Bioturbator-stimulated loss of seagrass sediment carbon stocks

Alexandra C.G. Thomson[1*], Stacey M. Trevathan-Tackett[1, 2], Damien T. Maher[3, 4], Peter J. Ralph[1], Peter I. Macreadie[2]

[1]Climate Change Cluster, University of Technology Sydney, 15 Broadway, Ultimo, NSW 2007, Australia.

[2]Deakin University, Geelong, School of Life and Environmental Sciences, Centre for Integrative Ecology, 221 Burwood Hwy, Burwood, VIC 3125 Australia.

[3]1 Southern Cross Geoscience, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia.

[4]National Marine Science Centre, Southern Cross University, PO Box 4321, Coffs Harbour, NSW 2450, Australia.

[*]alexandra.thomson@uts.edu.au

Key Words: Blue carbon • seagrass • bioturbation • microbial priming • carbon sequestration • remineralisation •

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 because microbial activity is decreasing with depth. However, the carbon sequestered in seagrass ecosystems may be susceptible to remineralisation via the activity of bioturbating fauna. Microbial priming is a process whereby remineralisation of sediment carbon (recalcitrant organic matter) is stimulated by disturbance, i.e. burial of a labile source of organic matter (seagrass). We investigated the hypothesis that bioturbation could mediate remineralisation of sediment carbon stocks through burial of seagrass leaf detritus.

We carried out a 2-month laboratory study to compare the remineralisation (measured as CO_2 release) of buried seagrass leaves (*Zostera muelleri*) to the total rate of sediment organic matter remineralisation in sediment with and without the common Australian bioturbating shrimp *Trypaea australiensis* (Decapoda: Axiidea). In control sediment containing seagrass but no bioturbators, we observed a negative microbial priming effect, whereby seagrass remineralisation was favoured over sediment remineralisation (and thus preserving sediment stocks). Bioturbation treatments led to a 2- to 5 -fold increase in total CO_2 release compared to controls. The estimated bioturbator-stimulated microbial priming effect was equivalent to 15% of the total daily sediment-derived CO_2 releases. We propose that these results indicate that bioturbation is a potential mechanism that converts these sediments from carbon sinks to sources through stimulation of priming-enhanced sediment carbon remineralisation. We further hypothesised that significant changes to seagrass faunal communities may influence seagrass sediment carbon stocks.

Introduction

Estuarine and coastal ecosystems, especially seagrass meadows, saltmarshes, and mangrove forests (known as "blue carbon" ecosystems), are global hotspots for carbon (C) sequestration and storage (Donato et al. 2011; Fourqurean et al. 2012; Mcleod et al. 2011). Investigating how marine ecosystems sequester and release C has been proposed as a scientific priority (Guenet et al. 2010). Gaining a comprehensive understanding of the microbial processes within these environments is essential in order to interpret the factors affecting C sequestration in coastal seagrass sediments. 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 (Blum and Mills 1991). The remaining POC can stay buried for centuries to millennia due to anoxic sediment conditions and low microbial activity (Burdige 2007; Mcleod et al. 2011). This burial results in 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 (Bianchi 2011; Fontaine et al. 2003; Guenet et al. 2010; Kuzyakov et al. 2000).

Burrowing macrofauna are a common feature in coastal benthic ecosystems (Dworschak 2005; Dworschak 2000; Kristensen et al. 2012), but recent studies (e.g. Atwood et al. 2015) suggest that the intensity of bioturbation and the population density of bioturbating fauna is increasing globally due to changes in food web structures, namely via the loss of top-down control. Furthermore, understanding the impact of these bioturbators on the longevity of coastal sediment C stocks is a current 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; Kristensen et al. 2012; Maher and Eyre 2011b; Papaspyrou et al. 2005; Papaspyrou et al. 2004; Papaspyrou et al. 2010). Direct impacts include mechanical re-working of sediment, which disperses C-rich deep sediment onto the sediment surface in oxygenated water. Indirect impacts include feeding processes which alter the microbial community within the deeper parts of the burrow (Kristensen 2008). According to the definition proposed by Kristensen et al. (2012) which is adopted here, bioturbation encompasses all of these activities, including the process of "bioirrigation" or burrow ventilation.

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. Callianassid shrimp, which include the study species *Trypaea australiensis* (Decapoda: Axiidea), are dominant bioturbators in both the sub-tropical and tropical regions of the World (Dworschak 2001; Rowden et al. 1998; Suchanek 1983), and often exist on the boundaries and within seagrass meadows (Berkenbusch and Rowden 2003; Boon et al. 1997; Kneer et al. 2013; Suchanek 1983). Callianassids are described as "upward conveyors" with regard to their bioturbating activity, meaning they transport deep sediment particles to the surface, a characteristic common to many other bioturbators, including the lugworm *Arenicola* (Kristensen et al. 2012). In Australia, Callianassids are dominant bioturbators, occupying both eastern and western coastlines and establishing burrows in a range of sediments, from mud to coarse sand (Poore 1975; Poore 2008; Sakai 1988). Indeed, Axiidean shrimp burrows are often lined with seagrass detritus, which is actively integrated into the sediment by reworking and faunal 'gardening' (Dworschak et al. 2006; Dworschak 2001; Kneer et al. 2008; Stapel and Erftemeijer 2000; Vonk et al. 2008). Bioturbator burrows also support extensive microbial populations, with up to 11-times higher microbial biomass found within the walls of burrows

compared to in surrounding sediment (Papaspyrou et al. 2005). This coupling of high microbial biomass and increased supply of fresh organic matter can stimulate CO_2 release. The magnitude of this sediment stimulatory effect depends on bioturbator activity and intensity of burrow irrigation, density of fauna, and most critically, the quality and quantity of OM (Banta et al. 1999; Hansen and Kristensen 1998; Papaspyrou et al. 2007).

Although seagrass ecosystems are ideal for rapid, permanent C storage, the preservation of these stocks is threatened by anthropogenic activity, including habitat loss (Fourqurean et al. 2012; Koller et al. 2006; Macreadie et al. 2015; Marbà et al. 2006), and loss of top-down predator control affecting food webs and trophic cascades (Atwood et al. 2015). Such disturbances could lead to microbial priming, which 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' (i.e. recalcitrant) 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 (Fontaine et al. 2007; Fontaine et al. 2003; Kuzyakov et al. 2000), in some cases increasing soil respiration rates up to 11-fold (Blagodatskaya and Kuzyakov 2008). The effects of bioturbation on sediments can vary widely depending on density, bioturbation type, and seagrass morphology and density (Kristensen et al. 2012). While in some cases it can cause major turnover of sediments to the point of seagrass habitat loss (Berkenbusch et al. 2007; Valdemarsen et al. 2011; Valentine et al. 1994), not all bioturbation is detrimental to seagrass sediments and in some cases fulfils essential functions in a meadow (DeWitt 2009).

While previous research has highlighted the microbial priming effect (MPE) in both terrestrial and marine systems (Aller 1994; Banta et al. 1999; López et al. 1998), the effect of priming on C cycling has recently garnered increased attention in marine systems (Gontikaki et al.

2015; Guenet et al. 2010; Steen et al. 2016), specifically with regard to identifying the sources of C remineralisation and release in coastal ecosystems (van Nugteren et al. 2009). Indeed, the stimulation of sediment metabolism by bioturbating macrofauna could be a trigger for remineralisation of recalcitrant C in deep sediment layers, which could be enhanced by the MPE. 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. Indeed, compared to sediment C stocks, seagrass detritus itself is relatively labile in nature. Seagrass detritus contains a large portion of labile OM, present as protein, hemicellulose and soluble carbohydrate compounds, making up as much as 30% of the total dry weight (Trevathan-Tackett et al. 2015).

The aim of this study was to determine the effect of (1) bioturbation, and (2) the burial of a labile organic matter (LOM) source (i.e. seagrass leaf detritus) into deeper sediment on remineralisation of sediment carbon stocks. We hypothesize that these disturbances will stimulate sediment metabolism, both separately and in combination. To do this we used an orthogonal laboratory experiment design, added Callianassids and seagrass to sediment, and measured the quantity and sources of net respired CO_2 using isotopically-enriched seagrass detritus and traced the evolution of ^{13}C-CO_2. We hypothesised that the incorporation of seagrass (LOM) detritus into the sediment via bioturbation would lead to higher fluxes of sediment-sourced CO_2 into the water column, and that a microbial priming effect (MPE) (as defined by Kuzyakov et al. 2000) would increase the overall remineralisation of sediment recalcitrant organic matter (ROM).

Methods

Sediment and seagrass collection

Sand consisting of a fine to medium grain (average grain size between 62.5 – 500µm) sediment (Trevathan-Tackett 2016), with an organic carbon (C_{org}) content of 1.5 – 3%, was collected from Fagans Bay, Brisbane Waters, NSW (-33.43˚, 151.32˚) in August 2015 (Fig. 1). Fagans Bay is the northern-most bay within 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 and a yearly water temperature range of 12 – 29°C (Gladstone 2006). *Zostera muelleri* is the dominant seagrass species within the bay, and at the time of sampling seagrass density was estimated as described in McKenzie et al. (2001) at approximately 75 – 80% cover, and ranged in canopy height from 10 cm in the shallows to approximately 50 cm in the deepest part of the meadow.

Collection occurred along the edges and within an existing shallow subtidal *Z. 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. Subsamples of the homogenised sediment were dried (60°C for 24 hours) and acidified following the procedure described below (see "sediment characteristics"). The subsamples (n = 3) were then analysed for $\delta^{13}C$ on an Isotope Ratio Mass Spectrometer (Thermo-Finnegan Delta V IRMS) to calculate the contribution of sediment to CO_2 efflux, and on an elemental analyser (Costech Elemental Analyser) to establish sediment organic carbon content, respectively.

Whole *Z. muelleri* (henceforth referred to as "seagrass") plants were collected from the same location. 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, while those showing signs of heavy epiphyte colonisation or senescence were excluded from the experiment.

