSW. Visible burrows were excavated, and sediment was sieved through a 0.5 cm mesh to retrieve Callianassids. Smaller-sized (1.2g ±0.3) adult Callianassids were used to ensure that the mesocosm size did not limit burrowing activity. Individuals were transported in seawater back to the laboratory, and left undisturbed in aerated seawater for 2–3 days to ensure that their guts were emptied before exposure to the sediment core. Individuals were weighed before introduction to experimental conditions.

Isotopic labelling of seagrass detritus

Epiphytes were gently removed from seagrass leaves, and plants were rinsed in the laboratory with distilled water to remove any sediment. They were then labelled with 99% atm ^{13}C-amended sodium bicarbonate (Novachem, VIC, Australia). Labelled sodium bicarbonate (1.2 g) was mixed with 40 L of artificial seawater (salinity 32). The

labelled seawater was transferred into an 80 L container, where the seagrass plants were submerged and incubated for 72 hours under light (150 μmol photons m^{-2} s^{-1}); 12:12 hour day and night cycles). Plants were then removed from the solution, and thoroughly rinsed with distilled water to remove salts and excess label. Leaves were isolated from roots and rhizomes, and chopped into uniform pieces < 15 mm. The prepared leaves were then separated into 4.8 g portions, and each portion was left to partially degrade in artificial seawater (32) for a further 72 hours. A subsample of the labelled seagrass was analysed for $\delta^{13}C$ and organic carbon content on an Isotope Ratio Mass Spectrometer (Thermo-Finnegan Delta V IRMS), and elemental analyser (Costech Elemental Analyser) respectively.

Experimental set-up

A laboratory experiment was carried out for 65 day to quantify the effect of Callianassid bioturbation on seagrass degradation and sediment CO_2 flux. Four treatments were established (n = 5), consisting of bioturbated and control (defaunated) high-carbon sediment cores, amended with and without the addition of $\delta^{13}C$ labelled seagrass leaves. Sediment cores were prepared by adding sediment into 20 acrylic (length = 30 cm; diameter = 8 cm) core tubes, to a depth of 22 cm. Cores were left to settle overnight (approximately 16 hours) at an ambient temperature.

The following day, 250 g sediment, corresponding to a 4 cm layer, was added to control cores (n = 10). The portions of seagrass detritus (4.8 g wet weight) were mixed with sediment (100 g wet weight) and added to the remaining cores as a 2 cm thick layer, thereafter an additional 2 cm of unamended sediment was added. All cores were left to settle for 12 hours, so that the final sediment depth was 24 cm. Each core was then topped up with additional artificial seawater, and all 20 sediment cores were transferred to four 90 L seawater tanks filled with artificial seawater (salinity of 32), with at least 5 cm of water above each core. Two tanks contained the Callianassid treatment cores (bioturbated) and two tanks contained the controls to ensure there was no faunal migration between cores or contamination with $\delta^{13}C$. Each tank was fitted with two air stones, for aeration and mixture of the surface water within tanks and cores. All tanks were covered and kept in the dark for the length of the experiment to restrict growth of benthic microalgae and contamination of non-seagrass amended treatments.

On day 1 of the experiment, Callianassids were added to each bioturbated core (10 cores in total, 1 individual corresponding to 0.6–0.7 g total w/w). The animals were allowed to construct burrows for 24 hours before the first flux incubation was initiated on day 2 of the experiment. Cores were discarded if Callianassids were not viable. At the conclusion of the experiment, n = 3–5.

Gas flux analysis

Benthic fluxes of O_2 and CO_2 (dissolved inorganic C; DIC concentration) were determined before and after Callianassids were added to cores (days 1, 3, 7, 15, 21, 28, 35, 42, 49, 56, and 63). Cores were sealed with gas-tight rubber bungs, and water circulation was ensured with stirring magnets (1.5 cm long) fitted to the core tubes, and driven by a rotating external magnet (~ 60 rpm). All cores were incubated in darkness at a constant temperature (22°C) for 2–3 hours. O_2 concentration within the water was measured at the beginning and at the end of the incubation using a Firesting Optical Microsensor, with a 100 µm retractable tip, connected to an Optical Oxygen Meter (FireSting, Pyro Science, Denmark). The exchange of O_2 (mmol m^{-2} d^{-1}) between sediment and water was calculated from changes in overlying core water concentration during incubations, the incubation time, volume of overlying water within each core, and surface area of the respective core using the following calculation:

$$O_2\text{Flux} = \frac{[O_2\ \text{Conc}\ T_1(mmol\ L^{-1}) - O_2\ \text{Conc}\ T_0\ (mmol\ L^{-1})]\ \text{x Water volume}\ (L^{-1})}{\text{Sediment surface area}\ (m^{-2})\ \text{x Time}\ (d^{-1})}$$

Initial and final water samples to determine CO_2 flux rate were taken with a 60 mL syringe and transferred to 40 mL gas tight glass vials and preserved with saturated $HgCl_2$ (300 µl). Total CO_2 (measured as change in DIC) samples were stored in darkness at 5°C and analysed by a DeltaV Infared Mass Spectrometer (IRMS) with precision of < 1‰, coupled to an OI TOC analyzer (Maher and Eyre 2011).

An isotope mass balance was used to determine the $\delta^{13}C$ contributions of sediment and seagrass DIC (CO_2) effluxed into the overlying water (Maher and Eyre 2011). The $\delta^{13}C$ values of accumulated DIC (represented below as x) concentrations were was calculated by:

$$x = \frac{(\text{DIC Conc}\ T_0\ \text{x}\ \delta^{13}\text{C}\ \ T_0) - (\text{DIC Conc}\ T_1\ \text{x}\ \delta^{13}\text{C}\ \ T_1)}{(\text{DIC Conc}\ T_0 - \text{DIC Conc}\ T_1)}$$

DIC Conc T_0 and DIC Conc T_1 represent the measured initial and final overlying water DIC concentrations respectively.

The results of the isotope mass balance were then used in a 2-end member mixing model, using the known $\delta^{13}C$ concentrations of both the seagrass and the sediment, to determine the proportion of seagrass-derived DIC in the effluxed DIC (represented below as $DIC_{seagrass}$). The equation was rearranged to calculate the proportion of sediment-derived DIC at each time point:

$$DIC_{\text{seagrass (proportion)}} = \frac{(\delta^{13}C \text{ measured} - \delta^{13}C \text{ sediment})}{(\delta^{13}C \text{ seagrass} - \delta^{13}C \text{ sediment})}$$

The $\delta^{13}C$ measured represents the calculated $\delta^{13}C$ concentration of overlying water DIC (CO_2) derived from seagrass. These calculated proportions were then multiplied by the rate of CO_2 flux (calculated using DIC accumulation over time) to establish the rate of both seagrass and sediment remineralisation within each treatment.

Priming effect (PE) estimations were calculated based on Kuzyakov et al. (2000). Additive interactions between bioturbation by Callianassids and sediment remineralisation (i.e. changes to CO_2 flux due to burrowing and Callianassid respiration) were calculated from control (no seagrass enrichment) treatments using the equation below, with CO_2 representing the measured rate of sediment-derived remineralisation:

$$\text{Callianassid } CO_2 = \text{Total Sediment } CO_{2\text{(faunated control)}} - \text{Total Sediment } CO_{2\text{(defaunated control)}}$$

As the remineralisation of seagrass was already accounted for, the PE in relevant treatments was calculated using the equation below, with CO_2 representing the calculated rate of remineralisation:

$$PE = \text{Total sediment } CO_2 \text{ - Sediment } CO_{2\text{(faunated control)}}]$$

The sediment-associated CO_2 release from control (defaunated) sediment was added to the calculated Callianassid interaction and both were subtracted from the measured rate of sediment C remineralisation in sediment containing both seagrass and Callianassids. Any additional CO_2 released was attributed to PE.

Sediment characteristics – measurement of organic carbon content

Upon completion of the experiment, each core was sectioned into 1 cm intervals to 5 cm depth, 2 cm intervals to 17 cm depth, and the final two intervals were 3 cm thick. Subsamples of each sectioned portion were taken for sediment bulk density and organic C analysis. Callianassids were removed whole (live) from respective treatments, and sediment from each core depth was homogenised then characterised. Sediment density was analysed gravimetrically with a 4 mL cut-off syringe. Samples were transferred to aluminium trays, and the wet weight of each sample was recorded. They were then dried at 60° C for 24 hours, ground with a mortar and pestle, and sub-samples of each section (0.5 g each) were acidified with HCl. The acid was then washed out of the sediment with double distilled water (and centrifuged at 1500 rpm for 8 minutes between each wash), and samples dried (60 °C for 48 hours) and ground. The C_{org} content of each sample was measured using high temperature combustion (Costech Elemental Analyser). Sediment sub-samples were analysed for$\delta^{13}C$ *via* IRMS.

Recovery of buried seagrass detritus from sediment cores

All large pieces of particulate detritus > 0.5 mm were recovered from each sediment slice of each core and the extent of degradation, quantity and distribution of seagrass material remaining in the sediment at the end of the experiment, determined. After sediment subsamples were taken, sediment from each slice was individually sieved (0.5 mm mesh) and all visible detritus was retained. The collected detritus was washed in distilled water, and dried at 60 °C for 24 hours. Seagrass detritus taken from each sediment depth were then re-weighed, and all material recovered within each core was pooled. The dried detritus was then ground using a clay mortar and pestle, and analysed for OM quality using thermogravimetry (Lopez-Capel et al. 2005). The ground detritus from each core was transferred into a platinum cup, and heated for 10°C min^{-1} to 600 °C under N_2 (SDT Q600, TA Instruments, New Castle, DE, USA). Quantification of mass loss within designated thermal intervals was identified using Universal Analysis software (TA Instruments, New Castle, DE, USA).

Allocation of thermal intervals was established based on the rate of change derivative (% mass loss $°C^{-1}$), indicating separate temperature-driven weight loss intervals. According to Trevathan-Tackett et al. (2015), the first OM mass loss interval (labile

OM, corresponding to soluble carbohydrates and hemicellulose) ranges from 200 °C to 300 °C, followed by recalcitrant OM (including cellulose and organic residues) from 300°C to 400°C. Refractory OM (including lignin and insoluble polysaccharide residues) mass loss occurs between 400°C to 600 °C. Estimations for detritus burial were based on the depth of recovery and comparison to initial addition weight.

Callianassid metabolism

The contribution of Callianassid metabolism to CO_2 and O_2 fluxes was determined in a separate experiment. Individual Callianassids were recovered from the sediment cores, and weighed before being transferred to sealed 500 mL glass vials filled with O_2 saturated seawater and left for approximately 24 hours to settle. Initial and final seawater samples for both O_2 and CO_2 were taken (as described above), before and after incubation for 2 hours. Vials were kept in darkness at a constant temperature (22 °C) and salinity (32) throughout the incubation. Samples were analysed for DIC and O_2 as described above.

Statistical analysis

The impact of Callianassid bioturbation on the degradation of the seagrass detritus was tested with separate one-way ANOVAs on both recovered detritus OM composition and total recovery. Two-way ANOVAs were used to analyse total core C_{org} (gm) content, using fauna addition and seagrass enrichment as fixed factors. Separate two-way ANOVAs based on faunal addition and seagrass enrichment were used to distinguish differences in time integrated release of net CO_2, as well as differences between integrated seagrass and sediment remineralisation. CO_2 flux, as well as seagrass and sediment remineralisation rates were additionally compared using separate two-factor repeated measures ANOVAs. In each analysis, enrichment (with or without seagrass enrichment), fauna (defaunated or Callianassid bioturbation) and time (10 time points) were used as fixed factors.

Levene's test for homogeneity of variance was performed on data prior to analysis. When appropriate, Tukey's post-hoc test was used to establish which variables produced a significantly different interaction. All tests were performed with a significance level of $\alpha = 0.05$, using IBM SPSS Statistics (Ver. 22).

Results

Recovery of seagrass detritus (> 0.5 mm)

Approximately 79% (± 5.51%) of the original detritus buried in control sediment was recovered after 65 days (Fig. 5.1a), representing approximately 29.1 g C m^{-2}. However, recovery of seagrass in bioturbated sediment was significantly reduced ($F_{1, 712.473}$ = 13.031, p = 0.023), with a total of 65% ± 2.55% of the original seagrass detritus recovered (Fig. 5.1b), reflecting approximately 20.5 g C m^{-2}.

Seagrass detritus in the control sediment remained close to the original burial depth (2–3 cm; Fig. 5.1a), while bioturbation by Callianassid shifted the distribution of seagrass deeper, to a maximum depth of 23 cm (Fig. 5.1b).

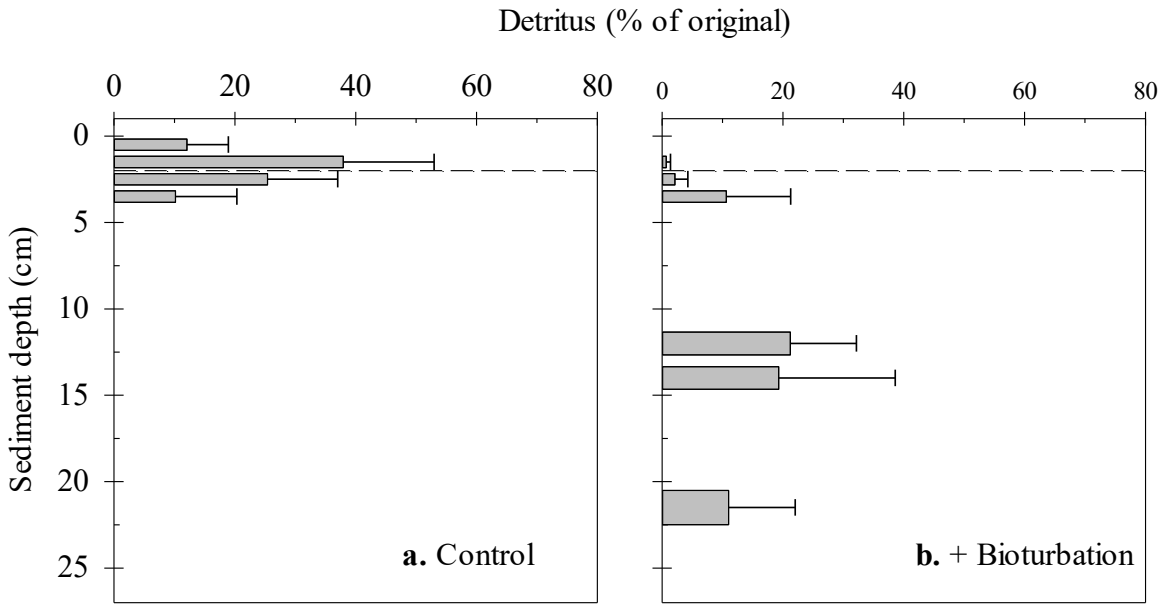

Fig. 5.1: Vertical profiles of seagrass detritus recovery in (a) Control (defaunated) sediment cores, and cores with the presence of Callianassid (b) + Bioturbation. Column bar width is indicative of the size of the sediment portion sampled. The dashed reference line (2.5 cm depth) indicates the original burial depth of seagrass detritus. Error bars: SE (n = 3–5).

Organic content of seagrass detritus recovered from sediment

Seagrass detritus recovered from bioturbated sediment contained a significantly lower proportion of soluble carbohydrate associated OM (labile OM, including proteins and hemicellulose) than detritus recovered from control sediment ($F_{1, 26.7} = 21.54$, $p = 0.01$; Fig. 5.2). There was no difference in the proportion of cellulose-associated OM (recalcitrant OM; $F_{1, 1.558} = 0.668$, $p = 0.46$) or lignin-associated OM (refractory OM; $F_{1, 6.90} = 2.333$, $p = 0.202$) in recovered detritus between bioturbated and control sediment.

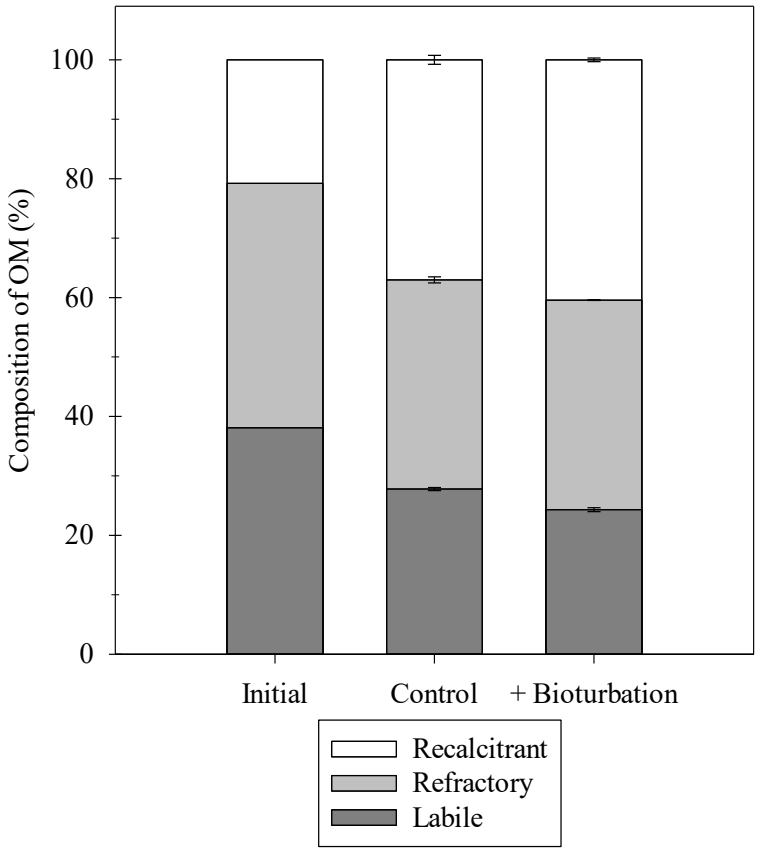

Fig. 5.2: Organic matter composition of seagrass (*Zostera muelleri*) leaf detritus, determined *via* thermogravimetric analysis. Specifically proportion of labile OM (mass lost between 200–400 °C), refractory, and recalcitrant OM (mass lost between 400–550 and 550–650 °C respectively), in seagrass leaves fresh from the field (initial), recovered from control sediment, and sediment populated with Callianassid (+ Bioturbation). Proportions of labile, refractory and recalcitrant OM are presented as a percent of total OM, Bars represent means ± 1 SE (n = 3). In some places error bars are too small to be visible.

Sediment carbon content

Sediment C_{org} in control sediment containing seagrass tended to be highest (1.08–1.54%; Fig. 5.3) in the top 3 cm of sediment which corresponded to the depth of seagrass burial, whereas the C_{org} content (0.77–1.05 % C_{org}) was less below this depth. C_{org} (0.73–1.27% C_{org}) was relatively inconsistent throughout the sediment in the control (defaunated) bare sediment.

