**Seagrass derived organic matter influences biogeochemistry, microbial communities,
and seedling biomass partitioning in seagrass sediments**

M.W Fraser^{1*}, J. Statton¹, R.K. Hovey¹, B. Laverock^{1,2#}, G.A. Kendrick¹

¹ School of Plant Biology & Oceans Institute, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

² Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, Ultimo, NSW 2007, Australia

*Corresponding author: matthew.fraser@uwa.edu.au

#Current address

Keywords: bacteria, decomposition, enzymes, *Posidonia australis*, roots, seeds

The final publication is available at Springer via <http://dx.doi.org/10.1007/s11104-015-2721-0>

Abstract

Aims Seedling establishment is a crucial life history stage in seagrasses, yet factors that affect seedling health are poorly characterized. We investigated if organic matter (OM) additions to sediments provided nutritional benefits for seagrass seedlings through microbial degradation.

Methods We tested the effects of sedimentary OM additions on *Posidonia australis* seedlings growing in tank cultures. We focussed on sediment biogeochemical processes and microbial communities that may impact seedling growth and physiology.

Results Enrichment of sediments with OM changed microbial community composition (DNA-ARISA) and a significant increase in hydrolytic enzyme expression. Total seedling biomass did not differ between OM treatments, but above:belowground biomass increased with OM enrichment. Nitrogen and phosphorus concentration of seagrass leaves was lower with increasing OM.

Conclusions Seagrass derived OM has been considered a refractory store of carbon, yet here we show its deposition into sediments significantly alters belowground conditions. Remineralization of the OM changes both physical and chemical nature of sediments that leads to greater biochemical activity, change in microbial communities and greater investment into above ground photosynthetic biomass. The presence of OM may assist

seagrass seedling survival during early development by enhancing root branching and stability in sediments, but is unlikely to provide nutritional benefits.

Introduction

Inorganic nutrient concentrations are often below required concentrations required to maintain primary productivity in marine sandy habitats where seagrasses dominate (McGlathery et al. 2001). The microbial breakdown of organic matter (OM) supplements primary productivity, and is especially important in low nutrient ecosystems such as tropical forests (Reed et al. 2011). Similarly, OM buried in marine sediments may represent an important source of nutrients to seagrasses (López et al. 1995; Evrard et al. 2005; Kilminster et al. 2006). The role of microorganisms in OM breakdown may be particularly important in seagrass sediments as these sediments contain high concentrations of seagrass derived OM (Kennedy et al. 2010). While this belowground pool has been widely investigated for its role in carbon (C) sequestration (Fourqurean et al. 2012), the ecological implications of these sediments have not been examined in great detail. Seagrass derived OM consists of a range of organic molecules that differ significantly in their biological availability, but most forms of OM are refractory and not readily available for direct uptake by seagrasses (Danovaro 1996). Seagrass therefore rely on the metabolic capabilities of their sedimentary microbes to convert complex molecules to more labile forms of dissolved OM and nutrients that are then available for uptake by seagrasses. Conversely, microbes may also reduce nutrient availability through immobilisation of available nutrients into microbial biomass.

Microbes primarily liberate nutrients from OM through the release of extracellular enzymes. Extracellular enzymes play a crucial role in all biogeochemical cycles by being proximate agents in crucial processes such as decomposition (Burns and Dick 2002; Burns et al. 2013). Enzymes play a complex role in the degradation of plant litter, with numerous enzymes needed to hydrolyse OM at different stages. Plant litter comprises a high proportion of recalcitrant C rich macromolecules such as cellulose, lignin, or hemicellulose. These macromolecules are broken down through the combined enzyme activities of a diverse range of microorganisms (Burns and Dick 2002; Burns et al. 2013). For example, β -glucosidase breaks down cellulose, while α -glucosidase hydrolyses starch (Dick 2011). Consequently, the release of nutrients via microbial enzyme production is partly dependent on the type and quantity of plant litter (Burns et al. 2013). For instance, expression of β -glucosidase and acid phosphatase (associated with C and phosphorus (P) release from OM) in soils was higher in soils amended with grass litter relative to soils without litter addition (Dornbush 2007).

Distinct shifts in the microbial community composition are also apparent during decomposition (Sinsabaugh and Follstad Shah 2012). These shifts can be directly (C availability, chemical composition of litter) or indirectly (redox, pH) related to the presence of OM. The addition of OM to sediments should therefore result in distinct belowground shifts in microbial communities and enzyme production.