Collection of Callianassids

T. australiensis (henceforth referred to as "Callianassids") is an upward conveyor 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). Callianassids typically live on a diet of diatoms and small particles of organic material, preferring particles < 63 µm in size (Stapleton et al. 2001).

Surveys for Callianassid density were performed at several sites in the Sydney region. At Careel Bay, NSW (-33.61˚, 151.32˚), and Narrabeen Lagoon, NSW (-33.71˚, 151.29˚), transect lines were run from the shoreline for 50 m through seagrass meadows dominated by *Z. muelleri*. At alternating sides of the transect line, 50 cm^2 quadrats were placed on the seagrass at 2 m intervals. 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). Callianassid density ranged from 2 – 248 per m^2, and in areas with seagrass density > 40%, there was an average of 36 active (mound) burrow openings per m^2.

Callianassids were collected from within an intertidal mixed *Z. muelleri* and *Halophila ovalis* meadow located within Narrabeen Lagoon, NSW (Fig. 1). Similar to Fagans Bay, Narrabeen

Lagoon has a high sediment and nutrient load (Roy et al. 2001), with the sediment characterised as a fine to medium (average grain size between 200 – 412µm) sand with an organic carbon (C_{org}) content of 0.5 – 3.9% (Dye and Barros 2005). At the time of sampling, seagrass density estimated following McKenzie et al. (2001) was patchy, with an estimated 40 – 50% seagrass cover. Visible burrows were excavated, and sediment was sieved through a 0.5 cm mesh to retain Callianassids. Smaller-sized (0.6 – 0.7 g total wet weight) adult Callianassids were used to ensure that the sediment volume did not limit burrowing activity. Individuals were transported in seawater back to the laboratory and were left undisturbed in aerated seawater for 2 – 3 days to ensure that their guts were emptied before exposure to the sediment mesocosms (described in the experimental set-up below). Individuals were weighed before their introduction to experimental conditions.

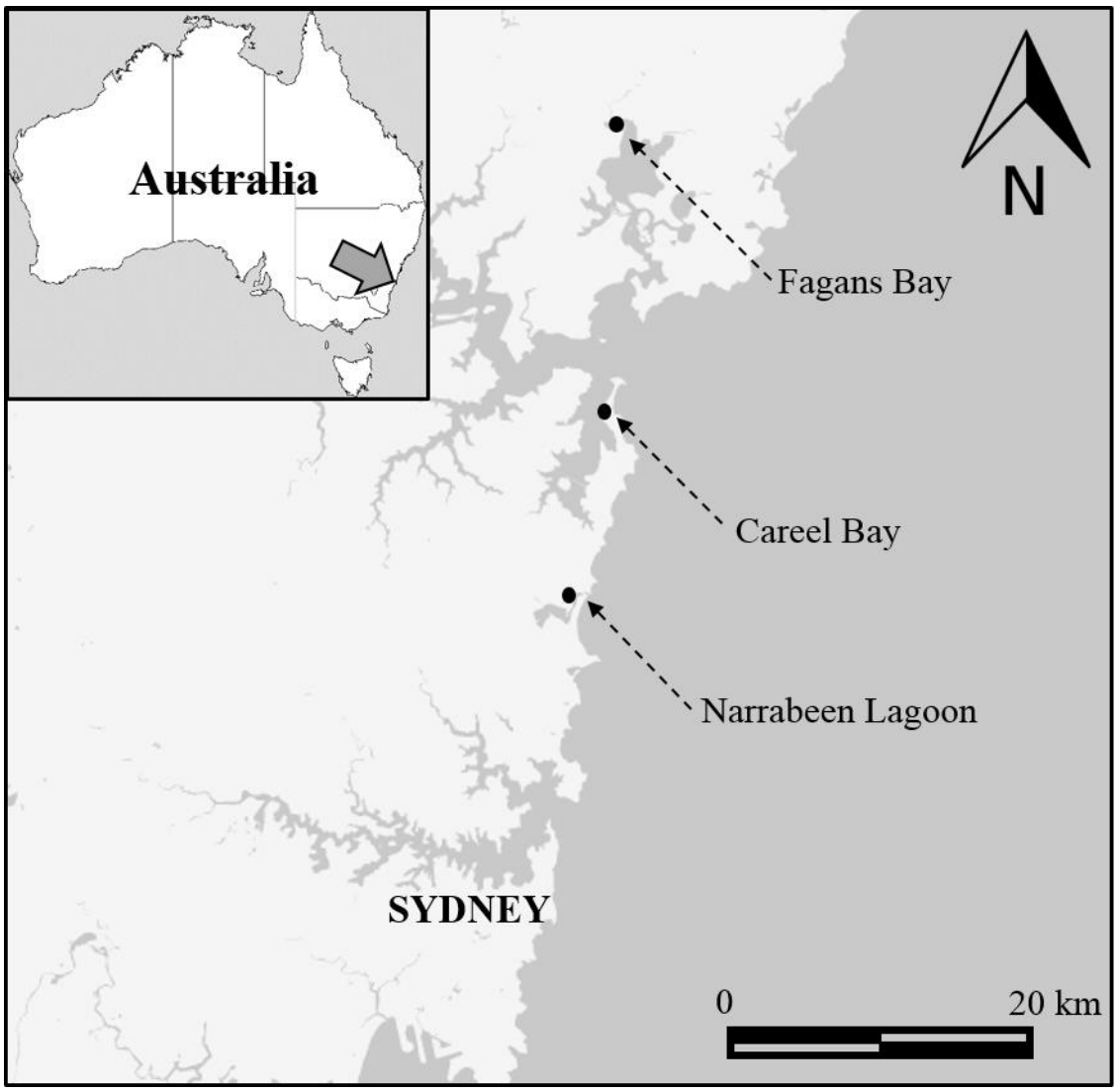


Fig.1: Map of the Central East Coast of New South Wales (NSW), showing the locations of the three sampling sites (Careel Bay, Narrabeen Lagoon, and Fagans Bay Lagoon) in relation to the NSW capital of Sydney.

Isotopic labelling of seagrass detritus

Epiphytes were gently removed from seagrass leaves, and plants were rinsed in the laboratory with artificial seawater (salinity 32) 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

196 and incubated for 72 hours under light (150 μmol photons m^{-2} s^{-1}); with 12:12 hour day and
197 night light cycles. Plants were then removed from the solution, and thoroughly rinsed with
198 distilled water to remove salts and excess label. This process and enrichment time likely
199 concentrated enrichment in the labile compounds (e.g. soluble carbohydrates) within the
200 seagrass leaves (Kaldy et al. 2013), however given the short time of this study (65 days),
201 decomposition was most likely linked primarily to these compounds. Leaves were isolated
202 from roots and rhizomes, and chopped into uniform pieces < 15 mm. The prepared leaves
203 were then separated into 4.8 g portions, and each portion was left to partially degrade in
204 artificial seawater (salinity 32) for a further 72 hours. Subsamples of both the labelled
205 seagrass (n = 3), and unlabelled seagrass (n = 3) were analysed for $\delta^{13}C$ and organic carbon
206 content on an Isotope Ratio Mass Spectrometer (Thermo-Finnegan Delta V IRMS), and
207 elemental analyser (Costech Elemental Analyser), respectively.

208 **Experimental set-up**

209 A laboratory experiment was carried out for 65 days to quantify the effect of Callianassid
210 bioturbation on seagrass degradation and sediment CO_2 fluxes. Four treatments with five
211 replicates each were established, consisting of bioturbated and control (no bioturbators added)
212 sediment mesocosms, with (amended) and without (unamended) the addition of ^{13}C-labelled
213 seagrass leaves. Sediment cylinders (referred to as “mesocosms”) were prepared by filling
214 sediment into 20 acrylic (length = 30 cm; diameter = 8 cm) tubes, to a depth of 22 cm, which
215 were sealed watertight (from the bottom) with rubber caps. The constructed sediment
216 mesocosms were left to settle overnight (approximately 16 hours) at ambient temperature
217 (approximately 22°C).

The following day, 250 g sediment, corresponding to a 4 cm layer, was added to control mesocosms (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 mesocosms as a 2 cm thick layer, thereafter an additional 2 cm of unamended sediment was added. All sediment mesocosms were left to settle for 12 hours, so that the final sediment depth was 24 cm. Each mesocosm was then topped up with additional artificial seawater, and all 20 sediment mesocosms were transferred to four 90 L seawater tanks filled with artificial seawater (salinity 32), with at least 5 cm of water above each mesocosm. Four tanks were used, to make sure all five replicates of the four treatments could be kept together in one tank to avoid faunal migration or contamination with ^{13}C. Each tank was fitted with two air stones for aeration and mixture of the surface water within tanks and mesocosms. All tanks were covered and kept in the dark inside the laboratory at a constant 22°C (reflecting the average yearly water temperature for the Sydney region of 18 – 23°C) 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 mesocosm (10 mesocosms in total, 1 individual in each 0.005 m^2 mesocosm, corresponding to 0.6 – 0.7 g total wet weight). Given the recorded natural densities of Callianassids, we suggest that this study represents an extreme case of bioturbation, such as that seen in the high densities observed in the field survey portion of this study. The animals were allowed to construct burrows for 24 hours before the first flux incubation was initiated on day 2 of the experiment. Mesocosms were discarded if Callianassids were not viable, with a final n = 3 (in mesocosms with bioturbation, amended with seagrass); n = 4 (in mesocosms with bioturbation, control sediment); and with the remaining treatments n = 5 at the conclusion of the 65 day experiment.

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 mesocosms, 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. During the incubation period, individual Callianassids were held in darkness, and were observed to be moderately active. 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. The resulting flux values for Callianassid metabolism were then subtracted from the relevant mesocosm fluxes.