C_{org} concentrations peaked (0.87–1.48%) in the upper 4 cm of bare sediment cores containing Callianassid, but declined to 0.91–1.23% with depth. In bioturbated sediment containing seagrass, the upper 5 cm was comparably lower in C_{org} (0.71–1.11%), and became more constant with depth (~ 0.99%).

Neither Callianassid bioturbation ($F_{1, 6.313} = 2.274$, $p = 0.157$), nor seagrass enrichment ($F_{1, 0.020} = 0.007$, $p = 0.934$) had a significant impact on total core sediment C_{org} (g C) content after the 2 month experiment.

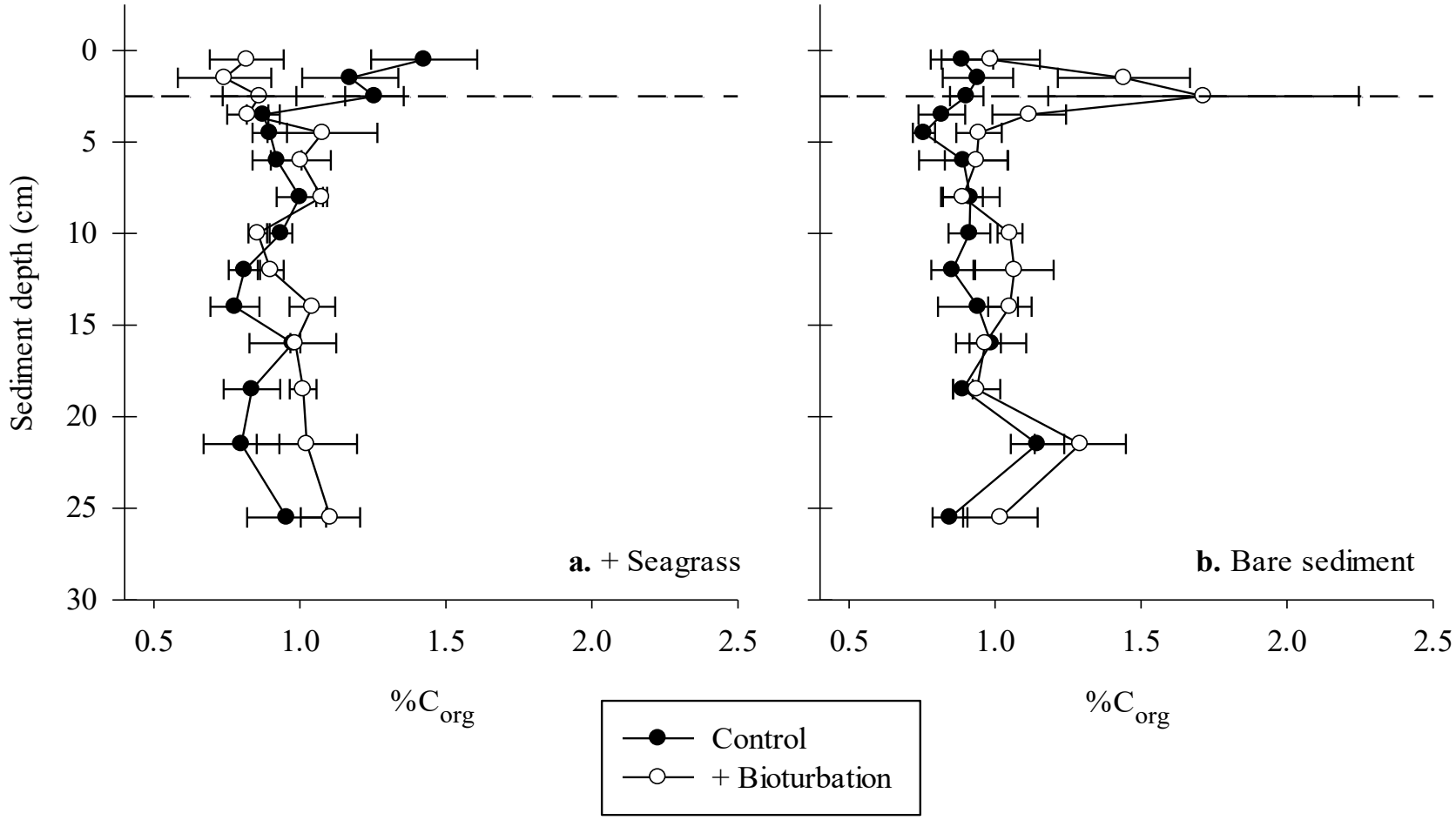

Fig. 5.3: Sediment organic carbon (%C_{org}) content in core sediments amended (a) with (+ Seagrass) and (b) without (Bare sediment) seagrass. The two curves on each graph show sediment cores populated with (+ Bioturbation; white symbols) and without (Control; dark symbols) bioturbating fauna. The dashed line (2.5 cm depth) indicates the approximate initial depth of seagrass burial. Values represent means ± 1 SE (n = 3–5).

Gas flux analysis

The addition of Callianassids had a clear impact on the O_2 uptake by the sediment (Fig. 5.4a, 4b), and TCO_2 release (Fig. 5.4c, 4d). Addition of Callianassids increased sediment O_2 demand consistently, with consumption of O_2 approximately 1.5–4 times that of control sediment.

O_2 uptake was initially similar at about 53–64 mmol m^{-2} d^{-1} in the control bare sediment and control sediment containing seagrass. Sediment containing seagrass displayed relatively consistent O_2 consumption over time, with an uptake rate of 46–56 mmol m^{-2} d^{-1} in sediment populated with Callianassids, and 33–41 mmol m^{-2} d^{-1} in control sediment. O_2 uptake in bare sediment was initially variable, with consumption becoming relatively stable after ~ 21 days. After this time, bare sediment populated with Callianassids consistently had an O_2 uptake rate higher that of control sediment than (as previously described).

Significantly more CO_2 was released from the sediment in the presence of Callianassid ($F_{1, 5203.802} = 23.972$, $p = 0.039$). The release of CO_2 in both Callianassid treatments was double that of control (defaunated) sediment. Callianassid bioturbation had a significant interaction on CO_2 release with time ($F_{9, 1738.092} = 2.646$, $p = 0.038$). The presence of seagrass also led to an increase in sediment CO_2 release ($F_{1, 24549.960} = 43.335$, $p = 0.021$), however the impact of seagrass enrichment did not have a significantly sustained interaction with time ($F_{1, 923.009} = 1.345$, $p = 0.283$).

In sediment containing seagrass, bioturbation by Callianassid lead to a 1.6–5.8 times increase in CO_2 release. A consistent decline in CO_2 release was observed in both bioturbated and control sediments throughout the experimental period. CO_2 release in bare sediments was variable: release in sediment populated with Callianassid being 1.1–6.4 times that of control sediment, until the final sampling point.

Callianassid individuals were responsible for an average of 18% (seagrass treatments) and 29% (bare sediment treatments) of the total CO_2 production; and 16% (seagrass treatments) and 21% (bare sediment treatments) of the total O_2 uptake, per day.

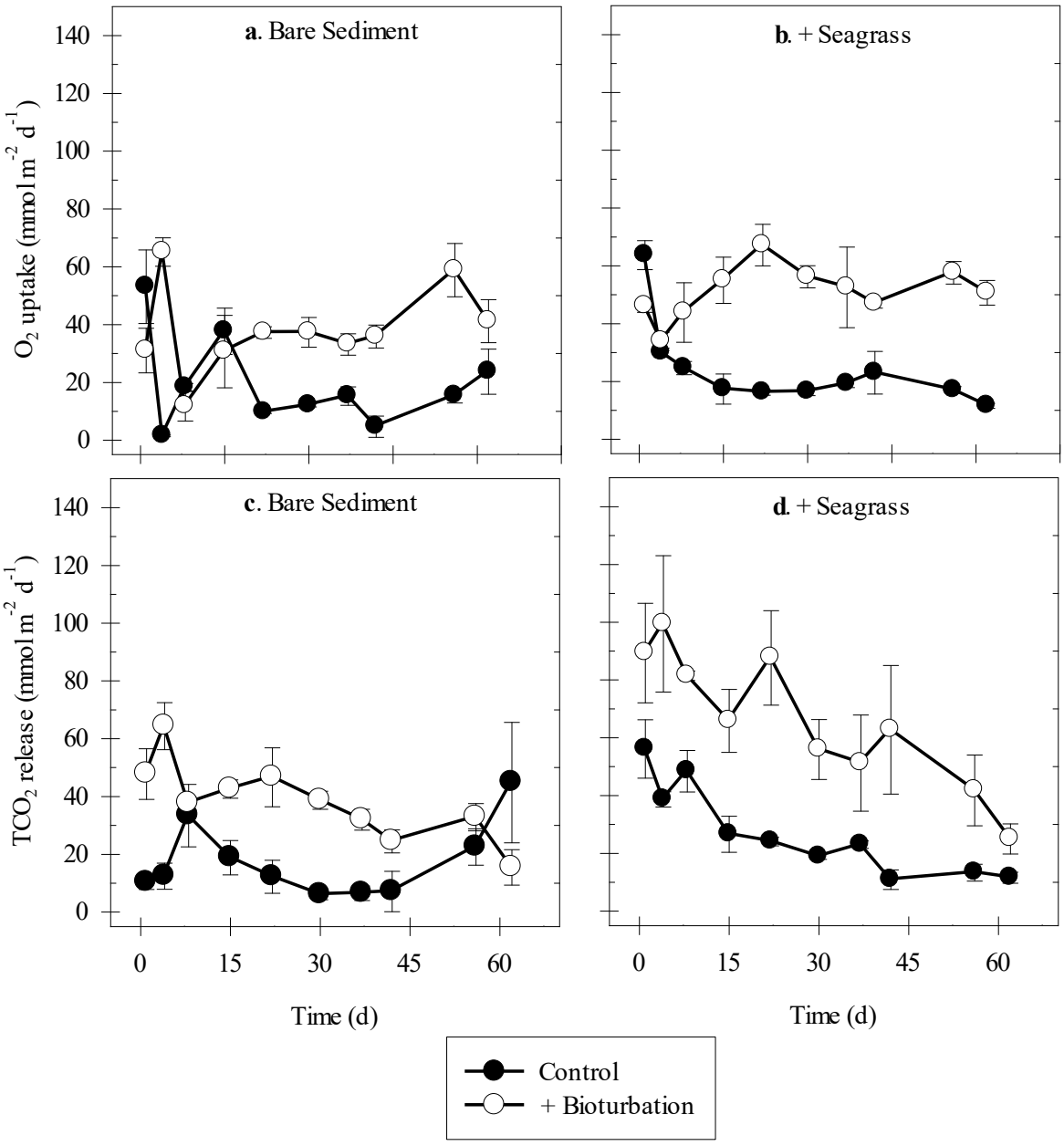

Fig. 5.4: Total oxygen (O_2; Fig. 5.4a, b) and carbon dioxide (CO_2; Fig. 5.4c, d) effluxes over time in sediment cores with (+ seagrass) and without (bare sediment) seagrass detritus ($mmol^{-2}\ d^{-1}$). The two curves in each graph show efflux profiles in sediment cores with (+ Bioturbation) and without (Control) Callianassid bioturbation. Values represent means ± SE (n = 3–5).

Sediment and seagrass remineralisation rates

Isotopic tracing of released CO_2 (DIC) revealed that Callianassid bioturbation had a clear impact on the rate of sediment remineralisation (Fig. 5.5a, 5.5b). Significantly more sediment organic matter (OM) was remineralised in the presence of Callianassids ($F_{1, 41580.479} = 351.071$, $p = 0.003$): the rate of sediment remineralisation was 2–5 fold greater than in control sediment (Fig. 5.5b). Bioturbation by Callianassids had a significant interaction with sediment remineralisation over time ($F_{9, 7922.545} = 139.047$, $p = 0.007$). The presence of seagrass detritus also had a significant impact on sediment remineralisation ($F_{1, 12724.607} = 28.958$, $p = 0.033$), with the rate of sediment remineralisation approximately 45% lower in control sediment containing seagrass compared to control sediment; however, the impact of seagrass did not have a significantly sustained interaction with time ($F_{9, 236.436} = 1.638$, $p = 0.166$).

There were no differences in the rates of seagrass remineralisation ($F_{1, 67.570} = 0.239$, $p = 0.673$) in both control and bioturbated sediments (Fig. 5.5a). Rates declined steadily for 3 weeks of the study, and becoming stable after the first month (~ 7 mmol m^{-2} d^{-1}).

A significant interaction was observed between Callianassid bioturbation and sediment amended with seagrass. Both conditions showed a significantly larger (1.3–6-fold greater) rate of sediment remineralisation (recalcitrant organic matter; ROM) than all other conditions ($F_{9, 13802.947} = 25.436$, $p = 0.037$).

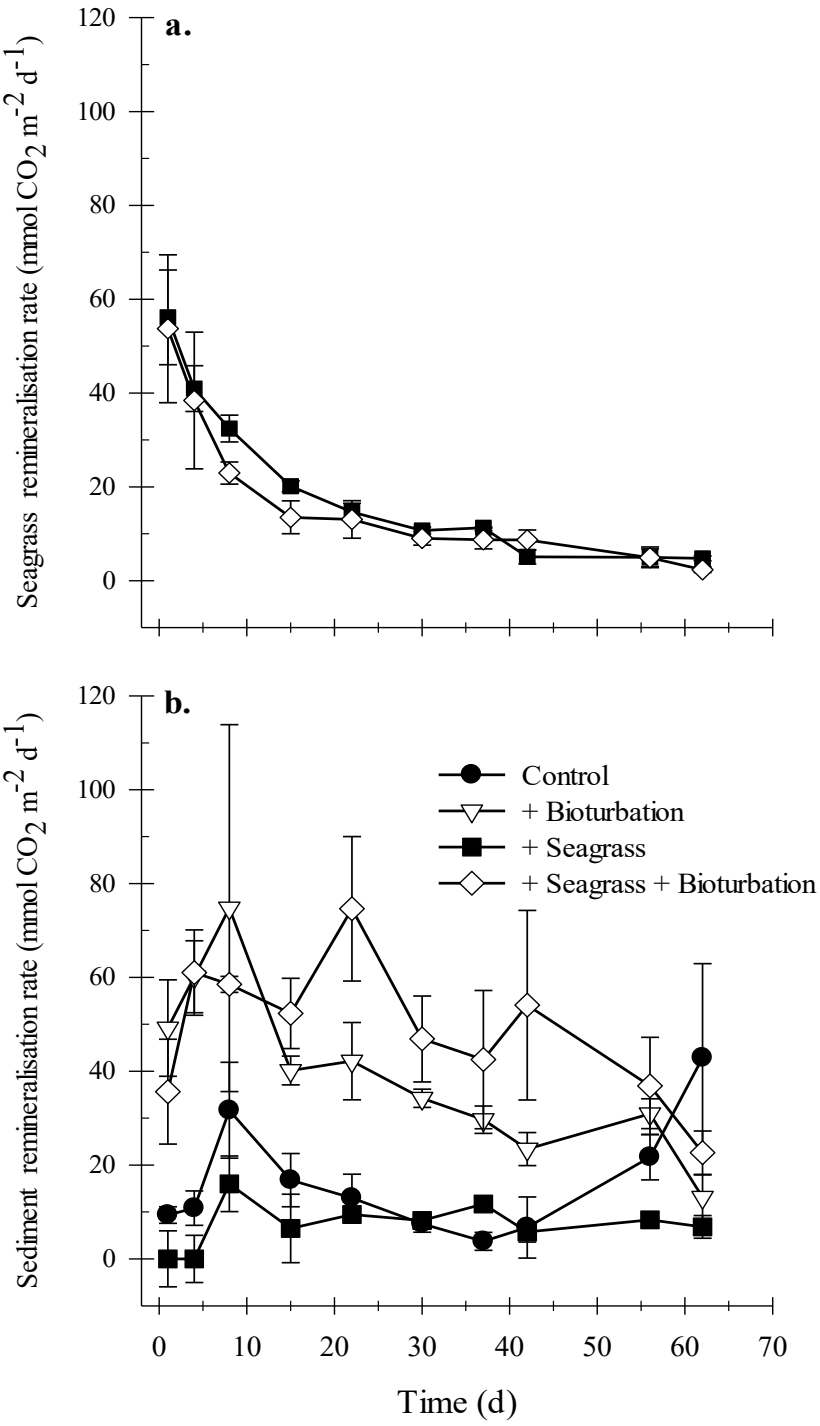

Fig. 5.5: Total calculated (a) seagrass and (b) sediment remineralisation rate in sediment cores containing seagrass (+ Seagrass), and without seagrass. The curves in the top graph (a) show seagrass remineralisation calculated from $\delta^{13}C$ concentrations measured in respective sediment cores with (+ Bioturbation; white symbols) and without (Control; dark symbols) Callianassid bioturbation. Curves in graph (b) reflect sediment remineralisation measured in all treatments. Values represent means ± SE (n = 3–5).

Estimated microbial priming effect

The relative contribution that the various OM sources to CO_2 release showed a discernable difference among sediment treatments (Fig. 5.6). A clear difference was observed in sediment exposed to Callianassid bioturbation (Fig. 5.6a), with an increased rate of sediment-based C release. While bioturbation was responsible for an average additional sediment-derived release of ~ 20.8 mmol CO_2 m^{-2} d^{-1} over the period of the study, a priming effect (PE) is observed in bioturbated sediments containing seagrass (Fig. 5.6b). Calculated rates of priming (PE) indicate that treatments containing seagrass, and exposed to Callianassid bioturbation, were responsible for a priming effect that was 15% that of the total C released in these sediments. A negative priming effect (preferential degradation of a more labile source of OM, i.e. seagrass, over a more recalcitrant source of OM, i.e. sediment) was apparent in the first week of the experiment, with positive priming beginning shortly after and peaking after about 1 month (36 mmol m^{-2}). A negative priming effect was observed in control sediment amended with seagrass: the sediment-derived CO_2 in amended sediment was less than half that observed in bare (control) sediment.