The successful establishment of seedlings is a crucial life history stage in seagrasses. Recolonization through seedling establishment may be particularly important in large degraded areas with high rates of seagrass mortality that limits recovery through asexual propagation. Successful recovery would therefore likely depend on new recruits entering from adjacent populations; with several biotic and abiotic bottlenecks to successful settlement and growth of seedlings (Orth et al. 2005; Rivers et al. 2011). However, ecological factors influencing seagrass seedling survival post settlement have received limited attention, despite their importance for maintaining populations (Kendrick et al. 2012). In particular, the nutrient requirements of recently settled seedlings are poorly understood. Additions of inorganic nutrients (N and P) did not increase seedling growth or productivity (Statton et al. 2014). Instead, the release of nutrients from decomposing OM may be an important source of nutrients to *P. australis* seedlings. *Posidonia australis* seedlings grown in tanks with additions of OM showed increased root growth and productivity compared to those grown in control sediments (Statton et al. 2013). However, the belowground processes that increase this root growth are not known.

Here, we tested the effects of OM additions on *P. australis* seedling growth in tank cultures. We also investigated sediment biogeochemical processes and microbial communities that could explain seagrass responses to OM additions. We hypothesised that (i) enzymes involved in C and P cycling would have higher concentrations at higher OM concentrations, (ii) microbial communities would be significantly different in pots with OM compared to pots without OM, and (iii) seagrass seedlings would display higher leaf, root and rhizome biomass and nutrient (N and P) concentrations in sediments with higher concentrations of OM.

Methods

Seedling collection

Posidonia australis fruit were collected from Woodman Point (32°08'10"S, 115°44'31"E) during November 2012. Fruit were transported in seawater back to the Fisheries WA Marine Research Facility at Hillarys, Western Australia. Fruit were left to dehisce naturally over 3

days in a holding tank containing constant flow-filtered seawater. Dehiscence of fruit resulted in the negatively buoyant *P. australis* seeds (with emerging radicle and prophyll) collecting in numbers on the bottom of the tank. We assessed seeds for viability based on the presence of an intact seed, developing prophyll, and radicle.

Experimental design

The effect of OM addition on seagrass seedling growth and sediment biogeochemistry was investigated using three tanks, each containing eight replicates of six organic matter treatments. Tanks received a 12h/12h light regime supplied by artificial metal halide lamps positioned above each tank ($250 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ or $10.8 \text{ mols photons d}^{-1}$), with constantly replaced seawater filtered to $0.2\mu\text{m}$. Replicate seedlings were planted into square pots (70 x 70 mm, 300 mm height) containing one of the six OM treatments (0, 0.5, 1, 1.5, 2, 2.5% sediment dry weight). Given the quantitative nature of our treatments, we used a ‘replicated regression’ design to increase the power and flexibility of our experiment (Cottingham et al. 2005). These OM concentrations represented levels common in natural seagrass sediments, and are comparable to previous *P. australis* aquaculture experiments (Statton et al. 2013). Sediment was collected from Woodman Point beach. This sediment was primarily calcareous, and contained negligible quantities of OM (<0.1% dry wt.) Sediment was dried at 60°C for 72 hours prior to addition to pots. Organic matter was sourced from Woodman Point beach wrack and consisted primarily of *Posidonia sp.* leaves, roots and fibrous leaf sheaths. Organic matter was dried at 60°C for 3 days and ground to <2mm using a coffee grinder. Sediments were homogenously mixed to a known mass of organic matter according to treatment (Table 1). A total of 144 replicate seedlings were each randomly planted in individual pots with a randomly assigned treatment. Seedlings were cleaned by hand every 2-3 days to ensure no algal overgrowth that could limit light availability.

Post-harvest measurements

All seedlings were harvested after six months. Prior to harvesting (7 days), five replicate shoots from each treatment within each tank were marked by punching a hole with a pin through all leaves at the top of each shoot sheath (Short and Duarte 2001). After harvesting, leaves were removed then separated into old and young leaves with ages based on position on shoot (oldest leaf with the lowest vertical position on a shoot, and successively younger leaves positioned in ascending order along the shoot). New leaf growth was defined as the