Sediment metabolism

Benthic fluxes of O_2 and CO_2 (dissolved inorganic C or DIC concentrations) were determined before and after Callianassids were added to mesocosms (days 2, 4, 7, 15, 21, 28, 35, 42, 49, 56, and 63). Mesocosms were sealed with gas-tight rubber caps, and water circulation was ensured with stirring magnets (1.5 cm long) fitted to the mesocosms, and driven by a rotating external magnet (~ 60 rpm). Stirring magnets were positioned in the middle of the water column (approximately 4 cm from the sediment surface) to prevent any sediment resuspension. All mesocosms were incubated in darkness within their respective tanks 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 by inserting a Firesting Optical Microsensor, with a 100 µm retractable tip, connected to an Optical Oxygen Meter (FireSting, Pyro Science, Denmark), directly into the overlying mesocosm water. The exchange of O_2 (mmol

m^{-2} d^{-1}) between sediment and water was calculated from changes in overlying mesocosm water concentrations during incubations, the incubation time, volume of overlying water within each mesocosm, and surface area of the respective mesocosm 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, transferred to 40 mL gas tight glass vials and preserved with saturated $HgCl_2$ (300 µl). Total CO_2 (measured as change in DIC, which also includes seawater carbonate components, HCO_3 and CO_3^{-2}) samples were stored in darkness at 5°C and analysed with a DeltaV Infared Mass Spectrometer (IRMS) with a precision of < 1‰, coupled to an OI TOC analyser (Maher and Eyre 2011b).

An isotope mass balance model was used to determine the contributions of sediment and seagrass DIC (CO_2) effluxed into the overlying water (Maher and Eyre 2011b). Over the course of this study, the $\delta^{13}C$ value of fresh unlabelled seagrass was not observed within DIC samples. Fresh unlabelled seagrass was therefore excluded from remineralisation calculations. The $\delta^{13}C$ values of accumulated DIC (represented below as x) were calculated by:

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

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 $\delta^{13}C$ values of both the seagrass (established via IRMS analysis of freshly

labelled seagrass subsamples) and the sediment (established via IRMS analysis of fresh sediment subsamples), 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:

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

The $\delta^{13}C$ measured represents the calculated $\delta^{13}C$ value 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.

Microbial priming effect (MPE) estimations were calculated based on the MPE equations given in 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 and metabolism) 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 MPE in relevant treatments was calculated using the equation below (Kuzyakov et al. 2000; Trevathan-Tackett et al. 2017), with CO_2 representing the calculated rate of remineralisation, and Total Sediment CO_2 representing the total sediment-derived CO_2 flux from respective treatments:

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

307 The sediment-associated CO_2 release from control (no bioturbators added sediment was added
308 to the calculated Callianassid interaction and both were subtracted from the measured rate of
309 sediment C remineralisation in sediment containing both seagrass and Callianassids. Any
310 additional CO_2 released was attributed to the MPE.

Sediment characteristics – measurement of organic carbon content

Upon completion of the experiment, the sediment within each mesocosm was sectioned into 1 cm intervals to 5 cm depth, 2 cm intervals to 17 cm depth, and two 3 cm intervals to the bottom of the mesocosm. 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 mesocosm depth was homogenised before analysis. 4 ml subsamples of sediment for gravimetric analysis were taken with a cut-off syringe. Samples were transferred to aluminium trays, and the wet weight of each sample was recorded. Samples were dried at 60° C for 24 hours and ground with a mortar and pestle, and sub-samples of each section (0.5 g each) were acidified with HCl overnight at room temperature. 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 (supplementary information) was measured using high temperature (950°C) combustion (Costech Elemental Analyser).

Recovery of buried seagrass detritus from sediment mesocosms

All large pieces of particulate detritus > 0.5 mm were recovered from each sediment slice of each mesocosm and the extent of degradation, quantity, and distribution of seagrass material remaining in the sediment at the end of the experiment was 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, visually assessed, and then dried at 60 °C for 24 hours. Seagrass detritus taken from each sediment depth was then re-weighed, and all material recovered from within each mesocosm was pooled. The dried detritus (n = 5, Initial and Control; and n = 3, + Bioturbation) 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 mesocosm 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.

Statistical analysis

First, a one-way Analysis of Variance (ANOVA) was used to test the impact of Callianassid bioturbation (independent variable) on the degradation of the seagrass detritus (dependent variables: recovered detritus OM composition and total biomass recovery). Next, two-way ANOVAs were used to analyse the effects of fauna and seagrass addition (independent) on total mesocosm sediment C_{org} (g) content (dependent). Lastly, two-way ANOVAs tested the effect of faunal and seagrass addition (independent) on the time integrated release of net CO_2 release (dependent) and CO_2 flux over time (dependent with repeated measures).

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

357 significantly different interaction. All tests were performed with a significance level of α =

358 0.05, using IBM SPSS Statistics (Ver. 22).

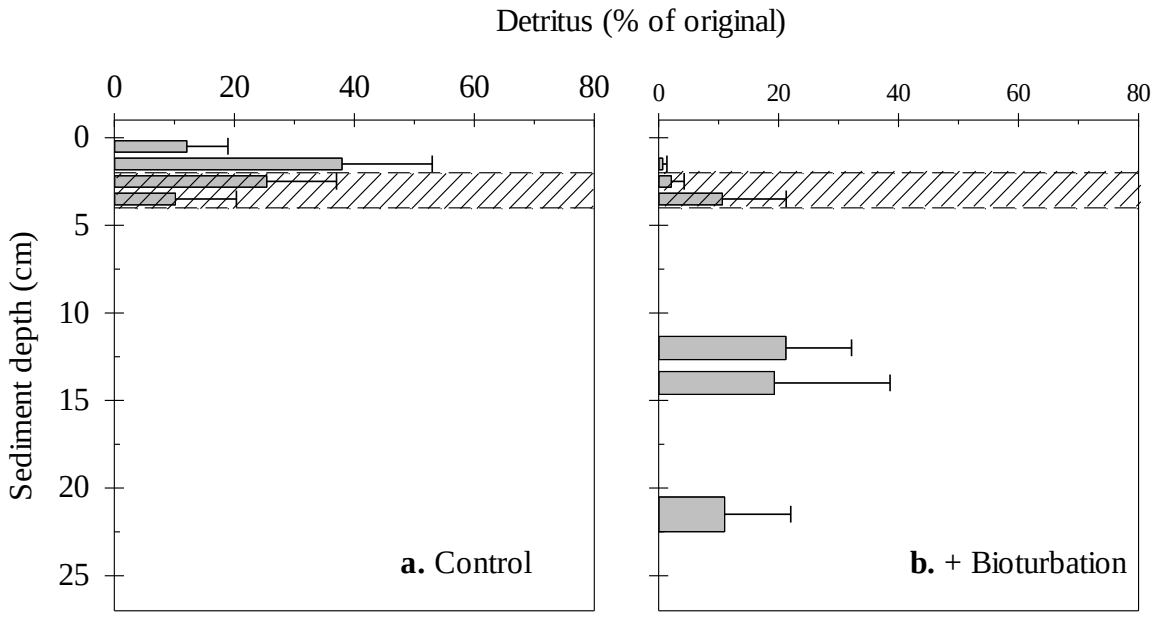
Results

Recovery of seagrass detritus (> 0.5 mm)

The initial sediment and seagrass detritus C_{org} content was 1.18 ± 0.09 % and 30.14 ± 1.11% dry weight (DW), respectively. After the ^{13}C labelling, the seagrass C_{org} content was 31.11 ± 0.23% DW.

Approximately 79% (± 5.51%) of the original detritus buried in control sediment was recovered after 65 days (Fig. 2a), corresponding to approximately 29.1 g C m^{-2}. However, recovery of seagrass in bioturbated sediment was significantly lower ($F_{1, 4}$ = 13.031, p = 0.023), with a total of 65% ± 2.55% of the original seagrass detritus recovered (Fig. 1b), representing approximately 20.5 g C m^{-2}. There was no observable visual difference in the seagrass detritus between the bioturbated and control sediments; specifically, there were no bite marks or signs of consumption on the detritus recovered from bioturbated sediments. Compared to the original detritus burial depth (2 – 3 cm; Fig. 2a), seagrass detritus in the control sediment appeared at a reduced depth relative to the sediment surface due to compression, while bioturbation by Callianassids shifted the distribution of seagrass to approximately 20 cm deeper (maximum depth of 23 cm; Fig. 2b).

All treatments exposed to bioturbation were found to have deposited sediment on the sediment surface of all mesocosms, equating to, on average, an additional 0.81 cm (± 0.80) of deposited sediment. Furthermore, assuming the total burrow length (including offshoot tunnels) was no more than 52 cm and burrow width an average of 0.75 cm, we conservatively estimate that the Callianassid burrows increased the original 50.3 cm^2 mesocosm sediment surface area in each mesocosm by up to 105 cm^2 (representing more than a 200% increase).