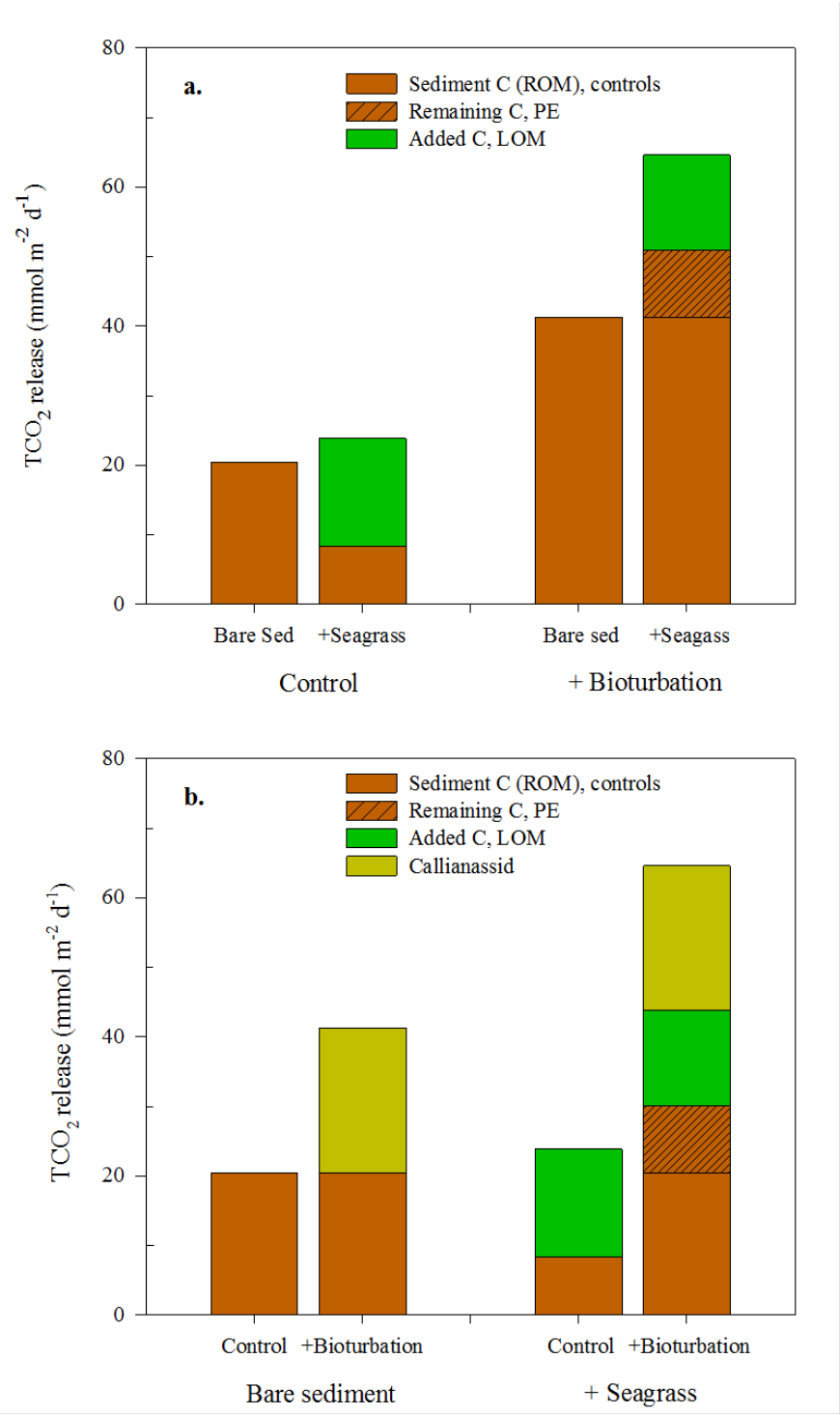

Fig. 5.6: Estimated contributions of labile organic matter (LOM; seagrass) and recalcitrant organic matter (ROM; sediment C) sources to respiration. The contribution of (a) Bioturbation and (b) Seagrass (LOM amendment) to microbial priming (PE) is compared. The fraction of sediment C (ROM) and added C (seagrass; LOM) contribution to the total CO_2 flux was calculated from stable isotope signatures using a 2-end member mixing model. Values represent means (n = 3–5). Error bars are absent due to the calculated nature of the values.

Discussion

Bioturbation is a prevalent process in coastal ecosystems globally (Woods and Schiel 1997, DeWitt 2009, Kristensen et al. 2012, Kneer et al. 2013, Garbary et al. 2014, Govers et al. 2014), and the activities of bioturbating macrofauna have a clear effect on sediment metabolism and carbon (C) remineralisation (Papaspyrou et al. 2004, Webb and Eyre 2004, Kristensen et al. 2012). The results obtained in this study indicate that the effect of Callianassid bioturbation on sediment CO_2 release is larger than that of seagrass presence, and that the integration of both factors could result in a significant sediment priming effect. These findings have implications for our understanding of the persistence of global seagrass C stocks.

Burial of seagrass detritus was observed in bioturbated sediments, resulting in seagrass detritus distributed an additional 2–18 cm into the sediment. Rapid burial of seagrass detritus has been observed in other Thalassinidean species (Papaspyrou et al. 2004), and it has been proposed that these animals actively do this to stimulate microbial productivity (i.e. gardening) (Dworschak 2001). Not only did we observe that bioturbation lead to an overall decrease in recovery of seagrass detritus within the sediment, but it was noted that a higher proportion of labile organic matter (OM) was lost from detritus buried in bioturbated sediments. It can be elicited that the observed decreasing rate of seagrass remineralisation was likely due to this loss of seagrass labile OM (proteins, soluble carbohydrates, etc). Loss of seagrass OM content of up to 1% d^{-1} (Harrison and Chan 1980) can be expected from leaf material, and are comparable to the rates of decay observed in this study, which were ~ 0.95–1.01% d^{-1}. Cellulose and lignin compounds within seagrass detritus generally impedes microbial degradation (Trevathan-Tackett et al. 2015), and retention of seagrass detritus in the sediment at the end of our two-month study, (which largely consisted of recalcitrant and refractory OM), suggests that there is evidence for future seagrass-derived C input and replenishment of sediment C stocks.

The addition of seagrass detritus increased sediment O_2 demand by 26%, while the presence of Callianassid effectively doubled sediment oxygen consumption (130%). In sediments containing both Callianassid and seagrass, O_2 consumption was enhanced substantially compared to control sediment. High sediment O_2 uptake rates between 40 to 144 mmol $m^{-2}d$ have been reported in sediment populated with Callianassids (Webb

and Eyre 2004, Eyre et al. 2011, Maher and Eyre 2011). Callianassids can stimulate burrow irrigation at a rate of ~0.5 l h^{-1} and increase sediment O_2 substantially, most of which is consumed by microbial and biogeochemical processes (Webb and Eyre 2004). Faunal-stimulation of sediment metabolism also includes an increased rate of CO_2 efflux, largely due to increases in sediment surface area (burrow), and stimulated microbial and biogeochemical processes (Banta et al. 1999, Kristensen 2000, Webb and Eyre 2004). Our results indicate that Callianassid is responsible for up to 50% of the total CO_2 release; however given that metabolism of the fauna alone is responsible for ~20% of the total CO_2 release, it can be elicited that the remaining ~30% is due to increased sediment surface area *via* burrows, and resulting stimulation of microbial metabolism. Similar increases in CO_2 efflux have been reported for Thalassinidean bioturbation and in the presence of seagrass (Papaspyrou et al. 2004), with a net overall increase in C remineralisation with the introduction of bioturbation.

In control sediment amended with seagrass, the observed negative priming effect in control sediment containing seagrass, was equivalent to a >50% reduction in ROM-derived CO_2 release, indicating a preferential remineralisation of seagrass (LOC) by the microbial community (Gontikaki et al. 2013). Negative priming in seagrass sediments may indeed promote the long-term preservation of sediment C_{org}, with the microbial community preferentially remineralising labile compounds (i.e. proteins, hemicellulose and soluble carbohydrates Trevathan-Tackett et al. 2015) and even some recalcitrant lignocellulose compounds (Gontikaki et al. 2015) within the seagrass detritus, over the sediment-bound C_{org}. However, the extent of the PE is also related to the added substrate C as a proportion of the microbial biomass (Blagodatskaya and Kuzyakov 2008). Once available C has been depleted, and subsequent microbial activity has subsided, remaining C may be more resistant to degradation (Blagodatskaya and Kuzyakov 2008). Comparing the amount of sediment C_{org} remineralised within bioturbated sediment to that remineralised in control sediment, it is clear that this negative PE is completely outweighed by the bioturbator stimulated PE.

Aerobic sediment respiration typically results in balanced respiratory quotients (RQ; CO_2 efflux to O_2 consumption) of 1.0 (Banta et al. 1999), which were observed in control sediment treatments, unbalanced RQ values were observed in all other treatments. An RQ value of 1.2 was obtained for sediment containing seagrass, however

in bioturbated sediment containing seagrass, an RQ of 1.4 was observed. RQ values favouring CO_2 release generally indicate substantial changes to metabolic processes, such as increased rates of anaerobic decomposition (Hargrave and Phillips 1981). We suggest that in this case, patchy burial of seagrass detritus, Callianassid metabolism, as well as subsequent disturbance of the sediment microbial community, are the likely causes of the unbalance (Kristensen 2000).

We estimate that PE was responsible for 15% of the total CO_2 output in bioturbated sediment amended with seagrass. Although in these treatments bioturbation by Callianassid lead to a lower rate of seagrass remineralisation (12%), which was likely due to detrital burial, these sediments also displayed a significantly higher rate of sediment remineralisation. Although not thoroughly explored in marine systems (Guenet et al. 2010), in terrestrial systems PE has been suggested as a result of the microbial competition for energy, stimulated by the input of a labile OM source, which ultimately results in loss of recalcitrant OM (Kuzyakov et al. 2000, Fontaine et al. 2003, Fontaine et al. 2007). The results of this study support that this process may also occur in bioturbated coastal sediments, as the observed increase in CO_2 was attributed to sediment (i.e. recalcitrant C) rather than seagrass remineralisation. We suggest that in this case, the measured PE and resulting CO_2 release, was largely stimulated by microbial activity attributed to bioturbation by Callianassids.

Assuming that in ambient conditions, the rate of C burial is reflective of control (defaunated) sediment; release of CO_2 into the water column will lie in the range of 3.29–3.84 Mg C ha^1 yr^1. However the addition of bioturbators (i.e. Callianassids) can increase the rate of C_{org} release 2-fold in sediment (6.65 Mg C ha^1 yr^1) and 3-fold in sediment containing seagrass (10.40 Mg C ha^1 yr^1), similar to increases observed in accordance with other Thalassinidean species (Papaspyrou et al. 2004, Webb and Eyre 2004). Even a conservative estimate of one bioturbator burrow per m^{-2} may induce a PE equivalent to 1.6 g C m^{-2} y^{-1}, a significant portion of the estimated annual Australian seagrass C sequestration of 10 .1 g C m^{-2} y^{-1} (Lavery et al. 2013). Meadows in the study area are populated by 10–30 Callianassid burrows per m^{-2} (see Chapter 2), representing a potential for a much larger loss of C and ultimately a net loss of sediment C. This has implications on the potential for seagrass meadows to store C for long periods of time. If stimulated sediment metabolism and labile OM burial is indeed creating hot-spots of

microbial priming to effectively leak CO_2, an increase in bioturbator populations could decrease seagrass (and other) blue carbon stocks (Atwood et al. 2015). The potential of bioturbators to effectively facilitate microbial priming warrants further investigation. We would encourage further research to encompass the full range of remineralisation by-products, including DOC and porewater analysis. Further investigation on different macrofauna species (i.e. representing different activity) will allow us to make predictions of the vulnerability of seagrass sediment C stock.

Based on the results of this study, we present a scenario whereby a seagrass C stock may be significantly affected by bioturbation. Atwood et al. (2015) outlined that predation could have significant impacts on communities of bioturbating macrofauna. We suggest that in an ecosystem where there is a lack of top-down control, bioturbating macrofauna may experience growths in population size. While burial of detritus in meadows is continuous (Mcleod et al. 2011) and may therefore supplement C loss to some extent, changes to population structure may lead to a short-term increase in both priming and CO_2 release (Kuzyakov et al. 2000). Accordingly, bioturbator-stimulated priming may pose a considerable threat to C stock longevity, particularly considering the sediment remineralised in this study had been stored for an extended (hundreds to thousands of years) period of time (see Chapter 2, Fig. 1.1). We suggest that bioturbator-stimulated priming could have a considerable effect on C sequestration and persistence in coastal ecosystems (Guenet et al. 2010).

Synthesis, Conclusion and Outlook

Bioturbation by burrowing macrofauna is a key process affecting the supply of oxygen, burial of organic matter (and other sources of carbon), as well as microbial activity within the sediment of seagrass meadows. A fundamental goal of this thesis was to generate information that will enable generalisations to be made about the mechanisms and overall impact that bioturbators can have on seagrass carbon (C) sequestration. Although projects within this thesis focussed on three different climatic regions and three different bioturbating macro-faunal species, each species studied represented one of the major bioturbating functional groups within each respective seagrass environment. Understanding the effect of different functional groups means that this knowledge can be extrapolated to provide general predictions.

Chapter one focussed on a field study, investigating the composition of the burrow walls of a dominant bioturbator - *Uca signata* (fiddler crabs) in a tropical Australian seagrass meadow. By assessing burrow C source and content, this study revealed that female fiddler crabs appeared to be storing seagrass detritus within their burrows, leading to increased C in terminal burrow depths. Conversely, male burrowing had no discernible impact on sediment C. Previously, it was assumed that the influence of bioturbation on sediment geochemistry was determined by their functional group; however, this novel finding ascertained that differences within a species (i.e. gender differences) could have as large of an impact on sediment C as the functional grouping.

Chapter two was centred on identifying the chemical and microbial differences in surface and deep sediment, focusing on the common Australian bioturbator *Trypaea australiensis* (Callianassid). A mesocosm experiment compared the vulnerability of seagrass surface sediment C stocks (young) with deep (ancient) sediment C stocks. Over two months, bioturbation reduced surface C stocks by more than a third, while deep stocks appeared to be stable when exposed to bioturbation. We determined that the reduction in surface C was likely due to be a function of both increased sediment oxygenation as well as increased microbial density. This result has implications for maintaining seagrass C stocks, with bioturbation putting at risk the persistence of C stocks over time (compared to undisturbed sediment).

Chapters three and four captured how bioturbation affects the degradation of buried vegetative material, the subsequent impact on benthic metabolism and anaerobic microbial activity. Mesocosm experiments were conducted to compare the degradation of a labile macroalgal (*Fucus vesiculosus*) detritus with the comparatively recalcitrant seagrass (*Zostera marina*) detritus, in sediment with and without the common bioturbating Northern-European polychaete *Arenicola marina* (henceforth referred to as '*Arenicola*'). Additionally, we investigated if there was any effect on degradation when the two types of detritus were buried together. The results indicated that degradation of seagrass and macroalgal detritus, and hence stability and replenishment of coastal blue carbon (BC) stocks, is strongly dependent on the dominant primary producer contributing to the sediment C stocks of a seagrass meadow. Additionally, we observed an interactive effect whereby macroalgae were preferentially degraded when buried in the presence of seagrass, an effect which was further increased with the introduction of bioturbation.

From our results it is evident that bioturbation by *Arenicola* promotes the anaerobic degradation of seagrass derived C. It is clear that the effects of *Arenicola* bioturbation have on-going impacts on the sediment biogeochemistry, microbial community, and C degradation for some time (weeks). These observations suggest that bioturbating fauna, in addition to vegetation and associated microbial activity, impact C storage in sediments.

Chapter five brought together much of the knowledge obtained in the previous chapters, and focussed on the changing microenvironment of the sediment and plant degradation, as well as the effect of microbial priming. A two month mesocosm study compared the remineralisation (measured as release of CO_2) of buried seagrass (*Zostera muelleri*) to the rate of sediment remineralisation, in the presence of the common Australian bioturbator *T. australiensis* (Callianassids). Specifically, we investigated whether bioturbation by Callianassids stimulated a microbial priming effect promoting remineralisation of sediment C (recalcitrant organic matter). It was observed that bioturbation by Callianassids lead to clear changes in benthic metabolism, and resulted in the burial of seagrass detritus deep in the sediment. Interestingly, bioturbation led to a 2-5 fold increase in the total CO_2 released, with the calculated priming effect equivalent to ~15% of the total daily sediment-derived CO_2 release. In defaunated sediment

enriched with seagrass, a negative priming effect was observed whereby seagrass degradation was favoured over sediment degradation. We concluded that if bioturbation is indeed stimulating transient hot-spots of microbial priming in seagrass meadows to effectively "leak" ancient sediment-held C as CO_2, changes to faunal communities may have negative impacts on seagrass C stocks.

This thesis encompassed both field and laboratory-based experiments to determine whether bioturbation intrinsically results in net C gain or loss in seagrass C stocks. By focussing on sediment dynamics, we were able to determine how changes to the sediment chemistry, microbial community and activity, as well as physical structure, can result in loss of sediment C. The chapters in this thesis addressed the different mechanisms of how bioturbation could change the sequestration of C in seagrass meadows. Although it is difficult to say whether bioturbation can turn a sequestering system into a net C source, the integrated results in this thesis certainly indicate that bioturbation can temporarily result in a loss of C from the C stocks. The following section will synthesise the results of the thesis, and draw some new conclusions.

Global bioturbation and *Zostera* meadows

In this thesis we investigated the effects of three different bioturbators (*Uca signata, Trypaea australiensis* and *Arenicola marina*) on seagrass C, each of which was the dominant bioturbating species within each of the respectively studied seagrass ecosystems. *Uca spp.* and Callianassid shrimp (i.e. *T. australiensis*) are both dominant bioturbators in the sub-tropics and tropical regions of the World (Dworschak 2000, Levinton and Mackie 2013); while *Arenicola* dominates temperate regions within the Northern Hemisphere (Croker et al. 1975, Beukema and De Vlas 1979, Packer et al. 1980, Valdemarsen et al. 2011). While each species is considerably different in terms of their morphology and burrowing behaviour, several similarities were observed when the effects of their respective bioturbation activities on the sediment environment and resulting seagrass C storage capacity were compared.

Previous studies have reported that with an increased abundance of bioturbators, the productivity and health of seagrass declines sharply (Suchanek 1983, Dumbauld and Wyllie-Echeverria 2003). When comparing the density of bioturbators within our observed Australian study sites (Chapters 1 and 2), there was a clear negative

correlation between seagrass cover and dominant bioturbator density ($r_{s\ 129}$ = -0.562, p < 0.001; Fig. C.1). This relationship was also observed in comparisons made between different species (*Uca signata* in Gladstone, and *T. australiensis* in the Sydney region) along the east coast of Australia.

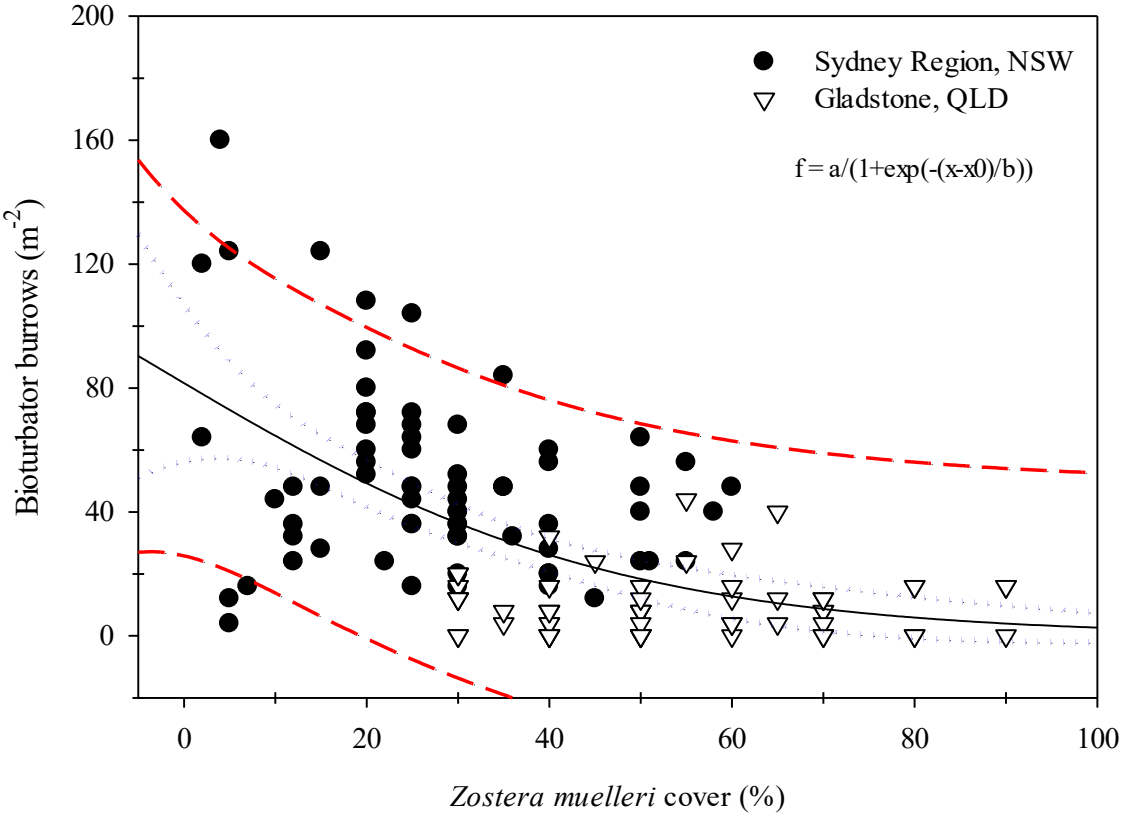

Fig. C.1: Non-linear regression of *Zostera muelleri* seagrass cover to density of active site-dominant bioturbator burrow openings, at two locations (Sydney region, NSW and Gladstone, QLD), showing an approximate 95% confidence envelope (blue dotted line) and predictor variable envelope (red dashed line). The 3-factor equation of the regression line is given.

In seagrass meadows within the Odense Fjord (study site; Chapters 3 and 4), densities of the common bioturbator *Arenicola marina* of up to 80 individuals m^{-2} have been observed (Valdemarsen et al. 2011). In some cases, such as *Arenicola*, Callianassid shrimp, and the stone crab (*Menippe* spp.), population increases have been directly linked to the cause of seagrass decline (Valentine et al. 1994, Berkenbusch et al. 2007,

Valdemarsen et al. 2011). It can be concluded that an increase in abundance of bioturbators, irrespective of species, can be negatively correlated to seagrass cover. It is important to consider that in addition to affecting the belowground C stocks, bioturbation may impede the ability of a seagrass meadow to sequester C by decreasing plant biomass and productivity (Suchanek 1983). There is clearly a factor of population density that dictates the effect of bioturbation on seagrass biomass, which is significant given that seagrass plant biomass is often a determining factor in the continued sequestration of C (Mcleod et al. 2011).

Recent studies have identified that climatic region can determine both the content of refractory C compounds (i.e. lignin) in, and the rate of degradation in seagrass species across the World (Irons et al. 1994, Couteaux et al. 1995, Silver and Miya 2001, Trevathan-Tackett 2016). As different species of bioturbators, and therefore their overarching functional groups dominate different biomes and climatic regions, it is important to consider how the relationship between seagrass refractory C and dominant bioturbating species may interact. For example, it has been observed that an increase in Callianassid density resulted in a decline in seagrass health and abundance in tropical regions; however in temperate regions, the opposite relationship was observed and an increase in seagrass recruitment was seen to lead to a decrease in Callianassid abundance (Berkenbusch et al. 2007). Climatic region, and therefore dominance of a particular bioturbating species and seagrass tissue structure, is potentially a factor determining the storage and flux of seagrass C stocks. These factors all have the potential to directly impact burial, retention, and microbial remineralisation of seagrass sediment C.

Bioturbation and burial of sources of carbon

The species of bioturbating macrofauna studied in this thesis belong to two bioturbating functional groups: (1) regenerators, i.e. *Uca* spp.; and (2) upwards conveyers including the lugworm *Arenicola* and Callianassid shrimp *T. australiensis* (Kristensen et al. 2012). Fauna in both of these functional groups expose or transport deep sediment to the substrate surface *via* their burrowing activities (Kristensen et al. 2012). Once on the surface, this deep sediment is exposed to an oxic, microbially active environment, where remineralisation of OM is comparatively higher (Kristensen and Holmer 2001). Conversely, groups such as downward conveyers ingest surface material, which is

typically rich in labile C (Fourqurean et al. 2012), and deposit it within deeper layers of the sediment (Kristensen et al. 2012). Due to the high C_{org} content found in seagrass sediments, the impact of particle mixing activities by groups of bioturbators is likely to have a diverse effect on C flux within seagrass sediments.

Among the three species studied, some commonalities arose in regards to their impact on the physical reworking of the sediment and resulting impact on OM burial. Burial of seagrass detritus by bioturbation, and often increased degradation, was observed in several chapters (Chapters 1, 3 and 5). Specifically, increased degradation of labile (i.e. carbohydrates) components of seagrass detritus was observed with bioturbation (Chapter 5), while burial of the remaining less reactive compounds (i.e. cellulose and lignin) in deeper sediment may aid in C stock replenishment. Recent research has, however, indicated that increased microbial activity in marine sediment can result in priming of lignocellulose (Gontikaki et al. 2015). These results suggest that while initially buried seagrass may aid in C storage, this C is at risk of being degraded much faster than undisturbed detritus, due to the changed microbial environment linked to bioturbation.

In chapter 2, we observed that bioturbation by the Callianassid shrimp T. aust*raliensis* resulted in a significant reduction of surface-held sediment C stocks. However, in a subsequent experiment described in Chapter 5, this observation was not made, despite using sediment from the same location. We have two alternative hypotheses as to what this lack of effect may have been due to: 1) Different "surface" sediment samples were used in each study: 1–10 cm in Chapter 2; 1–40 cm in Chapter 5. Characterisation of the age and C_{org} content of these sediments collected from the study site (Fagans Bay) indicated different profiles (see Fig. 2.1). 2) Different methods were used to slice and sample the sediment. Syringe cores were used in Chapter 2 to capture each *T. australiensis* burrow in its entirety with little surrounding sediment. In Chapter 5 however, large cores (8 cm diameter) were sliced in their entirety, and therefore there was more surrounding sediment in samples. We reaffirm that there was an impact of bioturbation on the C_{org} content of sediment in Chapter 5 due to the rates of sediment remineralisation measured *via* overlying water CO_2 content; however, we suggest that we were just unable to measure the extent of the effect within the sediment using the methods employed.

While burial of seagrass detritus could result in an increased storage of C_{org}, burial of different vegetative detritus sources may have the opposite effect. Our study showed that the burial of *Fucus* macroalgae by *Arenicola* lead to increased maceration of vegetative material, and almost the complete (80%) loss of all buried (detrital) OM (Chapter 3). Mixing of vegetative material similarly resulted in changed rates of degradation, with preferential degradation of the more labile C source (macroalgae) contributing to the preservation of seagrass C. While we can assume that bioturbation results in the burial of seagrass detritus, which is supported by the results of other studies (Papaspyrou et al. 2005, Papaspyrou et al. 2007, Vonk et al. 2008), labile sources of C (i.e. macroalgae) are unlikely to positively contribute to C storage. In regards to both macroalgae and seagrass, the tissue types used (blades and leaves, respectively) contain less refractory material than other macrophyte tissue (holdfasts, roots and rhizomes; Trevathan-Tackett et al. 2015). However, photosynthetic tissue, such as blades and leaves, are readily available as C donors within coastal ecosystems due to their proportionately higher biomass (Dudgeon and Johnson 1992, Duarte et al. 1998). Furthermore, they can easily detach and act as C donors to other habitats. These selected tissue types are therefore much more likely than, for example, rhizomes or holdfasts, to be in greater supply and be available within seagrass ecosystems. We should further highlight that in comparison to other macroalgae species, *Fucus* contains more refractory compounds and decays more slowly (Conover 2011). We can therefore expect that the contribution to the C pool will be lower in other macroalgal taxa that contain less refractory compounds compared to *Fucus* (i.e. *Ulva* spp.).

Macrofauna gender was identified as a factor affecting sediment C content (Chapter 1). Although there are clear gender morphological differences in *T. australiensis*, these did not appear to significantly impede or alter C sequestration capacity in our studies (Chapter 2 and 5). However, the crab species *Macrophthalmus* for example also inhabit seagrass environments, and similar to fiddler crabs, display clear differences in morphology and behaviour between genders (Schuwerack et al. 2006). Recruitment, and times of gender in-balance, may therefore temporarily impact the net C sequestration of a seagrass meadow.

Generalisations about the impact on the burial and degradation of seagrass detritus can be made for the upward conveying bioturbating fauna. While we cannot say with

certainty that regenerating species such as *Uca* spp. are actively burying seagrass detritus (detritus may be passively captured), burrowing activity certainly results in detritus being integrated into the sediment. Bioturbation by *Uca* spp. leads to an increased rate of detrital burial, and in some cases, detrital degradation. Ultimately, the source of OM (i.e. detritus), and therefore its composition and reactivity, is a determining factor of bioturbator-influenced C storage.

Bioturbation and the microbial community

Microbial activity and subsequent C mineralisation in coastal sediment is largely hindered by oxygen supply (Godshalk and Wetzel 1978, Burdige 2007), with buried plant detritus and deep (often ancient) sedimentary C generally viewed as relatively "stable" due to sediment anoxia. There is a clear and established positive relationship between bioturbators and microbial communities (Reichardt 1988, Mermillod-Blondin et al. 2004, Papaspyrou et al. 2007, Bertics and Ziebis 2009, 2010). Microbes are essential in the regulation of marine C cycling and resulting storage of coastal C (Arnosti 2011); however, the nature of the relationship between bioturbators and microbes has yet to be incorporated into the context of BC ecosystems. Several chapters in this thesis outline how bioturbation can increase sediment microbial biomass (Chapter 2) and mineralisation (Chapter 4 and 5), resulting in significant changes to C mineralisation and in some cases in the release of sediment-bound C. Not only did we observe substantial increases in microbial biomass and activity with bioturbation (Chapter 2 and 4), but the direct and indirect effects of bioturbation resulted in the rapid mineralisation of available sources of C. There was one clear common thread across the results of all chapters – that bioturbation stimulates environmental (namely sedimentary, i.e. increased sediment oxygenation, burial of detritus) conditions that favourable to increased microbial biomass and activity.

Bioturbation is known to stimulate microbial activity in sediment "micro-niches" (Bertics and Ziebis 2009, 2010), often determined by geochemical conditions. Increased microbial activity was noted corresponding to depths of detritus burial (Chapter 4) therefore suggesting that supply of fresh detritus (i.e. labile C) could be a determining factor in microbial activity. Although this is not a surprising finding, in comparison to the lower rates of microbial activity in undisturbed sediment, it certainly indicates that

detritus buried by bioturbation is more at risk of microbial remineralisation, than undisturbed buried detritus.

Previous studies showed that bioturbation altered the availability of electron donors, and potentially stimulated other mineralisation processes (i.e. fermentation) in addition to sulfate-reducing C mineralisation (Canfield et al. 2005, Valdemarsen and Kristensen 2010). We observed in our studies heterotrophic sediment processes stimulated by the presence of new detritus buried by bioturbation. We suggest therefore that patchy burial of seagrass detritus, sediment irrigation, as well as subsequent disturbance of the sediment microbial community, are the likely causes of these changes to remineralisation (Kristensen 2000).

In this thesis, we showed that bioturbator activity, and species-specific interaction with detritus, are a determining factor in the fate of detritus burial and C sequestration in bioturbated sediment. Consumption and maceration of *Fucus* macroalgae by *Arenicola* resulted in clear degradation and the subsequent release of CO_2. In Chapter 3, we observed that seagrass was not consumed, and therefore degradation was relatively hampered. Herbivory of detritus by macrofauna will potentially result in increased surface area to volume ratio of the detritus, creating both a larger space for interaction with oxygen, and increased microbial population due to defecation. It is therefore important to distinguish whether the major source of OM within an environment is likely to be consumed by burrowing macrofauna.

One of the most significant findings of this thesis was that bioturbation can stimulate a microbial priming effect (PE), a mechanism well-studied in terrestrial systems (Fontaine et al. 2007). Microbial priming has only recently begun to be investigated in marine systems, but injection of comparatively labile organic C (LOC) into deep sediments and the displacement of deep sediments by bioturbation has been suggested to stimulate PE in coastal sediment (Kuzyakov et al. 2000, Kristensen et al. 2012, Gontikaki et al. 2013). Not only did bioturbation stimulate preferential microbial degradation of macroalgae (negative priming; Gontikaki et al. 2013), resulting in the increased preservation of seagrass detritus within the sediment (Chapter 3), but injection of seagrass (LOC) by *T. australiensis* resulted in increased rates of sediment remineralisation (Chapter 5). The results of this thesis indicate that in bioturbator densities reflecting those observed in field conditions, PE stimulated by bioturbation

could cause significant leakage (efflux) of sediment-derived C as CO_2 out into the atmosphere (Kuzyakov et al. 2000).

Aerobic remineralisation of sedimentary C is unlikely to extend much past the sediment surface or burrow walls, as oxygen availability becomes deplete and aerobic microbial activity ceases (Chapter 2). Previously, research into the detritus burial by bioturbation has indicted that buried detritus may positively contribute to C stocks (de Vaugelas and Buscail 1990, Papaspyrou et al. 2005); however, in some cases when available OM has not become deplete, anaerobic reactions within the sediment may cause remineralisation and degradation of seagrass detritus (Chapter 4). Recent research also indicates that increased rates of microbial activity can stimulate the microbial priming of lignocellulose (Gontikaki et al. 2015), compounds which were previously thought to be relatively recalcitrant and less-reactive (Trevathan-Tackett et al. 2015). Sediment "memory" of microbial stimulation by bioturbation is also likely to result in increased rates of buried detritus degradation. Ultimately, both buried detritus and sediment-bound sources of C will be exposed to higher densities of microbial communities that display high rates of activity, and are potentially remineralised at a faster rate than undisturbed sediment or anoxically-held detritus.

Calculation of seagrass carbon loss over time

The addition of both bioturbation and fresh OM affected the release of CO_2 from seagrass sediment. However, it is not clear whether these factors are important in the context of meadow C sequestration. Our results suggest that in meadows rich with constant supply of OM (i.e. rapid burial of detritus), the impact on C sequestration will likely result in increased rates of detrital burial and degradation, and these rates will be dependent on the source of OM. In areas without a constant source of OM or low burial rates, remineralisation of surface-bound stocks (or more vulnerable sediment stocks) is possible.

So what is the mechanism behind this remineralisation? As uncovered in Chapter 5, burial of detritus by bioturbation can induce microbial priming. Microbial priming is certainly a key mechanism behind the increased rates of total CO_2 released from sediment (Chapter 5), as well as stimulated sediment remineralisation. However, is the effect of priming ecologically significant in the context of a whole ecosystem? Based on

our results, we suggest that in areas dominated by a bioturbator, whatever the most labile or easily degraded source of C, whether it be detritus (Chapters 3, 4 and 5) or sediment C_{org} (Chapter 2), is likely to be stimulated to degrade or be remineralised by the effects of bioturbation.

To put our results in the context of Australian and global seagrass, we reported our results into two categories (1) loss of CO_2 from increased remineralisation of OM (i.e. introduction of detritus), and (2) loss of C from sediment-held stocks. Globally, seagrass meadows sequester approximately 138 g C m^{-2} y^{-1} (Mcleod et al. 2011), with Australian meadows burying significantly less C at between 10.1 g C m^{-2} y^{-1} (Lavery et al. 2013) and 34 g C m^{-2} y^{-1} (Serrano et al. 2016). Certainly the field sites examined in this thesis had exceptionally slow burial rates (i.e. Fagans Bay, Chapters 2 and 5), and low C_{org} content (Gladstone, QLD, Chapter 1). Given that Australian seagrasses lack C burial capacity compared to meadows elsewhere in the world, maintenance of current stocks is imperative.

On a global scale, loss of C *via* bioturbation is still likely to have a meaningful and ecological impact (Fig. C.2). Species such as Callianassids will make a larger impact than *Arenicola* due to their larger biomass and higher activity (Kristensen et al. 2012). Release of sedimentary C *via* priming in moderate to high bioturbator density may also cause irrevocable change to C stocks. In terms of Australian ecosystems, bioturbation is expected to have a much larger impact, given the lower rates of sequestration. Without considering the increase in sediment C release due to the effect of burrowing, our results indicate that PE alone can potentially release 1.0–1.6 g C y^{-1} for each (*T. australiensis*) burrow in a meadow. Even if we consider PE as only a transient effect, and assuming that PE is the only mechanism responsible for net loss of C (i.e. loss of C *via* undisturbed sediment remineralisation is accounted for in published seagrass C budgets), one burrow per m^{-2} could still result in 7–20% of the sediment C stock lost to PE (Fig. C.2). Extrapolating these rates of loss based on densities of bioturbators observed in Chapter 2, an overabundance of bioturbators resulting in an unsustainable release of sediment C stocks, could result in site-specific seagrass C loss greater than that induced by anthropogenic-stimulated loss of seagrass biomass (Kennedy et al. 2010, Serrano et al. 2016). Furthermore, if we consider that rates of C mineralisation are not limited to sediment enriched with detritus (i.e. Chapter 2), remineralisation of surface-held sediment stocks also need to be accounted for.