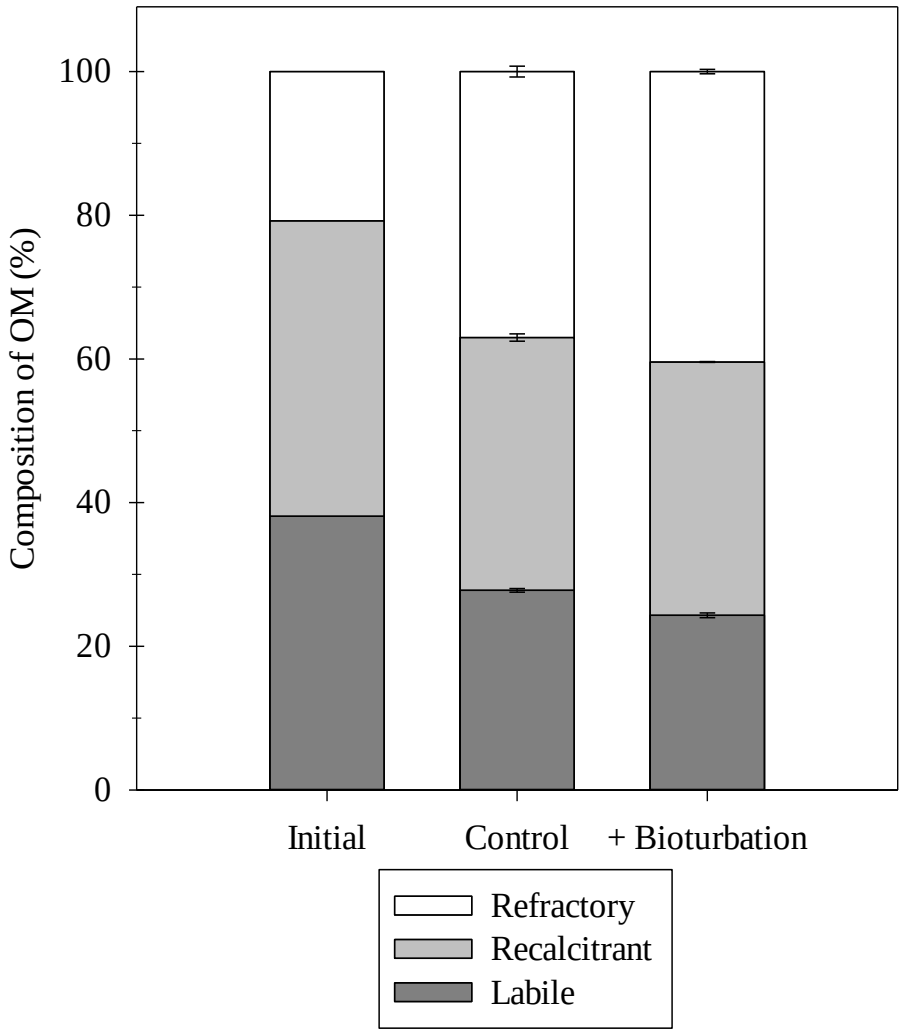
381

382 Fig. 2: Vertical profiles of seagrass detritus recovery in Control (no bioturbators added)
383 sediment mesocosms (a), and mesocosms with the Callianassid bioturbation + Bioturbation
384 (b). Column bar width is indicative of the size of the sediment portion sampled. The dashed
385 reference line (2 cm – 4 cm depth) indicates the original burial depth of seagrass detritus.
386 Error bars: SE. N = 5 (Control); and n = 3 (+ Bioturbation).

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 ($F_{1, 4} = 21.54$, $p = 0.01$; Fig. 3). The total organic matter (OM) of the seagrasses were 49.44 ± 8.71%, 17.84 ± 1.76% and 15.48 ± 1.80% for the initial, Control and + Bioturbation samples, respectively. The remaining biomass (> 600 °C) consisted of inorganics and ash/char.

There was no difference in the proportion of cellulose-associated OM (recalcitrant OM; $F_{1, 4} = 0.668$, $p = 0.46$) or lignin-associated OM (refractory OM; $F_{1, 4} = 2.333$, $p = 0.202$) in recovered detritus between bioturbated and control sediment.



397

398 Fig. 3: Organic matter composition of seagrass (*Zostera muelleri*) leaf detritus, determined
399 via thermogravimetric analysis. Specifically, the percentage of labile OM (mass lost between
400 200 – 300 °C), recalcitrant (mass lost between 300 – 400 °C), and refractory OM (mass lost
401 between 400 – 600 °C), in seagrass leaves fresh from the field (initial), recovered from
402 control sediment (Control), and sediment populated with Callianassids (+ Bioturbation), is
403 shown. The percentage of labile, refractory and recalcitrant OM are presented as a percent of
404 total OM. N = 5 (Initial and Control); and n = 3 (+ Bioturbation). Bars represent means ± 1
405 SE. In some places error bars are too small to be visible.

406 **Sediment metabolism**

407 The addition of Callianassids had a clear impact on the O_2 uptake by the sediment (Fig. 4a,
408 4b), and TCO_2 release (Fig. 4c, 4d). The addition of Callianassids increased sediment O_2
409 demand consistently, with consumption of O_2 approximately 1.5 – 4 times that of control
410 sediment over time.

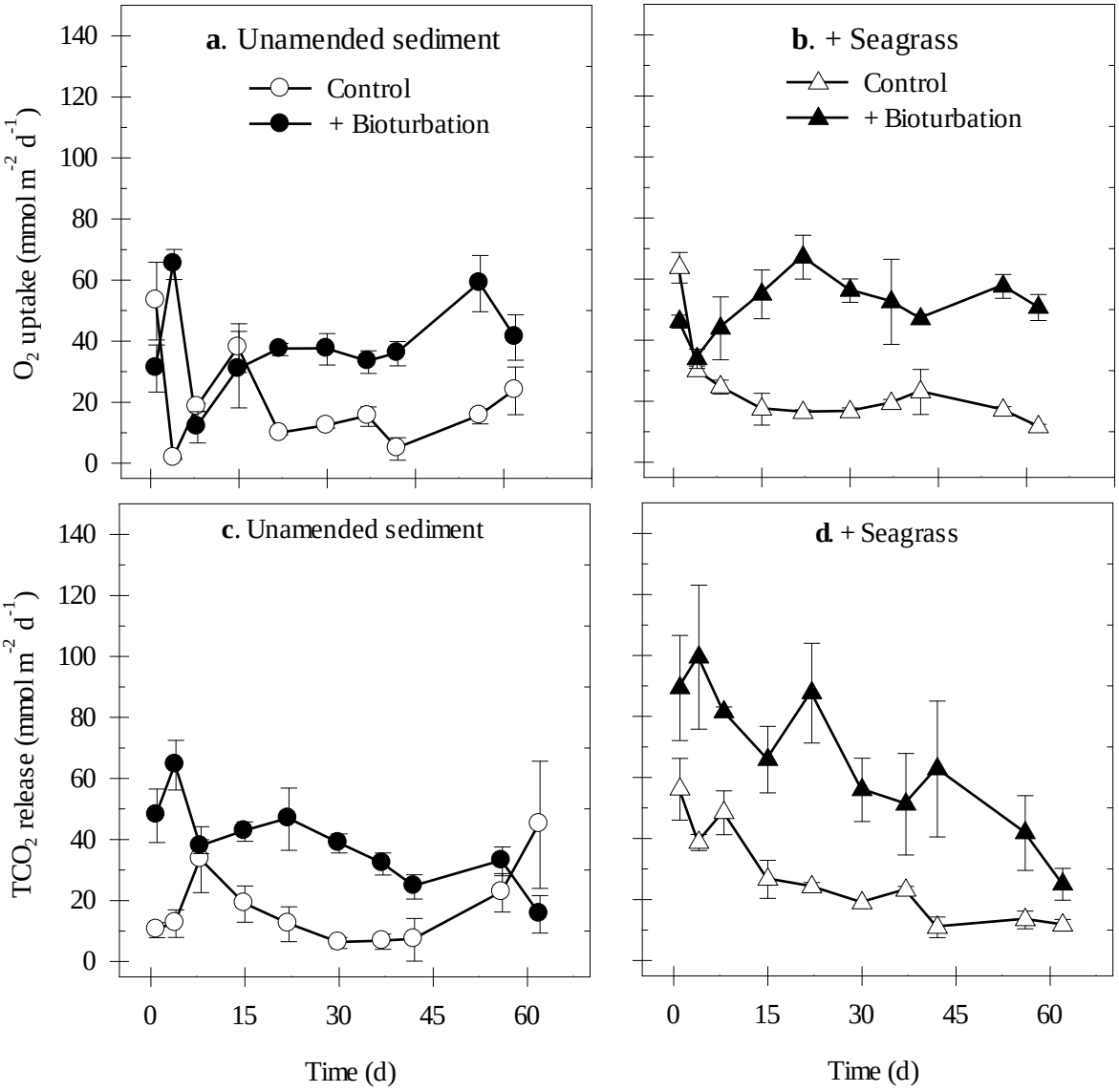
411 O_2 uptake was initially similar at about 53 – 64 mmol m^{-2} d^{-1} in the control unamended
412 sediment and control sediment containing seagrass. Sediment containing seagrass displayed
413 relatively consistent O_2 consumption over time, with an uptake rate of 46 – 56 mmol m^{-2} d^{-1} in
414 sediment populated with Callianassids, and 33 – 41 mmol m^{-2} d^{-1} in control sediment. O_2
415 uptake in unamended sediment was initially variable, and stabilised after ~ 21 days. After this
416 time, unamended sediment populated with Callianassids consistently had an O_2 uptake rate
417 higher than that of control (no bioturbators added) sediment (as described in the previous
418 paragraph).

419 Significantly more CO_2 was released from the sediment populated with Callianassids ($F_{1, 4}$ =
420 23.972, p = 0.039), compared to control sediment. The release of CO_2 in both Callianassid
421 treatments was double that of control (no bioturbators added) sediment. Callianassid
422 bioturbation had a significant interaction with CO_2 release over time ($F_{9, 1}$ = 2.646, p = 0.038).
423 The presence of seagrass also led to an increase in sediment CO_2 release ($F_{1, 2}$ = 43.335, p =
424 0.021), however the impact of seagrass enrichment did not have a significantly sustained
425 interaction over time ($F_{1, 10}$ = 1.345, p = 0.283).

426 In sediment containing seagrass, bioturbation by Callianassids lead to a 1.6 – 5.8 time
427 increase in CO_2 release. A consistent decline in CO_2 release was observed in both bioturbated
428 and control sediments throughout the experimental period. CO_2 release in unamended

sediments was variable, with release in sediment populated with Callianassids being 1.1 – 6.4 times that of control sediment, until the last measurement.