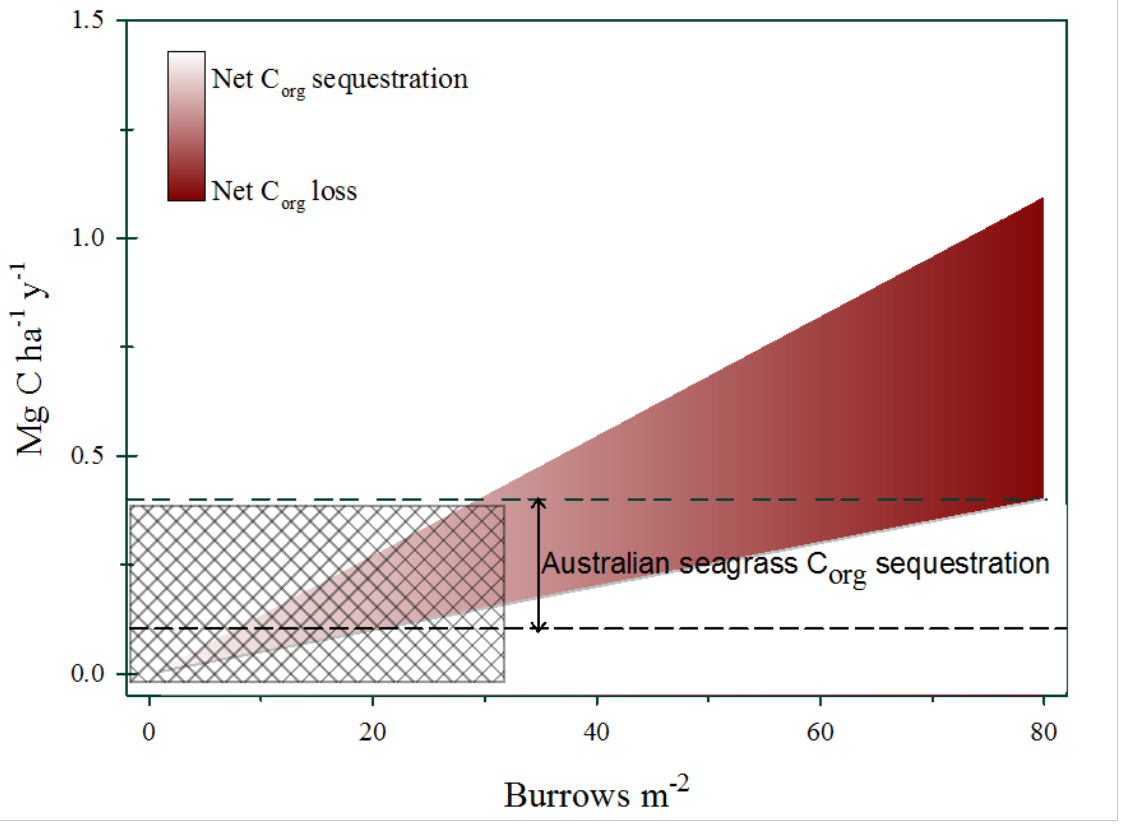

Fig. C.2: Projected loss of sediment C_{org} (as CO_2) stimulated by the microbial priming effect (PE), corresponding to bioturbator burrow densities from the Sydney region. Dotted horizontal reference lines indicate the range of annual sequestration rates for seagrass meadows in Australia (Lavery et al. 2013, Serrano et al. 2016). The cross-hashed reference box indicates the range of projected densities most likely to be sustainable (in terms of C flux) for the meadows tested in this thesis. The mean density of burrows in sites (with moderate to high seagrass density; see Fig. C.1) within the Sydney region (Chapter 2) was 36 m^{-2}. The range of projected PE stimulated C-loss values were calculated based on the (high, average and low) rates of observed sediment remineralisation (Chapter 5). The intensity of the range fill colour is indicative of the projected loss of sediment C due to PE.

Concluding remarks and research outlook

This thesis has contributed new knowledge to understanding how bioturbation affects the processes and factors contributing to C sequestration in seagrass meadows, from the burial of seagrass (and other macrophyte) detritus, to its decomposition in the sediment,

to the changes in benthic metabolism, and the mechanisms that stimulate remineralisation (Fig. C.3).

From this knowledge we can conclude that (Fig. C.3): (1) Burial of seagrass (or other vegetative sources of OM) *via* bioturbation will lead to changes in C stratification in the sediment (burrows); (2) burial of seagrass can contribute to changes in degradation rates of OM; (3) stimulation of benthic metabolism resulting in release of CO_2; and (4) increased microbial activity and stimulation by bioturbation may cause the release of sediment-bound C stocks, a process known as "microbial priming". Addressing whether bioturbation intrinsically results in net C gain or loss for seagrass C stocks is, however, difficult and complex.

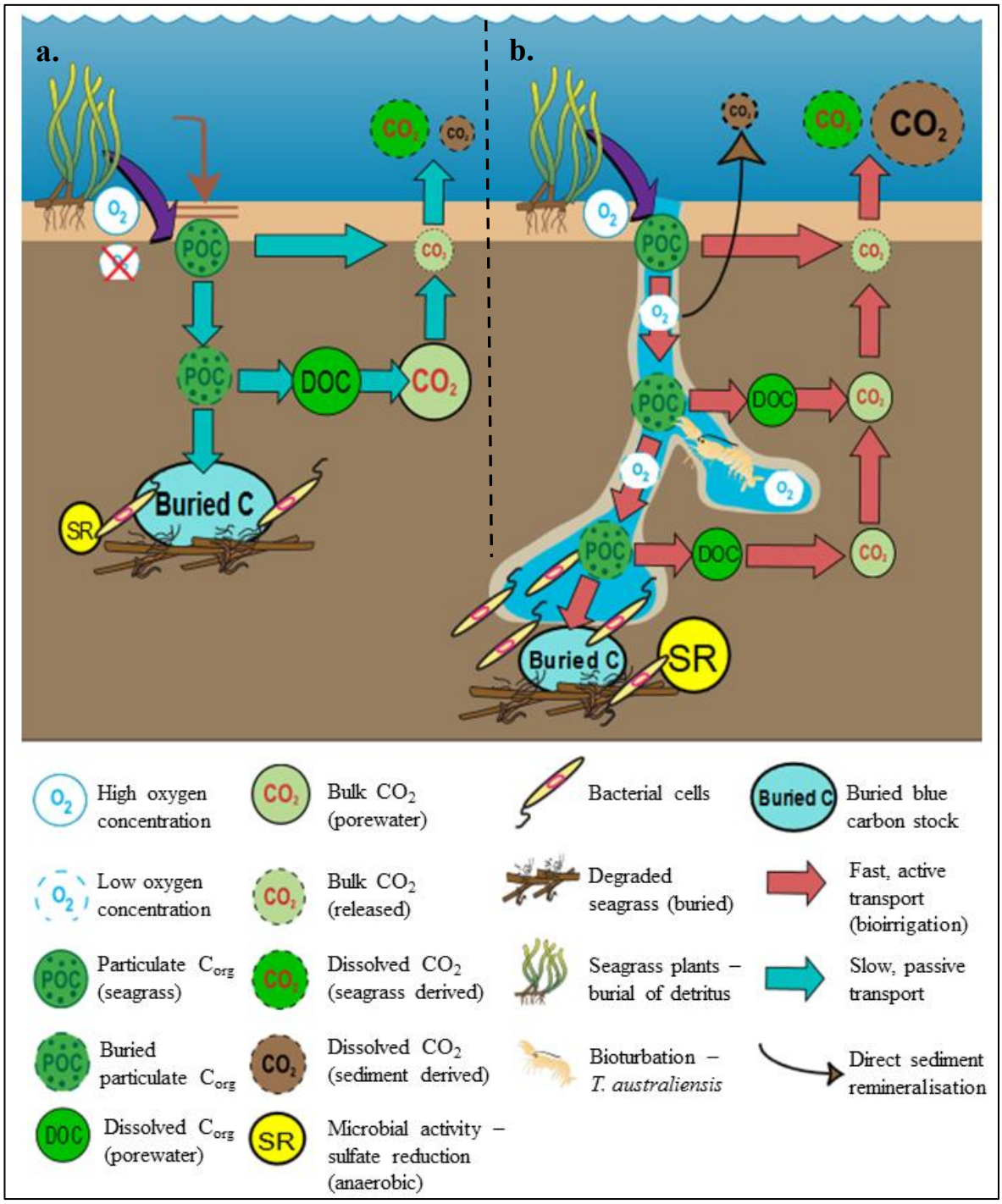

Fig. C.3: Conceptual diagram of the processes affecting seagrass detrital burial and C sequestration in seagrass meadows in (a) undisturbed sediment and (b) sediment populated with a bioturbator. Image created by A. Thomson using the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

While the results of this thesis suggest that bioturbation mixes sediment and vegetative material, resulting in "hot-spots" of C loss and potential PE, it is also evident that bioturbation has a larger scale impact on seagrass as a whole ecosystem. Burial of vegetative material may result in replenishment of sediment C stocks by supply of recalcitrant compounds (i.e. lignin or Alkyl compounds); but this is entirely dependent on the source of OM within a meadow. If a labile material is buried (i.e. macroalgae, containing no lignin), then C that is buried is likely to be lost. Furthermore, CO_2 that is released from sediment and seagrass remineralisation by bioturbators may indeed be absorbed by surrounding seagrass plants (i.e. biological C pump). What seems to be evident is that the impact of bioturbators on seagrass C stocks is a matter of density. Each seagrass meadow exists within a different range of abiotic and biotic conditions, and the efficiency of that meadow in storing C is in no way a constant. Eutrophication, disturbance, weather conditions, season, dispersal, die-back and disease all influence the trends of C accumulation and persistence of buried C stock (Mateo et al. 2006, Orth et al. 2006, Fourqurean et al. 2012, Macreadie et al. 2015). Therefore, there are likely to be thresholds of bioturbator density (and dominant species) that a meadow can support.

Macrofauna form part of a wider functioning ecosystem and provide many essential ecosystem services to seagrass meadows. All meadows may be subjected to times of changes to faunal species and population density that may impact both the burial of C (i.e. vegetation) and release of stored C (i.e. priming). We suggest that there will be instances where a meadow may experience net loss of C due to bioturbators. Thresholds of density and dominance by a particular bioturbator are expected to be a factor for the estimation of the C status of a meadow. However, thresholds of bioturbator density are likely to be ecosystem-dependant. How then do we account for and minimise any loss of C and seagrass productivity by bioturbation?

Managing global seagrass meadows and associated C stocks is essential. As we have mentioned, there is much more to be learned about the roles of different bioturbator functional groups and their effect on seagrass C stock. While some generalisations can be made pertaining to the burial of OM, bioturbator activity, and the conditions within a specific meadow are likely to be the largest impact on its C status. Investigating density thresholds and predominant sources of OM (i.e. detritus, terrestrial OC) should also be made a priority. Future research should not only focus on investigating the roles of

different functional groups and bioturbator density thresholds, but the scope should also widen to include the role of bioturbators in other BC habitats including mangroves and saltmarshes.

We would suggest creating coastal management plans that take a more holistic approach to ecosystem management. This would encompass monitoring the seagrass meadow as a whole ecosystem, rather than focussing solely on persistence of seagrass biomass. Development of the management model to encompass the impact of bioturbation should incorporate 4 key components: 1) Species of dominant bioturbator (including activity and rate of physical reworking); 2) density and seasonal flux of population; 3) state of existing seagrass meadow (including health and sediment C content); and 4) the dominant sources of OM within the meadow. In this way, ecosystem-dependent bioturbator thresholds, and budgets for representative rates of meadow C sequestration and loss, focussing on net C sequestration, can be established.

Implementing sustainable plans for bioturbator populations should also be made a priority. Re-instating the natural predatory trophic cascades within seagrass meadows where increased densities have been identified as an issue could assist in controlling bioturbator abundance (Atwood et al. 2015). In ecosystems where meso-predators have been excluded from within seagrass habitats, macro-invertebrate abundance have been seen to increase 3-10 times their natural density (Lewis and Anderson 2012). We suggest that in these situations, seagrass C sequestration would be affected. The reinstatement of top-order predators has been shown to have trophic cascade effects on ecosystem function (Hughes et al. 2013). Decreasing fishing pressure in areas where bioturbator densities have been identified as detrimental to seagrass stocks could provide both a long-term and sustainable solution. Fostering the growth of seagrass meadows could additionally assist the reduction of bioturbator populations. In studies where bioturbator densities have been altered and seagrass allowed to grow dense root systems, bioturbators struggle to burrow (Harrison 1987). By improving the conditions for seagrass growth, bioturbator densities may be kept in equilibrium with seagrass health and C sequestration (González-Ortiz et al. 2016).

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Appendix – Manuscripts submitted for publication

A Letter for Submission to Nature Climate Change

Microbial priming as a mechanism for enhanced CO_2 release in coastal sediments

Stacey M. Trevathan-Tackett[1*], Alexandra C.G. Thomson[1], Peter Ralph[1], Peter I. Macreadie[1,2]

[1]Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, NSW 2007 Australia.

[2]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3216 Australia.

Microbes are the 'gatekeepers' of the marine carbon cycle, yet the mechanisms for how microbial metabolism drives carbon sequestration in coastal ecosystems are still being defined[1]. The proximity of coastal habitats to runoff[2] creates ideal conditions for microbial priming, i.e., the enhanced remineralisation of stable carbon in response to fresh substrate availability, and poses a risk for enhanced the CO_2 release in these carbon sequestration hotspots [3-5]. Here we quantified the existence of microbial priming in coastal sediments and showed that the addition of fresh carbon stimulated a 1.7- to 2.7-fold increase in CO_2 release from recent and millennial carbon deposits. The intensity of priming was influenced by the quality of both the fresh inputs and the existing sediment carbon. We propose that microbial priming taking place at the sediment surface is a natural occurrence and can be minimised by the recalcitrant components of the fresh inputs (i.e., lignocellulose) and high rates of burial. Conversely,

in scenarios where physical disturbance reintroduces the deep sediments to the water column and to fresh carbon, the carbon that has accumulated over centuries or millennia would be rapidly remineralised over the course of weeks via microbial priming, representing damaging losses to coastal carbon stocks.

Coastal ecosystems, including seagrasses, mangroves and saltmarshes, have a disproportionately large concentration and accumulation rate of sediment carbon stocks considering their relatively small (0.1%) global ocean areal cover[6,7]. These ecosystems are currently at the forefront of research aimed at using natural environments in the sequestration of atmospheric CO_2[8]. Microbial metabolism ultimately determines the fate of this the carbon[1], thus the resulting the formation of millennial carbon deposits are enhanced by the rapid burial of organic carbon (OC) in predominantly anoxic, low-nutrients and high-refractory OC (ROC) conditions that limit microbial remineralisation rates [9]. Despite these ideal characteristics for permanent carbon accumulation and preservation, these ecosystems have been challenged by high rates of habitat losses in recent decades (e.g., seagrass loss is occurring at ~110 km^2 yr^{-1}), much of which has been attributed to anthropogenic activities, such as nutrient and organic matter runoff and physical disturbances[10-12]. Habitat destruction not only diminishes the OC-accumulation capacity of coastal habitats, but increases the risk of losing existing ROC[13-15].

The combination of fresh OC inputs and physical disturbances of deep ROC also creates ideal conditions for exacerbated ROC loss via the stimulation of microbial remineralisation, i.e., the 'microbial priming effect'. The priming effect is a phenomenon in which access to fresh, labile organic carbon (LOC) or inorganic nutrients stimulates sediment microbes to metabolise ROC that would not have been

otherwise utilised or would have been utilised at considerably slower rates[3]. This concept is widely accepted for terrestrial soil systems, and has been shown in some instances to double the amount of ROC metabolised by microbes[4,5,16].

The microbial priming effect has yet to be directly quantified in marine or coastal ecosystems, but there are some mechanisms that may stimulate priming and thus pose a significant threat to net ROC loss and CO_2 release: (1) the injection of LOC into deep sediments by bioturbation[17], (2) the addition of inorganic nutrients causing changes to porewater dynamics and bacterial nutrient limitations[18] and (3) the displacement of deep sediments[3] to the water column or sediment surface (and fresh nutrients or LOC) via physical disturbances like dredging and boating activities. The addition of LOC in the form of microalgae or seagrass detritus has also been shown to increase bacterial respiration and biomass in coastal sediments[19,20], indicating the potential for enhanced ROC remineralisation. Since the quality of LOC provided to microbes by microalgae and seagrass detritus produces differential microbial remineralisation patterns[21], different types of LOC sources would likewise induce different degrees of priming.

This study quantified the existence of the microbial priming effect in coastal seagrass sediments and revealed its potential role in weakening coastal ROC stocks. We added two common LOC sources: microalgae (*Chlorella*) to represent an algae bloom and *Zostera muelleri* seagrass to represent leaf inputs via senescence. Both LOC sources were enriched with ^{13}C before adding to the sediments, which were sampled at two depths to simulate undisturbed (surface) and disturbed (deep) sediments. To assess the microbial priming effect, we quantified the amount of CO_2 respired after LOC additions in comparison to controls. We also traced the OC source (LOC or ROC) being metabolised by microbes by analysing $\delta^{13}C$ values of CO_2. We hypothesise that the

addition of both forms of LOC will induce a priming effect, but microalgal LOC would be utilised more rapidly than detrital seagrass LOC due to higher relative lability. We also hypothesise that surface sediments may produce a larger priming effect due to a higher existing OC content available for remineralisation.

Incubation of the control sediments (ROC only) resulted in 2- to 5-fold greater respiration of CO_2 compared to CO_2 collected in the blank chambers, indicating there were active microbial populations in both surface and deep sediments (Fig. 1). The addition of LOC significantly increased the amount ($P \leq 0.001$) and rate ($P \leq 0.022$) of CO_2 respired for all samples (Figs. 1 and S1, respectively). The respiration rates for the microalgal LOC samples were significantly higher than the control surface and deep sediments ($P = 0.022$); however, little net priming for surface sediments occurred and became negligible less than one month into the experiment (Fig. 1a). In fact, when the sources of respired CO_2 were assessed for the surface treatment, the average total g C-CO_2 respired for microalgal LOC was less than the control sediments, with 17% of the total CO_2 derived directly from microalgal decomposition (Fig. 2). This is evidence for a negative or apparent priming effect, wherein, microbes preferentially utilise macroalgal LOC over ROC, resulting in little-to-no additional ROC lost from the system, i.e., 'preferential remineralisation'[3,4,9,22].