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



434

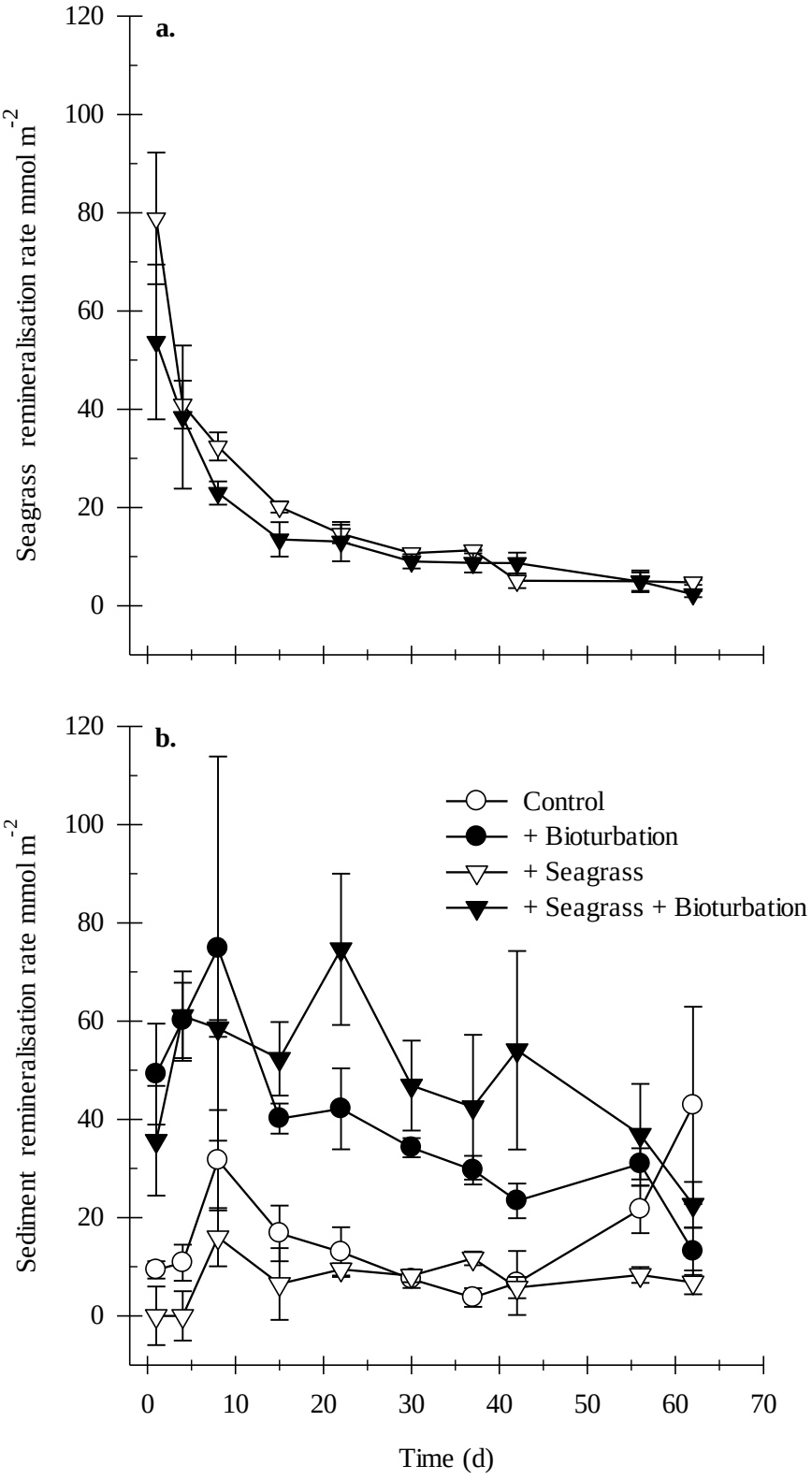
435 Fig. 4: Total oxygen (O_2; Fig. 4a, b) influxes, and carbon dioxide (CO_2; Fig. 4c, d) effluxes
436 over time in sediment mesocosms with (+ seagrass) and without (unamended sediment)
437 seagrass detritus ($mmol^{-2}$ d^{-1}). The two curves in each graph show efflux profiles in sediment
438 mesocosms with (+ Bioturbation) and without (Control) Callianassid bioturbation. Values
439 represent means ± SE. N = 3 (+ Seagrass, + Bioturbation; and + Seagrass); n = 4 (Unamended
440 sediment, + Bioturbation); and n = 5 (Control).

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. 5a, 5b). Significantly more sediment organic matter (OM) was remineralised in treatments with bioturbation by Callianassids ($F_{1, 15}$ = 351.071, p = 0.003): the rate of sediment remineralisation was 2 – 5 fold greater than in control sediment (Fig. 5b). Bioturbation by Callianassids had a significant interaction with sediment remineralisation over time ($F_{9, 1}$ = 139.047, p = 0.007). The presence of seagrass detritus also had a significant impact on sediment remineralisation ($F_{1, 10}$ = 28.958, p = 0.033), with the average rate of sediment remineralisation in control treatments and in sediment amended with seagrass calculated to be 17.2 ±1.1 and 8.2 ± 1.3 mmol m^{-2} d^{-1}, respectively. These remineralisation rates represent an approximately 45% lower sediment remineralisation rate in control sediment amended with seagrass compared to control sediment; however, the impact of seagrass addition did not have a significantly sustained interaction over time ($F_{9, 1}$ = 1.638, p = 0.166).

There were no differences in the rates of seagrass remineralisation ($F_{1, 15}$ = 0.239, p = 0.673) in both control and bioturbated sediments (Fig. 5a). Rates declined steadily for the first 3 weeks of the study, and became stable after the first month (~ 7 mmol m^{-2} d^{-1}).

A significant interaction was observed between sediment populated with Callianassids and sediment amended with seagrass. Both conditions showed a significantly increased (1.3 – 6-fold greater) rate of sediment remineralisation (recalcitrant organic matter; ROM) compared to all other conditions ($F_{9, 1}$ = 25.436, p = 0.037).



462

463 Fig. 5: Total calculated (a) seagrass and (b) sediment remineralisation rates in sediment

464 mesocosms amended with seagrass (+ Seagrass), and without seagrass. The curves in the top

graph (a) show seagrass remineralisation calculated from $\delta^{13}C$ values measured in respective sediment mesocosms with (+ Bioturbation; black symbols) and without (Control; white symbols) Callianassid bioturbation. Curves in graph (b) reflect sediment remineralisation calculated from $\delta^{13}C$ values measured in all treatments. Values represent means ± SE. N = 3 (+ Seagrass, + Bioturbation); n = 4 (+ Bioturbation); and n = 5 (Control, and + Seagrass treatments).

Estimated microbial priming effect

The addition of LOC and/or Callianassid bioturbators to sediments amended with seagrass caused differential CO_2 release based on the OM source (Fig. 6). In sediment exposed to Callianassid bioturbation, an increased rate of sediment-based C release was observed. 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 proportion of which was estimated to be attributed to Callianassid metabolism), a microbial priming effect (MPE) was observed in bioturbated sediments which contained seagrass. Using integrated daily rates of CO_2 remineralisation, the calculated rates of the microbial priming effect (MPE) indicate that treatments amended with seagrass, and exposed to Callianassid bioturbation, were responsible for a MPE that was 15% that of the total C released in these sediments. A negative MPE (preferential degradation of a more labile source of C, i.e. seagrass, over a more recalcitrant source of C, 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 MPE was observed in control sediment amended with seagrass: the sediment-derived CO_2 in amended sediment was less than half that observed in unamended (control) sediment.

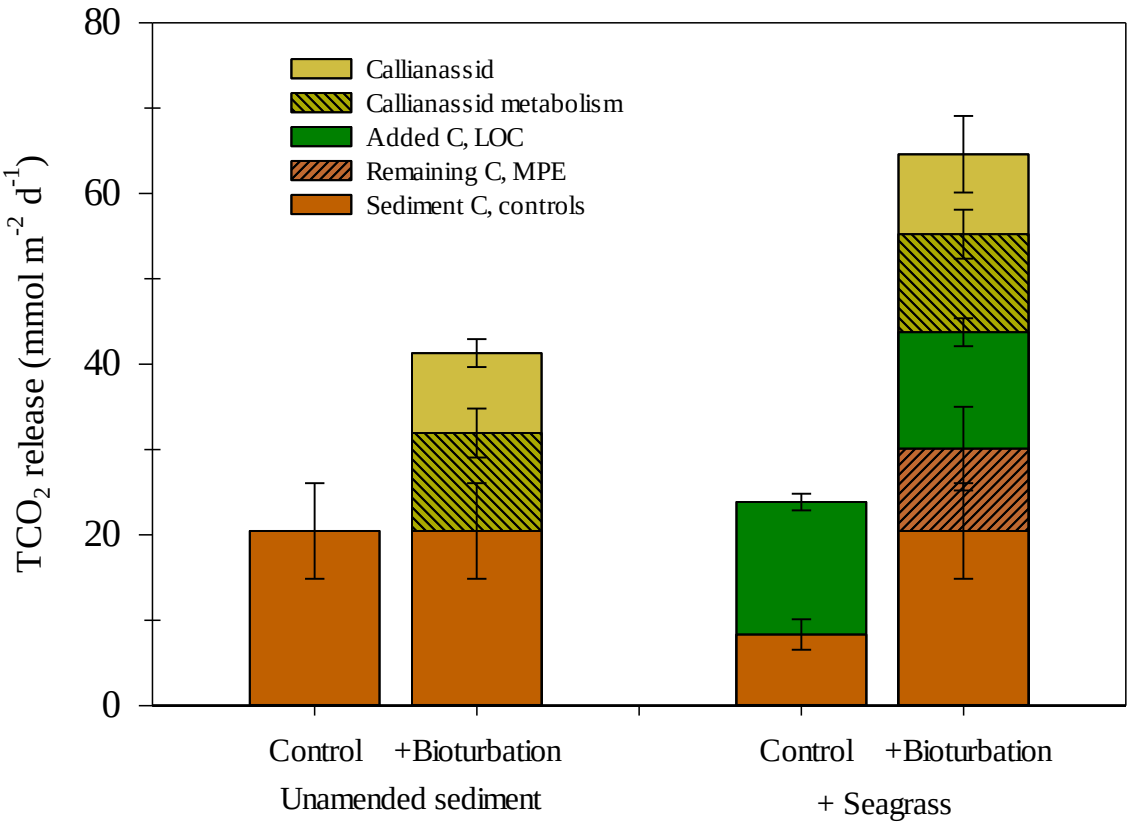


Fig. 6: Estimated contributions of labile organic matter (LOC; seagrass) and recalcitrant organic matter (sediment C) sources to respiration. The contribution of Bioturbation and Seagrass (+ Seagrass; LOC amendment) to microbial priming (MPE) is compared. The fraction of sediment C and added C (seagrass; LOC) contribution to the total CO_2 flux was calculated from stable isotope values using a 2-end member mixing model. The portion of TCO_2 release attributed to Callianassid metabolism (Callianassid metabolism), and the portion of TCO_2 release attributed to the effect of Callianassid activity (Callianassid) including burrow construction was estimated based on measured rates of Callianassid CO_2 flux. Values represent means ±SE. N = 3 (+ Seagrass, + Bioturbation); n = 4 (Unamended sediment, + Bioturbation); and n = 5 (Control treatments).