Seagrass detritus-amended sediments showed a very different response compared to microalgal LOC treatments. Both surface and deep sediments had significantly higher respiration rates than control sediments ($P = 0.001$), which were similar to respiration rates from *in situ* seagrass sediments[23]. Seagrass-amended sediments also had the larger priming effect between the two LOC treatments (Fig. 1b, d), which lasted for approximately 8 weeks for surface sediments (Fig. 1b). The time-frame of priming for these samples were within the range found of terrestrial soils and a

98 real priming effect (as opposed to apparent or negative priming, reviewed in
99 Blagodatskaya et al. and Kuzyakov et al.)[5,16,22]. These results suggest that after two
100 months, easily accessible LOC became deplete, resulting in a reduced rate of
101 remineralisation. Approximately 4 g C-CO_2 m^{-2} came from the remineralisation of the
102 newly introduced seagrass LOC, representing 1.7- and 2.7-fold more CO_2 respired as a
103 result of priming for surface and deep sediments, respectively (Fig. 2). Much of the
104 seagrass detrital biomass remained after the three-month experiment suggesting there is
105 potential for seagrass-based OC contributions to sediment ROC in the future, likely in
106 the form of lignocellulose[24].

107 The deep sediments had significantly lower respiration rates ($P = 0.002$) and
108 amount of CO_2 respired ($P = 0.024$) than surface sediments (Figs. 1c, d and S1). The
109 decreased activity could possibly be due to (1) the bacteria from deeper sediments
110 having lower metabolic and enzymatic activities and lower bacterial abundances,
111 causing reduced remineralisation rates[25,26], and (2) the deeper sediments having less
112 total OC, which was of higher recalcitrant quality compared to surface sediments (1%
113 vs 4% OC and 62% vs 52% recalcitrant/refractory OM, respectively; Fig. S2).
114 Therefore, in addition to having less ROC available for remineralisation, the deep ROC
115 would have likely already been heavily processed by microbes since burial[9], leaving
116 less easily available ROC to be primed. Regardless, the addition of LOC to the deeper
117 sediments still elicited 2- to 2.7-fold more CO_2 released as a result of priming compared
118 to the deep control sediments (Fig. 2). By the end of the experiment, microbial priming
119 was still occurring for both deep, LOC-amended sediments, which indicated that the
120 microbial populations in the deep sediments can sustain priming for longer than three
121 months (Figs. 1c, d).

122 The present results provide evidence that the microbial priming effect can exist
123 for coastal sediments and results in at least a doubling ROC-CO_2 release. The
124 magnitude of priming in this study was highly dependent on the quality and structure of
125 LOC, with seagrass LOC treatments producing the greater, more sustained priming
126 response than microalgal LOC. This enhanced response was likely attributed to the wide
127 diversity of organic substrates provided by the detrital LOC, and likewise support
128 greater diversity of microbial groups to remineralise the detritus[16]. These differences in
129 priming response could also be due to the differing physical structures of the two LOC
130 sources. Future experiments on more similarly structured LOC (e.g., macroalgae vs
131 seagrass), will allow us to better define the roles of diversity and lability of substrates on
132 the microbial priming effect.
133 Based on the results of this study, we present two scenarios for how microbial
134 priming is affecting ROC stocks in coastal ecosystems (Fig. 3). First, the rapid
135 accumulation and burial of fresh LOC in coastal ecosystems[6] is generally a natural and
136 continuous process. Therefore, the negative or apparent priming from microalgal
137 additions and likelihood of detritus preservation, suggest that such LOC additions will
138 outpace its remineralisation for surface sediments. In this case, priming would have a
139 small impact on long-term ROC losses. Alternatively, consider disturbance scenarios,
140 such as bioturbation, dredging and boating activities, that lead to the reintroduction of
141 deep sediments (and nutrient-limited microbes) to the water column or sediment
142 surface, followed by the interaction with LOC. In these scenarios, a priming effect can
143 be produced that will at least double the vulnerability of ROC to remineralisation.
144 Consequently, microbial priming poses a considerable threat to the longevity of stored,
145 millennial ROC and future carbon accumulation in coastal ecosystems undergoing
146 physical disturbances. With the high rate of coastal habitat disturbance and loss

147 worldwide, we identify microbial priming as a significant mechanism in weakening
148 ROC storage and a pathway to greenhouse gas release in disturbed marine ecosystems.
149
150 Methods
151 **Sediment Characteristics**
152 The sediment (i.e., the source of refractory organic carbon or ROC) used in this project
153 was from Fagans Bay in New South Wales, Australia (33.4306 S, 151.3211 E). The bay
154 was characterised as a high nutrient and sedimentation input environment, with patchy
155 seagrass *Zostera muelleri* Irmisch ex Ascherson meadows along the shore. We chose to
156 quantify priming for surface sediments (0-1 cm), which routinely come in contact with
157 LOC, and deep sediments (29-30 cm) to simulate the reintroduction to the surface after
158 a physical disturbance[27]. Age-dating of sediments from the site used in this study
159 confirmed that the surface sediments were recent (< 10 yrs) and deep sediments were
160 millennial deposits (> 1000 yrs) (Figs. S2 and S3). (See Supplementary Methods for
161 coring and age-dating details).
162
163 **Isotope Labelling and Incubation Experiment**
164 *Chlorella vulgaris* were used for the microalgal treatments due to its availability in ^{13}C-
165 enriched form (97.2% atm; IsoLife, Wageningen, Netherlands). For the seagrass detritus
166 treatment, whole *Z. muelleri* plants were collected from Fagans Bay and labelled with
167 enriched sodium bicarbonate on the same day.
168 The experimental design was fully-orthogonal (3 replicates) with two ROC
169 sediment depths (0-1 cm, 29-30 cm) and three LOC treatments: control (unamended),
170 microalgal addition (+ *Chlorella*), seagrass detritus addition (+ *Zostera*). A procedural
171 control (i.e., blank) was included to account for the background CO_2 concentrations

within the chamber (no sediment). LOC was added to the sediments immediately before closing the incubation chamber at the start of the experiment. For the microalgal LOC treatments, 30 mg DW (46.5% C) of lypholised *Chlorella* was mixed with 2 mL of sterile CO_2-free seawater at a salinity of ~22 before adding to the surface of the sediment. The concentration of algae used was based on benthic microalgal bloom concentrations in coastal sediments[28]. The seagrass LOC treatments received 200 mg DW (30% C) of labelled seagrass, which represented low detritus concentrations in east Australian coast *Z. muelleri* meadows[29].

The incubation chambers were made of sterilised 1.4 L plastic containers with an air-tight silicon seal and consisted of (1) sediment + treatment, (2) 40 mL 0.1 M sterile, CO_2-free sodium hydroxide (NaOH) to trap the CO_2 being respired and (3) 20 mL sterile CO_2-free ultrapure water to maintain moisture within the chamber[30]. Chambers were randomly placed into the incubator and maintained in the dark at 20°C. Repeated sampling occurred at 1, 3, 7, 14, 28, 56 and 84 days. At each sampling day, the NaOH vial was removed, capped, sealed with Parafilm M® and replaced with fresh NaOH. The NaOH samples containing the dissolved inorganic carbon (DIC) was stored at 4°C until analysis (See Supplementary Methods for isotope labelling and analysis details).

Calculations and Statistical Analyses

The average C captured as CO_2 (mg C-CO_2 L^{-1}) of the blank samples from each collection day was subtracted from the C-CO_2 content of the control and amended samples from the corresponding day. The resulting C-CO_2 content from each time point was normalised to the area of the sediment slice (g C-CO_2 m^{-2}). The priming effect was estimated by subtracting the C-CO_2 content in the control sediments from amended

sediments[5]. A mixing model was then used to incorporate the stable isotope values (% atm for microalgal treatments to accommodate for the highly enriched samples and per mil for seagrass treatments) to quantify the proportion of respired CO_2 from LOC and ROC: $P_{ROC} = (\delta_{Mixture} - \delta_{LOC})/(\delta_{ROC} - \delta_{LOC})$, where P is the proportion respired as C-CO_2. These proportions were applied to the total g C respired over the 84 day experiment to estimate g C respired as a result of the priming effect. A repeated measures 2-way ANCOVA (*treatment*, *depth*) with *time* as covariate was used to analyse the cumulative g C-CO_2 m^{-2} respired and respiration rates (mmol C-CO_2 m^{-2} d^{-1})[16]. A Bonferroni adjustment was used for pairwise comparisons.

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Supplementary information is available in the online version of the paper.

Contributions of Authors

S.T.T. and P.M. conceived and designed this study. S.T.T. and A.C.G.T. conducted the incubation and analysed the data. P.M, P.R and S.T.T. interpreted the results. S.T.T. wrote the manuscript. All authors took part in editing and commenting on the manuscript.

221 Author Information

222 The authors declare no competing interests.

223 *Corresponding Author, s.trevathan.tackett@gmail.com, ph: +61 03 9244 3899. Current
224 Address: School of Life and Environmental Sciences, Centre for Integrative Ecology,
225 Deakin University, Burwood, VIC 3216 Australia.

226

Figure 1: Cumulative microbial respiration from surface and deep sediments. Priming effect was calculated as the difference in cumulative CO_2 respired between amended and control sediments. Amended sediments had a microalgal (+ *Chlorella*) or a seagrass (+ *Zostera*) labile organic carbon (LOC) addition to the sediments. Controls consisted of sediments without LOC additions. Values represent mean ± S.E.M.

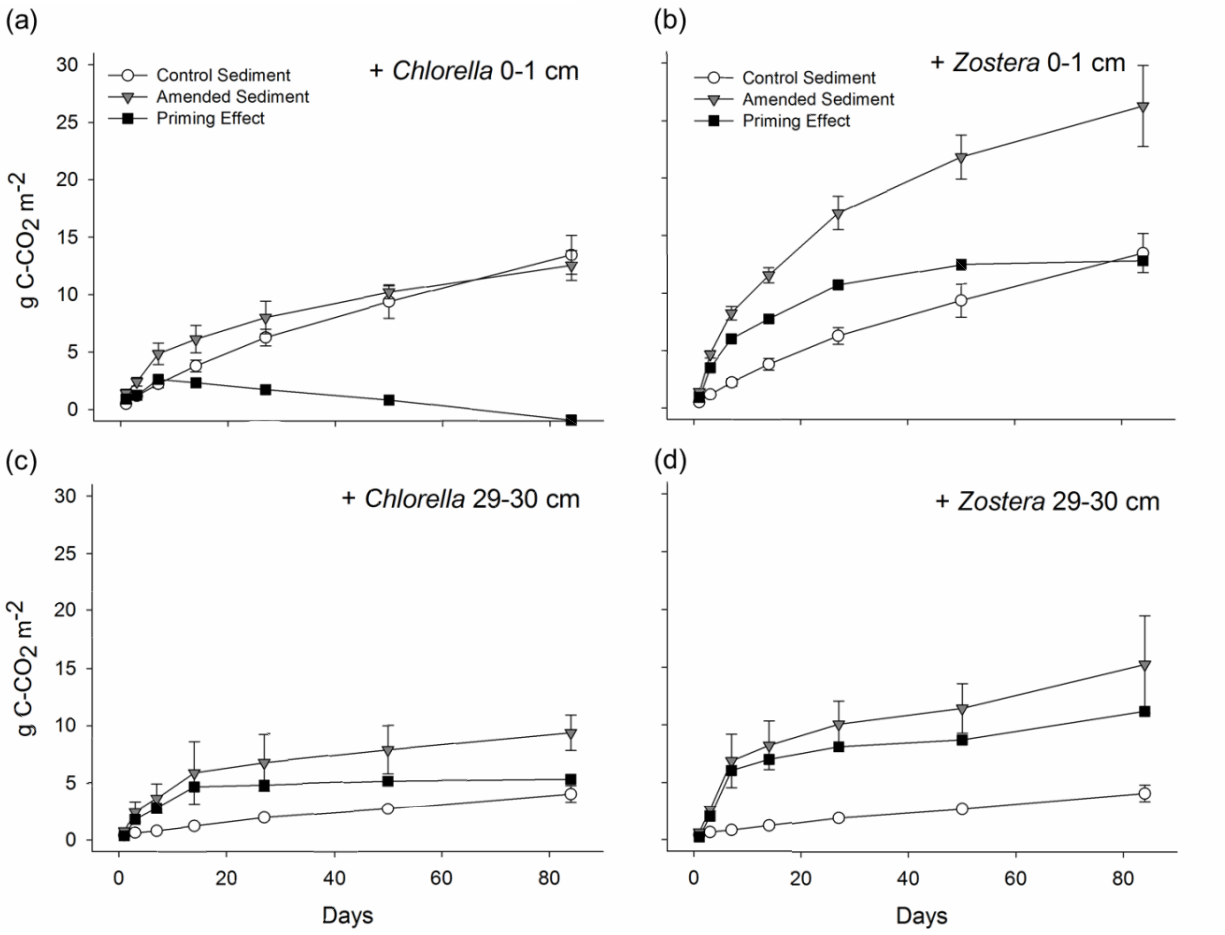


Figure 2: Estimated contributions of labile organic carbon (LOC) and refractory organic carbon (ROC) sources and a priming effect (PE) to respiration. LOC additions were either a microalgal (*Chlorella*) or a seagrass (*Zostera*) treatment. Controls consisted of sediments (ROC) without LOC additions. Each bar stack represents a fraction of the total C-CO_2 respired, with the fractions of ROC and LOC calculated from stable isotopes signatures using a mixing model (See *Methods: Calculations and Statistical Analyses*). Values represent means.

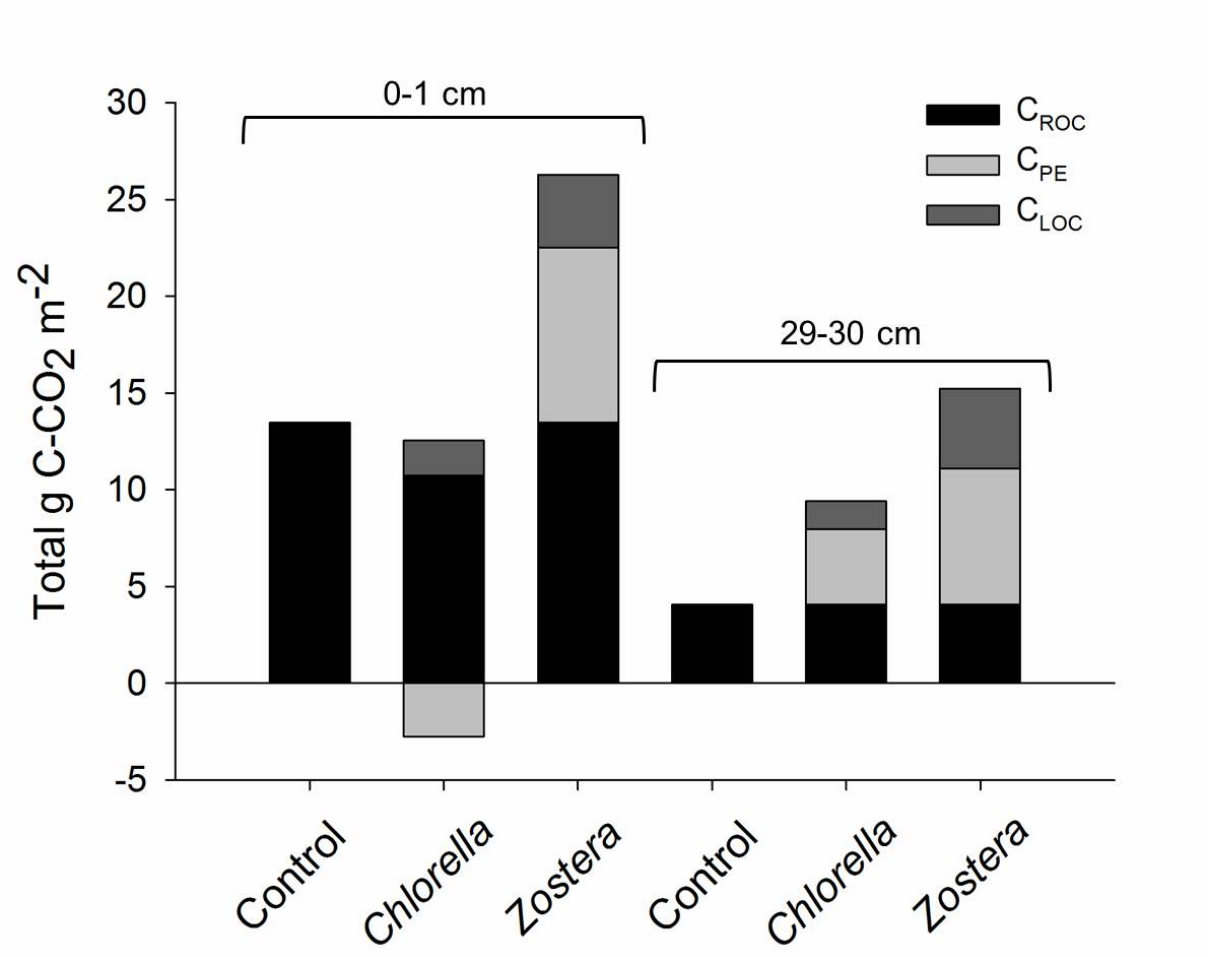
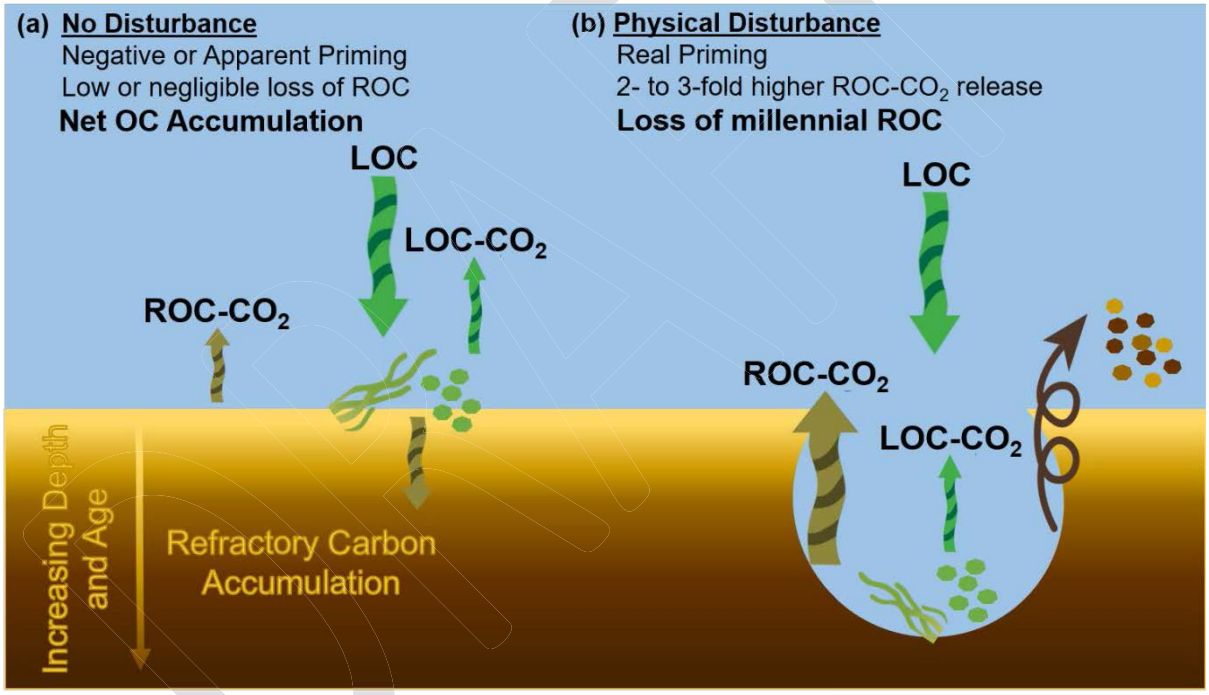


Figure 3: A conceptual model for how microbial priming influences organic carbon cycling in coastal ecosystems. In undisturbed conditions (a) the addition of labile organic carbon (LOC) input is a rapid, natural occurrence. The losses of recent refractory organic carbon (ROC) could be offset by residual OC accumulation from the fresh inputs, e.g., lignocellulose. In physically disturbed sediments (b) deep ROC is exposed to LOC and the resulting rapid loss of ROC that has accumulated of centuries or millennia. Residual OC accumulation from the fresh inputs cannot be offset the loss of old sediment ROC.



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Can we manage coastal ecosystems to sequester more blue carbon?

Running title: Coastal biosequestration

Peter I. Macreadie[1]*, Daniel A. Nielsen[2], Jeffrey Kelleway[2], Trisha Atwood[3], Justin Seymour[2], Katherina Petrou[2], Rod M. Connolly[5], Alexandra C. G. Thomson[2], Stacey Trevathan-Tackett[2], and Peter J. Ralph[2]

[1]School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Victoria 3216 Australia (p.macreadie@deakin.edu.au); [2]Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, 2007 Australia; [3]Department of Watershed Sciences and The Ecology Center, Utah State University, Logan, UT 84322 USA; [4]School of Life Sciences, University of Technology Sydney, 2007, Australia [5]Australian Rivers Institute – Coast & Estuaries, and School of Environment, Gold Coast campus, Griffith University, Queensland, 4222 Australia

Abstract: Protection and restoration of vegetated coastal habitats (seagrasses, saltmarshes and mangroves) are two well-known strategies for enhancing and maintaining blue carbon sequestration, whereas there has been much less attention to the importance of catchment-level management practices. Here we explore potential management strategies to optimize coastal blue carbon sequestration. From a broad list of environmental processes that influence blue carbon sequestration (warming, CO_2 levels, water depth, nutrients, runoff, bioturbation, physical disturbances, and tidal exchange), we identified three strategies that we believe are most amenable to resource managers: (1) reducing anthropogenic nutrient inputs; (2) re-instating top-down control of bioturbator populations; and (3) restoring hydrology. Drawing on case studies (albeit limited), we argue that these three strategies – or 'management levers' – have the potential to minimize blue carbon losses and maximize gains. The science behind these strategies is immature but growing rapidly. We offer suggestions for future research to address these knowledge gaps.

In a nutshell:

- Vegetated coastal habitats (seagrasses, saltmarshes, and mangroves) are significant global sinks of organic carbon - termed ‘blue carbon’
- Carbon markets tend to focus on ‘additionality’ through restoration, with much less attention on optimizing the efficiency of existing ecosystems
- We investigated key environmental processes that influence blue carbon sequestration and identified three catchment-level processes amenable to resource management: nutrient inputs, bioturbation, and hydrology
- We argue that reducing nutrient inputs, avoiding unnaturally high levels of bioturbation, and restoring natural hydrology (freshwater flows and tidal exchange) will maximize blue carbon sequestration and minimize blue carbon losses

The idea of managing ecosystems to enhance sequestration of carbon and thereby help offset rising atmospheric CO_2 levels is relatively recent (Post *et al.* 2004). Attempts to increase 'green carbon' sequestration in terrestrial ecosystems through land restoration have proved somewhat disappointing because the scale of land conversion required to make meaningful CO_2 emission reductions for most countries is unrealistically large (e.g. two-thirds of working cropland), and because most terrestrial lands have short carbon retention (permanence) times and finite capacity to store additional carbon due to saturation (Fissore *et al.* 2010).

Vegetated coastal habitats (VCHs) - seagrasses, saltmarshes and mangroves– are now recognized as globally significant sinks of organic carbon in the oceans with the potential to overcome some of the shortcomings associated with terrestrial carbon sinks (McLeod *et al.* 2011). VCHs can store carbon over millennial timescales, which they sequester up to 57 times faster than tropical rainforests, and can accumulate carbon without reaching saturation (McLeod *et al.* 2011). Globally, VCHs sequester a similar amount of carbon as forests each year, despite their total area representing less than 3% of forests (Duarte *et al.* 2013). As with important terrestrial carbon sinks (e.g. Amazonian forests, permafrost regions), ecosystem degradation can also shift VCHs from carbon *sinks* to carbon *sources* (Pendleton *et al.* 2012).

From a scientific and resource management perspective, we raise the question of whether *existing* VCHs could be better managed to sequester more blue carbon and minimize CO_2 emissions. There are many environmental processes that control carbon sequestration (stock accumulation and efflux) in VCHs (WebTable 1); we focus on those that are amenable to management intervention. While our focus is on managing *existing* VCHs, we stress that the first priority for managing VCHs is to stop their destruction and degradation, which is happening globally through a variety of human activities, such as dredging, harvesting, filling, dyking, and drainage (McLeod *et al.* 2011). It is only through 'additionality' that VCHs can bolster climate change mitigation efforts as well as secure carbon trading finances. Additionality can be achieved in two ways: 1) Actions that lead to emission reduction – i.e. preventing carbon losses due to 'business as usual' management activities; or 2) Removal of atmospheric emission – i.e. net sequestration gains via habitat expansion or management to enhance sequestration efficiency.

Key environmental processes that influence blue carbon sequestration

Organic carbon that is produced or captured by VCHs has three main fates: (1) it can be broken down (remineralized by microbes) and converted into atmospheric carbon dioxide - CO_2; (2) it can be assimilated into microbial biomass; or (3) it can become sequestered within sediments where it can be bound for millennia. If the processes that control breakdown of carbon within VCHs can be understood (Fig. 2), then it may be possible to manage coastal areas to maximize carbon gains and minimize carbon losses.

In WebTable 1 we summarize key environmental processes that influence blue carbon sequestration (incl. mechanisms and examples from the literature), and their amenability to being controlled by resource managers. The latter – 'amenability to management control' - is discussed from a theoretical/logistical viewpoint (i.e. if boosting blue carbon sequestration was the sole goal); but it is important to note that in most cases there are broader political, ethical, and environmental implications that could make such management intervention unwise or unfeasible (e.g. removal of hard barriers such as bund walls from the coastal zone may improve conditions for VCHs, but could lead to loss of coastal properties and other human infrastructure).

From the broader list of possible candidate processes identified in WebTable 1, we identified three - nutrient inputs, bioturbator activity, and estuarine hydrology (Fig. 3) - that are amenable to management intervention, and have the potential to make meaningful differences to global carbon sequestration by VCHs. These are discussed in the following sections and summarized in Panel 1.

Managing nutrients to increase carbon storage

Nutrients (particularly nitrogen and phosphorus) are elevated around much of the world's developed coastline, primarily due to the use of fertilizers for agriculture in coastal watersheds and discharge of human sewage effluent from coastal cities (Smith and Schindler 2009). A common assumption is that nutrient addition will improve coastal carbon sequestration by enhancing VCH plant productivity. The balance of evidence, however, is at this stage pointing to likely decreases in carbon storage under nutrient addition. For example, experimental evidence shows net losses of carbon either through plant mortality and gaseous efflux (e.g. in mangroves; Lovelock *et al.* 2009, Lovelock *et al.* 2014), or erosion and loss of sediment (e.g. in saltmarsh; Deegan *et al.*

2012). These effects of nutrient addition in mangrove and saltmarsh habitats also interact with porewater salinity and shifts in plant communities, and additional research is needed to better understand how the relationship varies regionally. Long-term studies of experimental nutrient impacts on seagrasses are mixed; López *et al.* (López *et al.* 1998) reported a one third decline in organic content in surface sediments with nutrient addition stimulating microbial activity and thus carbon remineralisation (Antón *et al.* 2011), whereas Howard *et al*. (2016) found no effect of nutrients on blue carbon stocks, but noted that working on small experimental spatial scales may have limited their ability to detect an impact.

On saltmarsh, a long-term study shows that nutrient addition results directly in community shifts in plant species assemblages, and that rates of sediment accretion, and concentrations of living and dead carbon in sediment, interact with the types of plant species present (Valiela 2015). Excess nutrient loading, when severe, can also cause a complete community shift in the dominant primary producers of shallow water ecosystems, such as from seagrass to micro- and macroalgae dominance (Antón *et al.* 2011). While this may not significantly change the gross primary productivity (ie. gross CO_2 fixation rate), the faster turnover of carbon incorporated into microalgae—as a result of lower structural complexity and higher overall nutrient content compared to seagrass rhizomes—along with the enhanced microbial activity, results in a smaller carbon reservoir (Antón *et al.* 2011). Community shifts can therefore have a profound impact on the fate of the production and total carbon sequestered. In addition, increased water column productivity from a nutrient-driven community shift also leads to light limitation for benthic plants and potentially hypoxia at the sediment surface as a result of increased microbial activity, negatively impacting sediment biogeochemistry (Howarth *et al.* 2011) and benthic organisms.

While more research is needed to quantify the long-term effect of nutrient loading on net carbon flux, particularly for saltmarshes and mangroves, the few existing studies (discussed earlier) suggest that nutrient reduction programs may have a favorable effect on carbon sequestration. Reducing nutrient loading in coastal systems should help to maintain the natural competition between macrophyte production, microalgae and bacterial activity, limiting the release of stored carbon and assisting VCHs to maintain carbon sequestration capacity. Substantial effort is already expended around the world, for other reasons, to limit or reverse eutrophication (Smith and Schindler 2009). Measures to control nutrient loads include reducing fertilizer use,

improving wastewater treatment and altering land practices (the handling of manure, soil conservation, wetland restoration and planting or protecting riparian buffers). Recent improvements in reducing phosphorus and nitrogen inputs through new technologies in wastewater treatment have proven particularly effective, although nitrogen inputs from diffuse catchment sources have been more difficult to rein in (Howarth *et al.* 2011).

The many nutrient reduction programs being implemented around the world provide an opportunity to rapidly refine our knowledge about the magnitude of changes in carbon sequestration rates that can ensue. Increased collaboration among biogeochemists and coastal managers can help to determine the scenarios and habitats offering the greatest gains in carbon storage relative to effort at reducing nutrients, which can be quite simple, such as avoiding the installation of stormwater pipes and outfalls near VCHs.

Controlling bioturbator populations to prevent carbon losses

Bioturbation is a fundamental ecosystem process that drives both inputs and outputs of a carbon reservoir (Kristensen *et al.* 2008). It can be defined as all transport processes carried out by animals that directly or indirectly affect the biology, geochemistry, and physical structure of sediments. Bioturbation in VCH is carried out by a diversity of organisms that live both above and below the sediments surface (e.g. crabs, shrimp, polychaete worms, and many others). These organisms influence carbon cycling in VCH through diverse, often competing processes and reactions that alter plant growth and the redistribution and release of gaseous (e.g., CO_2; Kristensen *et al.* 2008), particulate (Coverdale *et al.* 2014) or dissolved carbon.

Plant growth is important for soil carbon accumulation and preservation because plants provide structure for particle trapping and act as a fresh source of carbon to sediments. At relatively low densities, bioturbators often have positive effects on the growth of VCH plants (Kristensen *et al.* 2008). Feeding and burrowing activities (i.e. leaf litter processing, irrigation of burrows) of bioturbators help stimulate plant growth by enhancing sediment conditions through increasing nutrient and oxygen concentrations in the sediment (Smith *et al.* 2009). In mangroves, for example, studies have reported a positive correlation between tree growth and crab burrow density (Smith *et al.* 2009). Although some level of bioturbation is necessary for maintaining a

182 healthy VCH, at high densities bioturbators can have negative effects on VCHs with
183 cascading effects on soil carbon accumulation and preservation (Coverdale *et al.* 2014).
184 Many of the burrowing and feeding activities by bioturbators that enhance plant
185 growth can also stimulate microbial breakdown of soil carbon to CO_2. Sediment
186 reworking by bioturbators can increase microbial breakdown of carbon by refreshing
187 carbon supplies through the mixing of relatively young with ancient carbon during
188 burrowing (Papadimitriou *et al.* 2005). Sediment mixing during burrowing also
189 increases electron receptor (e.g. oxygen, nitrate) availability to deeper microbial
190 communities (Kristensen *et al.* 2008), with oxygen penetration increasing by several
191 orders of magnitude (Ziebis *et al.* 1996). As a result, microbial abundance can be >10-
192 fold higher (Papaspyrou *et al.* 2005) and CO_2 production 2-fold greater in sediments
193 with bioturbators compared to those without (Kristensen *et al.* 2008). Furthermore,
194 intense burrowing can alter the physical properties of the sediment and weaken plant
195 roots, resulting in bank erosion and the loss of carbon associated with those sediments
196 (Coverdale *et al.* 2014).
197 Increasing evidence of trophic cascades in VCHs has altered the previous belief
198 that consumer control in these communities is insignificant, and raises concerns that
199 trophic cascades may be influencing the sequestration capacity of these ecosystems
200 (Atwood *et al.* 2015). For example, in Cape Cod, USA, overharvesting of predatory fish
201 has led to a 4-fold increase in *Sesarma* crab populations, causing widespread saltmarsh
202 die-off from overgrazing and erosion, and an estimated release of 248.6 ± 4.8 Gg of
203 below-ground C (Coverdale *et al.* 2014). Across global salt marshes, overabundance of
204 bioturbators and large-scale bank erosion could be leading to an estimated release of~
205 2100-8500 tonnes CO_2 yr^{-1} (Coverdale *et al.* 2014); although the fate of carbon, and
206 thus its potential to be remineralized to CO_2, after such disturbances is unknown.
207 Similarly, the loss of top-down control has resulted in documented cases of overgrazing,
208 defoliation, and extreme bioturbation events in seagrasses and other salt marsh systems,
209 and there is indirect evidence of their occurrence in mangroves (Atwood *et al.* 2015).
210 Once VCHs are transformed to bare sediment, revegetation can be hindered by
211 propagule-eating bioturbators and the reworking of sediment from burrowing and
212 feeding activities, which can reduce the global CO_2 uptake by natural ecosystems by
213 several million tonnes (Atwood *et al.* 2015). As a result, the effects of trophic cascades
214 on carbon stocks in VCHs have the potential to be large and long-lasting.

Within the ecosystem restoration field there is a common perspective that enabling the recovery of natural ecological processes leads to successful long-term results. In the case of bioturbators in VCHs, re-instating top-down control of bioturbator populations may have the most sustainable outcomes, and studies from other ecosystems suggest that this tactic can lead to increases in carbon sequestration (Wilmers *et al.* 2012). However, we acknowledge that in some cases increases in bioturbator populations is the result of vegetation loss, not the initial cause (Valdemarsen *et al.* 2011). In these cases, a more successful management strategy may be to address the root cause of vegetation loss (we address some of these actions under our sections on restoring hydrology and managing for nutrients); although some direct actions on bioturbators may still be necessary if they inhibit re-vegetation.

In many cases, controlling for trophic cascades in VCHs is complicated by insufficient knowledge about trophic structure and above- and below-ground linkages. Thus, detailed studies that can elucidate the potential for trophic cascades to occur should be a research priority. However, in cases where the bioturbator is an invasive species and lacks a native predator (Malyshev and Quijon 2011), or in cases where action is required immediately, harvesting of bioturbators may be required to reduce their densities quickly. For example, along the western and eastern coasts of the USA, several agencies have already implemented trapping and saltmarsh transplanting programs to combat the invasive European green crab (Malyshev and Quijon 2011). When bioturbators are native species, however, it is important to identify target bioturbator densities that are conducive to high carbon accumulation and preservation rates for each site, and to what extent plant growth and recovery can be used as a proxy for the success of blue carbon sequestration strategies. This lack of information can be improved by incorporating plant growth, seedling recruitment, and bioturbator and predator population monitoring into coastal VCH management.

Restoring hydrology to increase carbon accumulation

Globally, there is a long history of human modification to coastal waterways through intentional draining of estuarine wetlands, the artificial opening or closing of intermittent estuary entrances, and the building of dams, weirs, barrages and flood gates. In this section, we identify strategies that utilize or modify existing water management structures (as opposed to new geo-engineering works) to maximize carbon

sequestration by VCHs. Whilst these measures are theoretically simple to implement, their execution may impact differentially upon the VCH types present (i.e., seagrass vs. mangrove vs. saltmarsh). For example, changes to hydrological and sedimentary regimes may promote the expansion of one VCH at the expense of another. Their implementation should therefore be based on their potential net sequestration outcome, and will require careful consideration of costs and benefits on a case-by-case basis.

Restoring allochthonous inputs

Much of the world's VCH occurs within estuaries (or are at least influenced by coastal rivers), where they capture mineral sediments important for maintaining surface elevation (Craft 2007, Lovelock *et al.* 2015) and may receive up to 50% or more of their carbon from allochthonous sources (Zhou *et al.* 2007, Kennedy *et al.* 2010). The refractory nature of terrestrial carbon transported by rivers, along with the rapid mineral sedimentation rates often concomitant with runoff, can limit remineralization of this carbon by microbes, resulting in rapid carbon sequestration by VCHs. However, in-stream barriers such as dams and weirs reduce these important allochthonous inputs - globally, 20% of suspended sediment loads are now retained in reservoirs (Syvitski *et al.* 2005). Under river regulation, catchment-derived plant materials and riverine carbon are likely to accumulate behind impoundments. This accumulation of organic matter can lead to CO_2 and CH_4 release within the dammed, freshwater reservoir (Friedl and Wüest 2002).