Discussion

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

Burial and degradation of seagrass leaf detritus

Burial of seagrass leaf detritus was observed in bioturbated sediments, resulting in the detritus being distributed an additional 2 – 18 cm into the sediment. Rapid burial of seagrass detritus has been observed due to the activity of another Callianassid 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 led to an overall decrease in recovery of seagrass leaf detritus within the sediment, but it was noted that a higher proportion of the labile fraction of organic matter (OM) was lost from detritus buried in bioturbated sediments. Due to the short labelling time within this study, the primary source of enriched ^{13}C-CO_2 is likely from labile and some recalcitrant compounds of the seagrass detritus. It is possible that the observed decreasing rate of seagrass remineralisation was likely due to this loss of seagrass labile OM (proteins, soluble carbohydrates, etc.). The $\delta^{13}C$ values of the seagrass recovered from the mesocosms were not

521 measured in this study and limits our ability to produce a mass balance for ^{13}C. While it is
522 typical for isotope mixing models to use fresh substrate for end-members, including post-
523 decay isotope values in the calculations would have provided more accurate estimates of the
524 changes in the isotope values during decomposition (Trevathan-Tackett et al. 2017;
525 Trevathan-Tackett et al. 2018). Loss of seagrass OM content of up to 1% d^{-1} (Harrison and
526 Chan 1980) can be expected from leaf material, and is comparable to the rates of decay
527 observed in this study, which were ~ 0.95 – 1.01% d^{-1}. Cellulose and lignin compounds within
528 seagrass detritus generally reduce the rates of microbial degradation, and retention of seagrass
529 detritus in the sediment at the end of our two-month study (which largely consisted of
530 recalcitrant and refractory seagrass OM) suggests that there is evidence for future seagrass-
531 derived C input and replenishment of sediment C stocks. Although we were unable to detect a
532 significant impact of bioturbation on sediment C_{org} content (supplementary material), we
533 reaffirm that bioturbation did influence sediment remineralisation based on the rates of
534 sediment remineralisation measured via overlying water CO_2 content. We suggest that we
535 were unable to measure the extent of the effect within the sediment using the methods
536 employed within the timeframe of this study.

537 **Sediment fluxes**

538 The addition of seagrass detritus increased sediment O_2 demand by 26%, while bioturbation
539 by Callianassids effectively doubled sediment oxygen consumption (increased by 130%). In
540 sediments containing both Callianassids and seagrass, O_2 consumption was enhanced
541 substantially compared to control sediment. High sediment O_2 uptake rates between 40 and
542 144 mmol $m^{-2}d$ have been reported in sediment populated with Callianassids (Eyre et al.
543 2011; Maher and Eyre 2011a; Maher and Eyre 2011b; Webb and Eyre 2004). Callianassids
544 can irrigate their burrows at a rate of ~0.5 l h^{-1} and increase sediment O_2 substantially, most of

545 which is used in microbial and biogeochemical processes (Webb and Eyre 2004). Faunal
546 stimulation of sediment metabolism also causes an increased rate of CO_2 efflux, largely due to
547 increases in sediment surface area (burrow), and further stimulates microbial and
548 biogeochemical activity (Banta et al. 1999; Kristensen 2000; Webb and Eyre 2004).
549 Furthermore, deposition of sediment onto the sediment surface and an increase in sediment
550 surface area due to burrow creation and maintenance, will further stimulate OM
551 remineralisation (Heilskov and Holmer 2001). Our results indicate that Callianassid
552 bioturbation is responsible for up to 50% of the total CO_2 release; however, given that
553 metabolism of the fauna alone is responsible for ~20% of the total CO_2 release, it can be
554 elicited that the remaining ~30% is due to increased sediment surface area via burrow
555 formation, resulting in a stimulation of microbial metabolism. However, due to changes in
556 Callianassid activity and resulting metabolism, the portion of CO_2 flux that Callianassid
557 metabolism alone is responsible for may be underestimated.

558 Similar increases in CO_2 efflux have been reported for Axiidean bioturbation in the presence
559 of seagrass (Papaspyrou et al. 2004), and resulted in a net overall increase in C
560 remineralisation with the introduction of bioturbation.

561 **Potential microbial priming effect**

562 By definition, MPE occurs when moderate changes or disruptions in environmental
563 conditions stimulate microbes to metabolise stored or 'stable' sediment C (Kuzyakov et al.
564 2000). These changes may arise due to decomposition of OM by microbes, which is an
565 essential part of the MPE; however priming can also manifest from physical disturbance, such
566 as that observed as a result of agricultural tilling (Bell et al. 2003). Due to the observed
567 increase in sediment-derived CO_2 flux after the addition of seagrass detritus, we believe that

568 there were two pathways that resulted in a MPE; the observed microbial decomposition of
569 seagrass, and the physical disturbance caused by Callianassid bioturbation. More recent
570 studies have identified an MPE with addition of seagrass and microalgae to sediments
571 (Trevathan-Tackett et al. 2017), but we believe this study is an important contribution to the
572 literature because we were able to trace the source of CO_2 flux, while highlighting the
573 potential of bioturbation to further stimulate this priming process and affect seagrass C
574 sequestration.

575 In control sediment amended with seagrass, the observed potential negative MPE was
576 equivalent to a >50% reduction in ROM-derived CO_2 release, indicating a preferential
577 remineralisation of seagrass (LOM) by the microbial community (Gontikaki et al. 2013).
578 Negative priming in seagrass sediments may indeed support the long-term preservation of
579 sediment C_{org} with the microbial community preferentially remineralising labile (i.e. proteins,
580 hemicellulose and soluble carbohydrates; Trevathan-Tackett et al. 2015) and some recalcitrant
581 lignocellulose seagrass compounds (Gontikaki et al. 2015), over the sediment-bound C_{org}.
582 However, the extent of the MPE is also related to the added substrate C as a proportion of the
583 microbial biomass (Blagodatskaya and Kuzyakov 2008), and some of this calculated negative
584 MPE may also be attributed to changes in redox conditions within the sediment, resulting in
585 temporarily higher remineralisation rates (Burdige 2007). Once available C has been depleted
586 and subsequent microbial activity has subsided, remaining C may be more resistant to
587 degradation (Blagodatskaya and Kuzyakov 2008). Comparing the amount of sediment C_{org}
588 remineralised within bioturbated sediment to that remineralised in control sediment, it is clear
589 that this negative MPE is negated by the bioturbator-stimulated MPE.

590 We estimate that the MPE may be responsible for up to 15% of the total CO_2 output in
591 bioturbated sediment amended with seagrass. Within this treatment bioturbation (and

associated bioirrigation) by Callianassids lead to a lower rate of seagrass remineralisation (12%), which was likely due to detrital burial. The sediments in this treatment also displayed a significantly higher rate of sediment remineralisation, however, underestimation of Callianassid metabolism, and remineralisation of unlabelled seagrass fractions (i.e. some recalcitrant and refractory compounds) may be responsible for some of the CO_2 flux attributed to sediment remineralisation. Although identified within marine systems (Aller 1994; Guenet et al. 2010; López et al. 1998), in terrestrial systems MPEs have been further investigated and suggested to be 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 (Fontaine et al. 2007; Fontaine et al. 2003; Kuzyakov et al. 2000). The results of this study support that this process can 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 scenario, the measured MPE and resulting CO_2 release was largely stimulated by microbial activity attributed to bioturbation (and associated bioirrigation) by Callianassids.

Potential loss of C stocks

Assuming that in ambient conditions, the rate of C burial is reflective of control (no bioturbators added) sediment, the release of CO_2 into the water column will range between 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}), which is similar to increases observed when studying other Callianassid species (Papaspyrou et al. 2004; Webb and Eyre 2004). While this study represents a case of extreme bioturbation (> 200 Callianassids per m^{-2}), extrapolating from the rates of MPE and resulting sediment C loss observed in this study attributed to bioturbation, even a conservative estimate of one bioturbator burrow per m^{-2} may

induce a MPE equivalent to 1.6 g C m^{-2} y^{-1}, a large (approximately 16%) 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 geographic area of this study are populated by 2 – 248 (with an average of 36) Callianassid burrows per m^{-2}, representing a potential for a much larger loss of C and ultimately a net loss of sediment C. This has implications for 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, in effect, leak CO_2, an increase in bioturbator populations could decrease seagrass (and other) blue carbon stocks (Atwood et al. 2015). Changes to the natural predatory trophic cascades within seagrass meadows, i.e. loss of meso-predators, could result in increases to bioturbator populations (Atwood et al. 2015). In ecosystems where meso-predators have been excluded from within seagrass habitats, macro-invertebrate abundance has been seen to increase 3 – 10 times compared to their natural density (Lewis and Anderson 2012). We suggest that in these situations, seagrass C sequestration would be negatively affected, whereby sediment C is remineralised via bioturbator-stimulated MPE. Conversely, decreases in bioturbator populations could increase C stocks and sequestration in some instances, especially in environments where C sequestration is reduced by faunal-induced MPE.