The trapping of fluvial inputs behind impoundments may starve downstream VCHs of sedimentary materials (inorganic and organic) they require for vertical accretion, helping to cope with sea level rise. An example is the regulation of the Mississippi River, which has led to significant reductions in rates of sedimentation to saltmarshes throughout the river delta and in the northern Gulf of Mexico (DeLaune *et al.* 2003). The resulting subsidence, combined with sea-level rise, continues to cause loss of saltmarsh and its associated ecosystem services.

In regulated catchments, reinstatement of freshwater inputs via strategic environmental flows may be a feasible method to return VCH carbon sequestration. Changes in common environmental indicators such as VCH extent, open water area, vegetation composition and elevation (or its inverse, inundation depth and frequency) may alert coastal managers to sediment deficiency. Flow may then be used to enhance

sedimentation in downstream habitats, and to increase VCH surface elevation. Freshwater inputs may also increase belowground production as many estuarine plants are facultative halophytes (i.e. they have a physiological requirement for freshwater). Such a response is likely to be species and setting dependent; however, it has been found that belowground root growth declines under increasing salinity for some mangroves (Krauss *et al.* 2014). Where belowground production is increased, there are likely to be positive outcomes for both surface elevation gain (through root production) and belowground carbon storage. Finally, in some cases an increase in sedimentation rates may also allow rapid burial of labile surface carbon (e.g. benthic algae and detritus) such that it bypasses the 'normal' oxidative processes and therefore escapes microbial remineralization.

Restoring hydrology and physico-chemical conditions

Tidal constriction through the operation of barriers such as flood barrages may convert estuaries from tidal saline systems into brackish lakes, with consequent changes to biota, including VCHs (Fig. 3). In southeast Australia, for example, there are 4,300 barriers to tidal flow in estuaries and coastal rivers - of these, a significant number (1,388) have been identified as being easily removable or modifiable to allow the re-establishment of tidal exchange (Williams and Watford 1997). Reinstatement of tidal waters has the potential to increase vertical soil accretion resulting from a) VCH biomass and litter production; b) increased access to particulate carbon transported during tidal inundation; and c) restoration of physico-chemical conditions which maximize carbon sequestration (Anisfeld 2012).

The conversion of coastal ecosystems through tidal flow restriction can disrupt carbon sequestration by coastal ecosystems and may switch these ecosystems from being net sinks to net sources of carbon (McLeod *et al.* 2011). For example, draining wetland soils is likely to result in the loss of soil carbon stocks through oxidation and enhanced decomposition rates associated with a shift to terrestrial conditions and altered microbial consortia. In contrast, increasing soil moisture has been shown to reduce surface soil CO_2 efflux in VCHs in the short term (minutes to weeks) by as much as 65% as soils become anoxic (Lewis *et al.* 2014). Tidal restoration has also been shown to enhance rates of surface carbon accumulation in the early stages (e.g. 10-14 years) after tidal restoration (Howe *et al.* 2009). Intervention should therefore be prioritized in

tidally restricted sites which currently exhibit high CO_2 and CH_4 efflux and/or slow surface accumulation rates (relative to suitable reference conditions).

Reintroduction of tidal exchange through removal of existing structures is also likely to assist with VCH migration under rising sea level, thereby facilitating future carbon sequestration. Recent modeling of an estuary in southeastern Australia suggests that opening of floodgates currently in place would allow for effective retreat of VCH wetlands, with potential carbon burial gains of up to 280,000 tonnes by 2100 (Rogers *et al.* 2013). Sites which have historically been VCH (but have since been altered through tidal restriction) and sites which offer the greatest opportunity for VCH expansion in terms of elevation and extent should be prioritized for such works.

A major challenge for coastal biogeochemists and managers is to combine the process-level research discussed above with biophysical models to provide quantitative estimates of carbon sequestration capacity through restoring hydrology. This, combined with monitoring of restoration trials and demonstration projects, will improve understanding of the feasibility and effectiveness of hydrology restoration for enhancing carbon sequestration.

Conclusions

These significant, but feasible, management strategies offer the potential to profoundly alter carbon accumulation and retention within VCH, providing new and previously underestimated strategies for mitigating climate change. The state of the science varies among the three management strategies suggested, with hydrology being the area of research that is most supported by robust science. In Panel 1 we have provided suggestions for future research. As for the fundamental question around manipulating ecosystems - i.e. should we do it? - we argue that each of the proposed strategies push coastal ecosystems towards a less-impacted state and will offer ecosystem benefits beyond carbon sequestration. Moreover, these strategies already feature in most coastal management plans (with the main exception being the monitoring of bioturbator populations), making the path towards their broad-scale implementation much smoother.

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Figure and panel captions

Figure 1. The most important vegetated coastal habitats for 'blue carbon' sequestration are the (a) seagrass, (b) saltmarsh and (c) mangrove. Photo credit: Peter Macreadie.

Figure 2. Key factors that mediate sequestration of blue carbon within vegetated coastal habitats (left to right: seagrass, mangroves, saltmarsh). Within the surface layer of sediments – the 'oxic zone' – aerobic heterotrophs gain energy by oxidizing carbon, using O_2 as the electron acceptor. Below that, in the 'anoxic zone', diverse microbial populations use alternative electron acceptors for catabolic reactions. Physical, chemical, and biological processes can influence which microbial communities and respiration processes dominate the sediments at different depths, with concomitant implications for the rate of carbon breakdown. Notes: The inset showing the electron acceptors and respiration processes is stylized; the depth and overlap of the various zones can vary widely in nature. Also, the distribution of the habitat types can vary – for example, saltmarshes can occupy the entire intertidal space in areas where there are no mangroves.

Figure 3. Managing catchment-level processes to enhance blue carbon sequestration. Three strategies are suggested: (1) reducing nutrient inputs; (2) re-instating top-down control of bioturbator populations; and (3) restoring hydrology by removing tidal flow restrictions and restoring fluvial inputs. Each of these will directly or indirectly control the burial efficiency and longevity of carbon within sediments through their effects on microbial activity. Though the goal is carbon enhancement, there are broad conservation benefits.

Panel 1. Key strategies for managing vegetated coastal habitats for blue carbon outcomes.

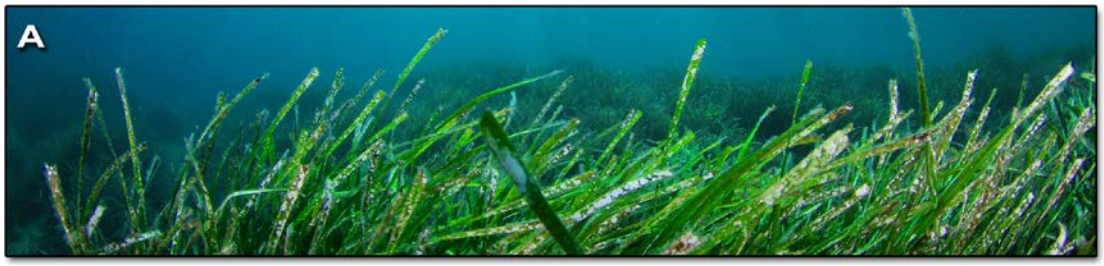


Fig. 1a

Fig. 1b

Fig. 1c

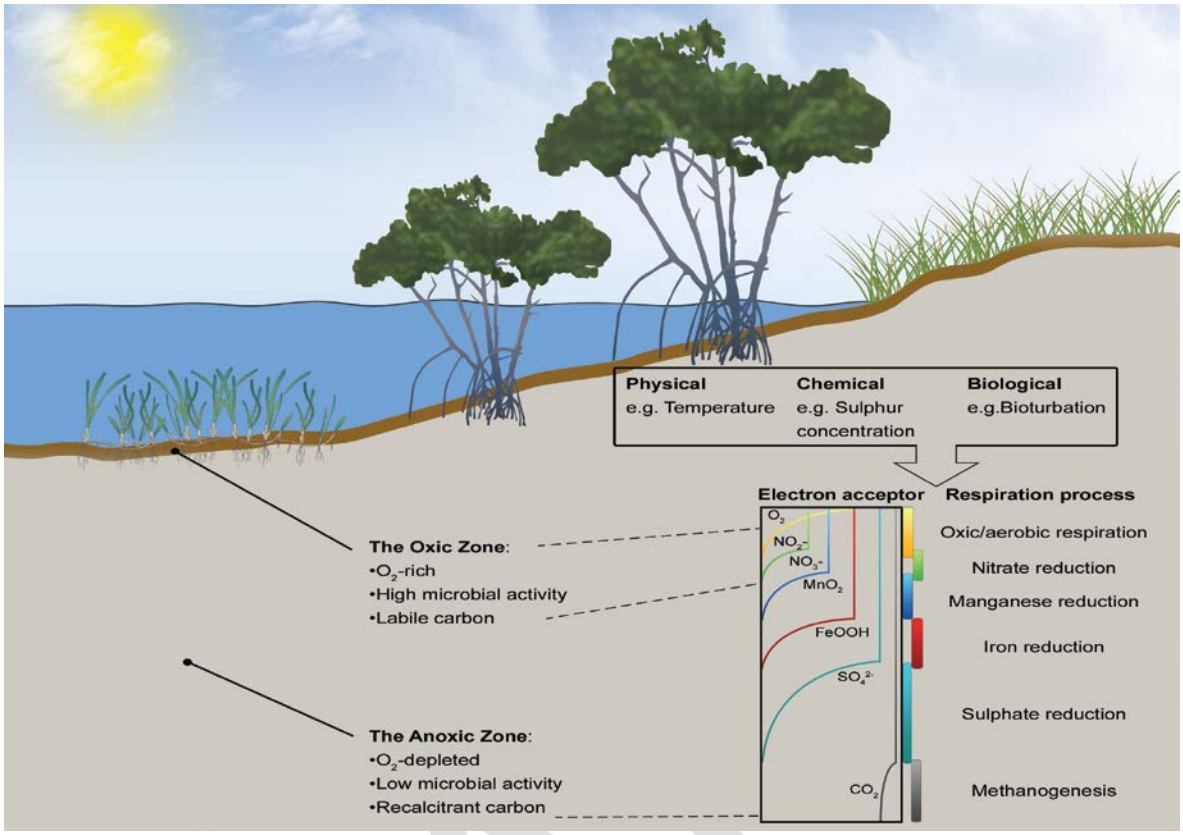
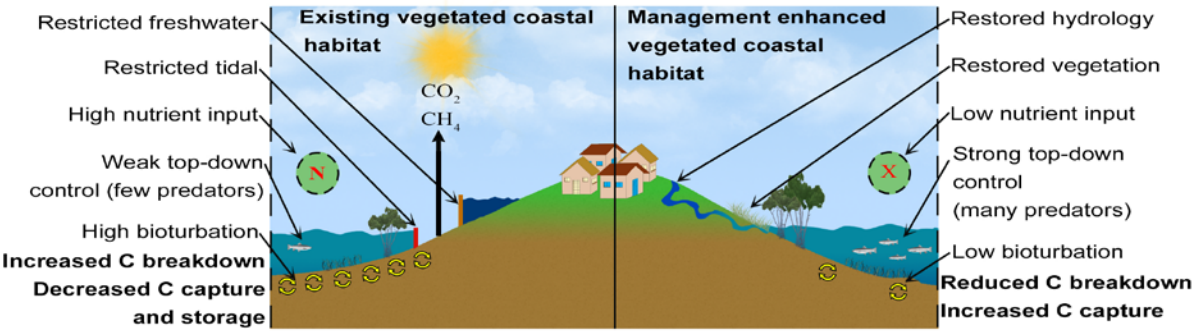

Physical
e.g. Temperature
Chemical
e.g. Sulphur concentration
Biological
e.g.Bioturbation
Electron acceptor
Respiration process
The Oxic Zone:
•O_2-rich
•High microbial activity
•Labile carbon
The Anoxic Zone:
•O_2-depleted
•Low microbial activity
•Recalcitrant carbon
O_2
NO_2^-
NO_3^-
MnO_2
FeOOH
SO_4^{2-}
CO_2
Oxic/aerobic respiration
Nitrate reduction
Manganese reduction
Iron reduction
Sulphate reduction
Methanogenesis

Fig. 2

492



493

494 **Fig. 3**

WebTable 1: Summary of the key environmental processes influencing blue carbon sequestration in vegetated coastal habitats (seagrasses, saltmarshes, and mangroves), how they influence blue carbon sequestration (incl. examples from the literature), and their amenability to being altered by resource managers for the purpose of improving blue carbon sequestration.

Key environmental processes influencing blue carbon sequestration	**Positive or negative** influence on blue carbon sequestration and mechanism(s)[1] of influence	Amenability to management control
Warming	**Negative** • Increased microbial metabolism leading to enhanced decomposition of blue carbon stocks and their conversion into CO_2, particularly during initial stages of warming prior to acclimation or when an optimal temperature is reached (Lovelock 2008, Pedersen et al. 2011, Kirwan and Mudd 2012) **Positive** • Latitudinal mangrove expansion leading to increased blue carbon sequestration capacity (Kelleway et al. 2016) • Depending on light regime, increased photosynthetic efficiency (resulting in greater plant productivity – higher autochthonous carbon production and greater carbon trapping capacity) for some species and locations up to a thermal optimum, after which a decline in growth could occur (Bulthuis 1987, McKee et al. 2012)	**Low** Cooler conditions would reduce benthic microbial activity, help regulate 'normal' temperatures for plant photosynthesis, and limit poleward range shifts by mangroves, but this would require the slowing of global warming (by reducing climate change) and cooling the planet (e.g. geo-engineering); these are currently among humanities greatest and most bold pursuits.
Increasing CO_2	**Positive** • Increased plant productivity and carbon assimilation for seagrasses and C3 mangrove and saltmarsh plants, while some C4 plants like *Spartina* sp. show mixed responses (McKee et al. 2012, Russell et al. 2013) • Increase below-ground biomass that may ultimately contribute to the blue carbon stock and aid in elevation gains with sea-level rise[2] • Increase above-ground plant material available for trapping allochthonous blue carbon	**Low** CO_2 levels have increased by ~36% during the past 150 years due to human activities. Continued burning of fossil fuels will further increase atmospheric and aqueous CO_2 levels. Facilitating CO_2 increases for the purpose of blue carbon sequestration counters against management control of warming.
Water column depth	**Negative**	**Low**

Key environmental processes influencing blue carbon sequestration	**Positive or negative** influence on blue carbon sequestration and mechanism(s)[1] of influence	Amenability to management control
	• Increased sea-level rise faster that land-ward colonisation of blue carbon species • Shading and low production by seagrass (Orth et al. 2006) • Erosion and inundation of mangrove roots (Gilman et al. 2007) and saltmarshes (Donnelly and Bertness 2001)	By the end of the 21st century, sea-level is predicted to increase from 0.18-0.59 m (Solomon et al. 2007). As a consequence to global warming, solutions to this global issue are complex and arduous.
Nutrient addition	**Negative** • Potential stimulation of decomposition of sediment blue carbon stocks (López et al. 1998, Antón et al. 2011) • Increased epiphyte load on seagrass leaves leading to reduced production (Burkholder et al. 2007)	**Moderate** Restricting anthropogenic nutrient inputs in eutrophication management efforts is complex, but feasible. The number of 'success stories' is rapidly increasing (Schindler 2006, Smith and Schindler 2009). Perhaps the greatest challenge from a management standpoint is getting infrastructure and policies in place to regulate nutrient inputs. From a science perspective, the challenge is defining what level of nutrient input is 'too much'.
Allochthonous organic carbon via runoff	**Positive** • Source of both particulate and dissolved organic carbon (POC and DOC) to blue carbon habitats • Refractory allochthonous inputs resistant to decomposition (Hedges et al. 1988, Canfield 1994, Nebbioso and Piccolo 2013) **Negative** • Potential increased exposure to sedimentation, heavy metals and other contaminants • Blue carbon species at risk to heavy metal toxicity (Kennish 2002)	**High** Restoration of fluvial inputs, e.g., environmental flows and/or dam removal could be a relatively simple way to increase allochthonous input of carbon (Arthington and Zalucki 1998, Tharme 2003). However, these structures often serve a social or economic purpose which complicates their modification/removal for environmental intentions.

Key environmental processes influencing blue carbon sequestration	**Positive or negative** influence on blue carbon sequestration and mechanism(s)[1] of influence	Amenability to management control
Bioturbation	**Positive** • Burial of detritus and blue carbon biomass (Papaspyrou et al. 2005) • Removal of contaminants (Hedman et al. 2008) • Promote nutrient cycling (Hansen and Kristensen 1998, Kristensen 2000) **Negative** • Loss of ancient blue carbon to the sediment surface or water column (Kristensen 2001, Wang et al. 2010) • Oxygenation of deeper sediments facilitating metabolism of refractory blue carbon (Bertics and Ziebis 2009) • Damage of below-ground plant tissues (DeWitt 2009)	**High** Managing bioturbator populations is highly achievable either directly or indirectly. In cases where infauna are detrimental to blue carbon stocks, the re-instatement of predatory trophic cascades have been found successful (Lewis and Anderson 2012, Hughes et al. 2013). Conversely when they are beneficial to blue carbon habitats, 'no-take' zones for harvested bioturbators can be implemented. However, for the latter option, the impact on the local fishery industry will need to be considered.
Hydrological Management-Tidal Exchange	**Positive** • Removal of flood gates or tide gates or the reduction of management for intermittently closed and open lakes and lagoons (ICOLLs) leading to expansion of blue carbon habitats, particularly in the face of sea-level rise (Howe et al. 2009, Rogers et al. 2013) **Negative** • Increased methane emissions through dilution of sulphate in the upper estuary (Weston et al. 2014) • High flow resulting in increased erosion of plants or soil carbon and reductions in submerged plant production through increased turbidity (Cabaco et al. 2008)	**High** Similar to restoring fluvial inputs, re-establishing tidal exchange is highly amendable, maybe even more so, since a significant number of these flood/tide gates have already been assessed as easily modifiable (Williams and Watford 1996, 1997).

Key environmental processes influencing blue carbon sequestration	**Positive or negative** influence on blue carbon sequestration and mechanism(s)[1] of influence	Amenability to management control
Physical Disturbance (e.g., dredging, boat activities, deforestation, etc.)	**Negative** • Habitat loss and resuspension of sediments causing buried OC to be lost from the systems or subjected to increased decomposition (Graca et al. 2004, Donato et al. 2011) • Increased respiration and oxygen demand (Wainright and Hopkinson Jr 1997) and decreased light levels for submerged habitats	**High** The reduction in anthropogenic disturbances is quite achievable with the implementation and continued use of "best-practices", particularly in regions near blue carbon habitats.

[1] The list of possible mechanisms and examples here is not meant to be exhaustive. Rather, we have listed the key mechanisms.
[2] More relevant to vegetated coastal habitats that contribute high amounts of plant structural material to blue carbon stocks, such as salt marshes.