Scenarios for microbial priming in bioturbator populated seagrass environments

The potential of bioturbators to effectively facilitate microbial priming warrants further investigation. This study is restricted in its estimates of seagrass sediment carbon stocks due to the use of above-ground seagrass tissue (leaves), rather than total plant biomass (including both above- and below-ground tissue). It is also important to consider that in many seagrass meadows, much of the above-ground seagrass biomass is exported (Duarte and Krause-Jensen 2017). To ensure a complete assessment of seagrass carbon stocks, we would encourage

further research to encompass the full range of remineralisation by-products, including DOC and porewater analysis, as well as incorporating below-ground biomass (i.e. roots and rhizomes) as a contributor to C sequestration. Further investigation on different macrofauna species (i.e. representing different activity) will allow us to make predictions of the vulnerability of seagrass sediment C stocks.

Based on the results of this study, we present a scenario whereby seagrass sediment C stocks 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 (Trevathan-Tackett et al. 2017), changes to population structure and density 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 sediment C stock longevity. We suggest that bioturbator-stimulated priming could have a considerable effect on C sequestration and persistence in coastal ecosystems (Guenet et al. 2010).

Acknowledgements

For technical and field assistance we thank Graeme Poleweski and Paul Brooks from the University of Technology Sydney. For providing comments and feedback on the manuscript, we thank Sabina Belli from the University of Technology Sydney. For assistance with experimental concepts, processing of samples, and providing insightful comments, we thank Professor Isaac Santos from Southern Cross University. We also thank the many volunteers from the University of Technology Sydney for assistance in both the field and the laboratory.

We acknowledge the support of an Australian Research Council (ARC) Discovery Early Career Researcher Award DE130101084 (PIM), and an Australian Postgraduate Award (ACGT). This project was undertaken as a part of the CSIRO Marine and Coastal Carbon Biogeochemistry Cluster (Coastal Carbon Cluster) initiative.

References

Aller, R. C. 1983. The importance of the diffusive permeability of animal burrow linings in determining marine sediment chemistry. Journal of Marine Research **41:** 299-322.

---. 1994. Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. Chemical Geology **114:** 331-345.

Atwood, T. B. and others 2015. Predators help protect carbon stocks in blue carbon ecosystems. Nature Climate Change **5:** 1038-1045.

Banta, G. T., M. Holmer, M. H. Jensen, and E. Kristensen. 1999. Effects of two polychaete worms, *Nereis diversicolor* and *Arenicola marina*, on aerobic and anaerobic decomposition in a sandy marine sediment. Aquatic Microbial Ecology **19:** 189-204.

Bell, J. M., J. L. Smith, V. L. Bailey, and H. Bolton. 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils **37:** 237-244.

Berkenbusch, K., and A. Rowden. 2003. Ecosystem engineering—moving away from 'just-so'stories. New Zealand Journal of Ecology**:** 67-73.

Berkenbusch, K., A. A. Rowden, and T. E. Myers. 2007. Interactions between seagrasses and burrowing ghost shrimps and their influence on infaunal assemblages. Journal of Experimental Marine Biology and Ecology **341:** 70-84.

Bianchi, T. S. 2011. The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. Proceedings of the National Academy of Sciences **108:** 19473-19481.

Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils **45:** 115-131.

Blum, L., and A. Mills. 1991. Microbial growth and activity during the initial stages of seagrass decomposition. Marine Ecology Progress Series**:** 73-82.

692 Boon, P. I., F. L. Bird, and S. E. Bunn. 1997. Diet of the intertidal callianassid shrimps
693 *Biffarius arenosus* and *Trypea australiensis* (Decapoda: Thalassinidea) in Western
694 Port (southern Australia), determined with multiple stable-isotope analyses. Marine
695 and Freshwater Research **48:** 503-511.
696 Burdige, D. J. 2007. Preservation of organic matter in marine sediments: controls,
697 mechanisms, and an imbalance in sediment organic carbon budgets? Chemical
698 Reviews **107:** 467-485.
699 Butler, S. N., and F. L. Bird. 2008. Temporal changes in burrow structure of the
700 thalassinidean ghost shrimps *Trypaea australiensis* and *Biffarius arenosus*. Journal of
701 Natural History **42:** 2041-2062.
702 Butler, S. N., M. Reid, and F. L. Bird. 2009. Population biology of the ghost shrimps,
703 *Trypaea australiensis* and *Biffarius arenosus* (Decapoda: Thalassinidea), in Western
704 Port, Victoria. Memoirs of Museum Victoria **66:** 43-59.
705 DeWitt, T. H. 2009. 10.0 The Effects of Bioturbation and Bioirrigation on Seagrasses.
706 Seagrasses and Protective Criteria: A Review and Assessment of Research Status.
707 Donato, D. C., J. B. Kauffman, D. Murdiyarso, S. Kurnianto, M. Stidham, and M. Kanninen.
708 2011. Mangroves among the most carbon-rich forests in the tropics. Nature
709 Geoscience **4:** 293-297.
710 Duarte, C. M., and D. Krause-Jensen. 2017. Export from seagrass meadows contributes to
711 marine carbon sequestration. Frontiers in Marine Science **4:** 13.
712 Dworschak, P. 2005. Global diversity in the Thalassinidea (Decapoda): an update (1998–
713 2004). Nauplius **13:** 57-63.
714 Dworschak, P., H. Koller, and D. Abed-Navandi. 2006. Burrow structure, burrowing and
715 feeding behaviour of Corallianassa longiventris and Pestarella tyrrhena (Crustacea,
716 Thalassinidea, Callianassidae). Marine Biology **148:** 1369-1382.

717 Dworschak, P. C. 2000. Global diversity in the Thalassinidea (Decapoda). Journal of
718 Crustacean Biology **20:** 238-245.
719 ---. 2001. The burrows of *Callianassa tyrrhena* (Petagna 1792)(Decapoda: Thalassinidea).
720 Marine Ecology **22:** 155-166.
721 Dye, A., and F. Barros. 2005. Spatial patterns of macrofaunal assemblages in intermittently
722 closed/open coastal lakes in New South Wales, Australia. Estuarine, Coastal and Shelf
723 Science **64:** 357-371.
724 Eyre, B. D., A. J. Ferguson, A. Webb, D. Maher, and J. M. Oakes. 2011. Metabolism of
725 different benthic habitats and their contribution to the carbon budget of a shallow
726 oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia).
727 Biogeochemistry **102:** 87-110.
728 Fontaine, S., S. Barot, P. Barré, N. Bdioui, B. Mary, and C. Rumpel. 2007. Stability of
729 organic carbon in deep soil layers controlled by fresh carbon supply. Nature **450:** 277-
730 280.
731 Fontaine, S., A. Mariotti, and L. Abbadie. 2003. The priming effect of organic matter: a
732 question of microbial competition? Soil Biology and Biochemistry **35:** 837-843.
733 Fourqurean, J. W. and others 2012. Seagrass ecosystems as a globally significant carbon
734 stock. Nature Geoscience **5:** 505-509.
735 Garbary, D. J., A. G. Miller, J. Williams, and N. R. Seymour. 2014. Drastic decline of an
736 extensive eelgrass bed in Nova Scotia due to the activity of the invasive green crab
737 (*Carcinus maenas*). Marine Biology **161:** 3-15.
738 Gladstone, W. 2006. Final report of the Brisbane Water estuary biodiversity study. NSW
739 Government.
740 Gontikaki, E., B. Thornton, T. Cornulier, and U. Witte. 2015. Occurrence of Priming in the
741 Degradation of Lignocellulose in Marine Sediments. PLoS One **10:** e0143917.

742 Gontikaki, E., B. Thornton, V. A. Huvenne, and U. Witte. 2013. Negative priming effect on
743 organic matter mineralisation in NE Atlantic slope sediments. PloS one **8:** e67722.
744 Govers, L. L., T. Pieck, T. J. Bouma, W. Suykerbuyk, A. J. Smolders, and M. M. van
745 Katwijk. 2014. Seagrasses are negatively affected by organic matter loading and
746 *Arenicola marina* activity in a laboratory experiment. Oecologia **175:** 677-685.
747 Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap
748 between terrestrial and aquatic ecology. Ecology **91:** 2850-2861.
749 Hansen, K., and E. Kristensen. 1998. The impact of the polychaete *Nereis diversicolor* and
750 enrichment with macroalgal (*Chaetomorpha linum*) detritus on benthic metabolism
751 and nutrient dynamics in organic-poor and organic-rich sediment. Journal of
752 Experimental Marine Biology and Ecology **231:** 201-223.
753 Harrison, P. G., and A. Chan. 1980. Inhibition of the growth of micro-algae and bacteria by
754 extracts of eelgrass (Zostera marina) leaves. Marine Biology **61:** 21-26.
755 Heilskov, A. C., and M. Holmer. 2001. Effects of benthic fauna on organic matter
756 mineralization in fish-farm sediments: importance of size and abundance. ICES
757 Journal of Marine Science: Journal du Conseil **58:** 427-434.
758 Kaldy, J. E., C. A. Brown, and C. P. Andersen. 2013. In situ 13C tracer experiments elucidate
759 carbon translocation rates and allocation patterns in eelgrass *Zostera marina*. Marine
760 Ecology Progress Series **487:** 27-39.
761 Katrak, G., and F. L. Bird. 2003. Comparative effects of the large bioturbators, *Trypaea*
762 *australiensis* and *Heloecius cordiformis*, on intertidal sediments of Western Port,
763 Victoria, Australia. Marine and Freshwater Research **54:** 701-708.
764 Kneer, D., H. Asmus, and J. Jompa. 2013. Do burrowing callianassid shrimp control the
765 lower boundary of tropical seagrass beds? Journal of Experimental Marine Biology
766 and Ecology **446:** 262-272.

767 Kneer, D., H. Asmus, and J. A. Vonk. 2008. Seagrass as the main food source of *Neaxius*
768 *acanthus* (Thalassinidea: Strahlaxiidae), its burrow associates, and of *Corallianassa*
769 *coutierei* (Thalassinidea: Callianassidae). Estuarine, Coastal and Shelf Science **79:**
770 620-630.
771 Koller, H., P. C. Dworschak, and D. Abed-Navandi. 2006. Burrows of *Pestarella tyrrhena*
772 (Decapoda: Thalassinidea): hot spots for Nematoda, Foraminifera and bacterial
773 densities. Journal of the Marine Biological Association of the United Kingdom **86:**
774 1113-1122.
775 Kristensen, E. 2000. Organic matter diagenesis at the oxic/anoxic interface in coastal marine
776 sediments, with emphasis on the role of burrowing animals. Hydrobiologia **426:** 1-24.
777 ---. 2001. Impact of polychaetes (*Nereis* spp. and *Arenicola marina*) on carbon
778 biogeochemistry in coastal marine sediments. Geochemical Transactions **2:** 92-103.
779 ---. 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes.
780 Journal of Sea Research **59:** 30-43.
781 Kristensen, E., M. H. Jensen, and T. K. Andersen. 1985. The impact of polychaete (*Nereis*
782 *virens* Sars) burrows on nitrification and nitrate reduction in estuarine sediments.
783 Journal of Experimental Marine Biology and Ecology **85:** 75-91.
784 Kristensen, E., G. Penha-Lopes, M. Delefosse, T. Valdemarsen, C. O. Quintana, and G. T.
785 Banta. 2012. What is bioturbation? The need for a precise definition for fauna in
786 aquatic sciences. Marine Ecology Progress Series **446:** 285-302.
787 Kuzyakov, Y., J. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of
788 priming effects. Soil Biology and Biochemistry **32:** 1485-1498.
789 Lavery, P. S., M.-Á. Mateo, O. Serrano, and M. Rozaimi. 2013. Variability in the carbon
790 storage of seagrass habitats and its implications for global estimates of blue carbon
791 ecosystem service. PloS one **8:** e73748.

792 Lewis, L. S., and T. W. Anderson. 2012. Top-down control of epifauna by fishes enhances
793 seagrass production. Ecology **93:** 2746-2757.
794 Lopez-Capel, E., S. P. Sohi, J. L. Gaunt, and D. A. Manning. 2005. Use of
795 thermogravimetry–differential scanning calorimetry to characterize modelable soil
796 organic matter fractions. Soil Science Society of America Journal **69:** 136-140.
797 López, N. I., C. M. Duarte, F. Vallespinós, J. Romero, and T. Alcoverro. 1998. The effect of
798 nutrient additions on bacterial activity in seagrass (*Posidonia oceanica*) sediments.
799 Journal of Experimental Marine Biology and Ecology **224:** 155-166.
800 Macreadie, P., M. Baird, S. Trevathan-Tackett, A. Larkum, and P. Ralph. 2014. Quantifying
801 and modelling the carbon sequestration capacity of seagrass meadows–a critical
802 assessment. Marine Pollution Bulletin **83:** 430-439.
803 Macreadie, P. I. and others 2015. Losses and recovery of organic carbon from a seagrass
804 ecosystem following disturbance. Proceedings of the Royal Society of Biology **282:**
805 20151537.
806 Maher, D., and B. D. Eyre. 2011a. Benthic carbon metabolism in southeast Australian
807 estuaries: Habitat importance, driving forces, and application of artificial neural
808 network models. Marine Ecology Progress Series **439:** 97-115.
809 Maher, D., and B. D. Eyre. 2011b. Insights into estuarine benthic dissolved organic carbon
810 (DOC) dynamics using δ 13 C-DOC values, phospholipid fatty acids and dissolved
811 organic nutrient fluxes. Geochimica et Cosmochimica Acta **75:** 1889-1902.
812 Marbà, N., R. Santiago, E. Díaz-Almela, E. Álvarez, and C. M. Duarte. 2006. Seagrass
813 (*Posidonia oceanica*) vertical growth as an early indicator of fish farm-derived stress.
814 Estuarine, Coastal and Shelf Science **67:** 475-483.
815 McKenzie, L., S. Campbell, and C. Roder. 2001. Seagrass-Watch: Manual for mapping and
816 monitoring seagrass resources by community (citizen) volunteers. QFS, NFC **2:** 100.

817 Mcleod, E. and others 2011. A blueprint for blue carbon: toward an improved understanding
818 of the role of vegetated coastal habitats in sequestering CO2. Frontiers in Ecology and
819 the Environment **9:** 552-560.
820 Papaspyrou, S., T. Gregersen, R. P. Cox, M. Thessalou-Legaki, and E. Kristensen. 2005.
821 Sediment properties and bacterial community in burrows of the ghost shrimp
822 *Pestarella tyrrhena* (Decapoda: Thalassinidea). Aquatic Microbial Ecology **38:** 181-
823 190.
824 Papaspyrou, S., E. Kristensen, and B. Christensen. 2007. *Arenicola marina* (Polychaeta) and
825 organic matter mineralisation in sandy marine sediments: in situ and microcosm
826 comparison. Estuarine, Coastal and Shelf Science **72:** 213-222.
827 Papaspyrou, S., M. Thessalou-Legaki, and E. Kristensen. 2004. Impact of *Pestarella tyrrhena*
828 on benthic metabolism in sediment microcosms enriched with seagrass and
829 macroalgal detritus. Marine Ecology Progress Series **281:** 165-179.
830 ---. 2010. The influence of infaunal (*Nereis diversicolor*) abundance on degradation of
831 organic matter in sandy sediments. Journal of Experimental Marine Biology and
832 Ecology **393:** 148-157.
833 Poore, G. C. 1975. Systematics and distribution of Callianassa (Crustacea, Decapoda,
834 Macrura) from Port Phillip Bay, Australia, with descriptions of two new species.
835 ---. 2008. Thalassinidean shrimps (Crustacea: Decapoda) from north-western Australia,
836 including five new species. Records of the Western Australian Museum, Supplement
837 **73:** 161-179.
838 Rowden, A., M. Jones, and A. Morris. 1998. The role of Callianassa subterranea
839 (Montagu)(Thalassinidea) in sediment resuspension in the North Sea. Continental
840 Shelf Research **18:** 1365-1380.

841 Roy, P. and others 2001. Structure and function of south-east Australian estuaries. Estuarine,
842 Coastal and Shelf Science **53:** 351-384.
843 Sakai, K. 1988. A new genus and five new species of Callianassidae (Crustacea: Decapoda:
844 Thalassinidea) from northern Australia. Beagle: Records of the Museums and Art
845 Galleries of the Northern Territory, The **5:** 51.
846 Stapel, J., and P. Erftemeijer. 2000. Leaf harvesting by burrowing alpheid shrimps in a
847 Thalassia hemprichii meadow in South Sulawesi, Indonesia. Biologia Marina
848 Mediterranea **7:** 282-285.
849 Stapleton, K. L., M. Long, and F. Bird. 2001. Comparative feeding ecology of two spatially
850 coexisting species of ghost shrimp, *Biffarius arenosus* and *Trypaea australiensis*
851 (Decapoda: Callianassidae). Ophelia **55:** 141-150.
852 Steen, A. D., L. N. Quigley, and A. Buchan. 2016. Evidence for the priming effect in a
853 planktonic estuarine microbial community. Frontiers in Marine Science **3:** 6.
854 Suchanek, T. H. 1983. Control of seagrass communities and sediment distribution by
855 Callianassa (Crustacea, Thalassinidea) bioturbation. Journal of Marine Research **41:**
856 281-298.
857 Trevathan-Tackett, S. M. 2016. Dynamics of refractory carbon in seagrass meadows.
858 University of Technology Sydney.
859 Trevathan-Tackett, S. M., J. Kelleway, P. I. Macreadie, J. Beardall, P. Ralph, and A.
860 Bellgrove. 2015. Comparison of marine macrophytes for their contributions to blue
861 carbon sequestration. Ecology **96:** 3043-3057.
862 Trevathan-Tackett, S. M., A. C. G. Thomson, P. J. Ralph, and P. I. Macreadie. 2017. Fresh
863 carbon inputs to seagrass sediments induce variable microbial priming responses.
864 Science of the Total Environment **621:** 663-669.

865 Trevathan-Tackett, S. M., C. Wessel, J. Cebrián, P. J. Ralph, P. Masqué, and P. I. Macreadie.
866 2018. Effects of small-scale, shading-induced seagrass loss on blue carbon storage:
867 Implications for management of degraded seagrass ecosystems. Journal of Applied
868 Ecology **55:** 1351-1359.
869 Valdemarsen, T., K. Wendelboe, J. T. Egelund, E. Kristensen, and M. R. Flindt. 2011. Burial
870 of seeds and seedlings by the lugworm *Arenicola marina* hampers eelgrass (*Zostera*
871 *marina*) recovery. Journal of Experimental Marine Biology and Ecology **410:** 45-52.
872 Valentine, J. F., K. L. Heck, P. Harper, and M. Beck. 1994. Effects of bioturbation in
873 controlling turtlegrass (Thalassia testudinum Banks ex König) abundance: evidence
874 from field enclosures and observations in the Northern Gulf of Mexico. Journal of
875 Experimental Marine Biology and Ecology **178:** 181-192.
876 van Nugteren, P., L. Moodley, G.-J. Brummer, C. H. Heip, P. M. Herman, and J. J.
877 Middelburg. 2009. Seafloor ecosystem functioning: the importance of organic matter
878 priming. Marine biology **156:** 2277-2287.
879 Vonk, J. A., D. Kneer, J. Stapel, and H. Asmus. 2008. Shrimp burrow in tropical seagrass
880 meadows: an important sink for litter. Estuarine, Coastal and Shelf Science **79:** 79-85.
881 Webb, A. P., and B. D. Eyre. 2004. Effect of natural populations of burrowing thalassinidean
882 shrimp on sediment irrigation, benthic metabolism, nutrient fluxes and denitrification.
883 Marine Ecology Progress Series **268:** 205-220.
884 Woods, C. M., and D. R. Schiel. 1997. Use of seagrass *Zostera novazelandica* (Setchell,
885 1933) as habitat and food by the crab *Macrophthalmus hirtipes* (Heller,
886 1862)(Brachyura: Ocypodidae) on rocky intertidal platforms in southern New
887 Zealand. Journal of Experimental Marine Biology and Ecology **214:** 49-65.

888