distance between the initial marking scar and base of the leaf (young and old leaves), plus any new unmarked leaves (young) formed during the seven day period. New leaf growth was dried (see above) and weighed for leaf productivity estimates. Seagrass biomass was determined from five replicates in each tank. Seedlings were cleaned of epiphytes and separated in component parts (individual leaves, roots, and rhizomes). These components were then dried at 60°C for 72 hours and weighed to obtain biomass. Due to low biomass, leaves from each treatment in each tank were pooled before nutrient and stable isotope analyses. Leaves were ground to a fine powder using a steel ball-mill. Carbon (C) and N concentrations were determined using an Automated C/N Analyser-Mass Spectrometer consisting of a 20/22 mass spectrometer connected to an ANCA-S1 preparation system (Sercon, Crewe, UK) at the Western Australian Biogeochemistry Centre at the University of Western Australia. All samples were standardized using multi-point normalization against a secondary reference of Radish collegate (3.167% N, 41.51% C), which was in turn standardized against primary analytical standards (International Atomic Energy Agency, Vienna) (Paul et al. 2007). The external error of analyses (one standard deviation) was no more than 0.1 for C:N ratio. Phosphorus contents of seagrass and sediment samples were determined using a dry-oxidation and acid hydrolysis technique, followed by a colourimetric analysis of the phosphate concentration of the extract (Fraser et al. 2012). Phosphate concentrations were compared with Australian Soil and Plant Analysis Council Standards 102 (*Pinus radiata*) and 105 (*Eucalyptus* sp.), which were treated accordingly. We found this technique to yield 94-101% of the reported P content of ASPAC Standards 102 and 105. Elemental concentrations of seagrass and sediment samples were calculated as a percentage of dry weight, and converted to elemental ratios.

Sediment biogeochemistry

The activities of four hydrolytic enzymes were measured using standard colourimetric methods (Dick 2011). The enzymes and substrates used were (i) α -glucosidase assayed with 4-Nitrophenyl α -D-glucopyranoside, (ii) β -glucosidase assayed with 4-Nitrophenyl β -glucopyranoside, (iii) acid phosphatase and (iv) alkaline phosphatase both assayed with 4-nitrophenyl phosphate disodium hexahydrate. Briefly, 1.00 g of sediment was added to a 50 ml Erlenmeyer flasks with 4 mL of modified universal buffer (pH=6.00 for α -glucosidase and β -glucosidase, pH=6.50 for acid phosphatase and pH=11.00 for alkaline phosphatase) and 1 mL substrate, and were incubated at 37 °C for one hour. Following incubation, 1 mL of 0.5M

CaCl₂ and 4 ml of 0.5M NaOH (for phosphatases) or 4 ml 10mM tris(hydroxymethyl) aminomethane (pH=12, for glucosidases) were added to stop the reaction. The final nitrophenol concentrations were determined photometrically at 400 nm. Resin extractable P was measured using anion exchange membranes that are a good representation of P that is available for uptake by plants (Cooperband and Logan 1994; Cheesman et al. 2010; Bünemann et al. 2013). Membranes were cut into 4 cm x 1 cm strips, and four were inserted into sediments in each pot. The membranes were left for four weeks to reach equilibrium with porewaters, before being removed, rinsed in DI water, and placed in 30 ml 0.5M HCl. Resin extractable P was then measured from HCl extracts using the Murphy Riley method for colourimetric determination of P concentrations (Murphy and Riley 1962). Sediment redox potential was measured six months after sediments were installed using a platinum electrode connected to a pH electrode and measured as millivolts. Platinum electrodes were left in sediments for 48 hours to allow biogeochemical profiles to be re-established after initial disturbance. No data was collected for redox potential in 2.5% OM treatments due to logistical difficulties. Sediment penetration resistance was measured using a Pocket Penetrometer (Humboldt Mfg. Co., Illinois, US).

Microbial molecular community identification

Surface sediment samples (2 ml, 0-2 cm depth) for DNA extraction and genetic analysis were collected from pots using a 3 ml syringe. Samples were transferred to a 2 ml cryovial, and immediately frozen in liquid nitrogen (N) before storage at -80°C. Sediment samples were then homogenized using a sterile metal spatula, and 0.5 g was added to a 2 ml Eppendorf tube. DNA was extracted from sediments using the MP Bio Fast DNA SPIN kits for soil (MP Bio, California, United States of America) with some modifications (Abell et al. 2011). DNA was eluted in nuclease-free water and DNA extraction was confirmed by agarose gel electrophoresis.

DNA was amplified using universal bacterial primers 16S-1392F (5'-GYACACACCGCCCGT-3') and a 5'HEX labelled version of the reverse primer 23S-125R (5'-GGGTTBCCCCATTCRG-3') (Hewson and Fuhrman 2004). 50 µL reactions containing 1 µL DNA, 25 µL of GoTaq Green Master Mix (Promega, Australia), 2 µL of Bovine Serum Albumin (Promega), 1 nM of each primer were amplified as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min 30 sec and 72°C for 1 min 30 sec, and a final extension step of 72°C for 10 min. Successful amplification was confirmed by gel electrophoresis, and PCR products were purified using

AMPure magnetic beads (Agencourt, Beckman Coulter Life Science, USA) according to the manufacturer's instructions, resuspended in 40 µL of TE buffer and quantified using a Nanodrop 8000 spectrophotometer (ThermoScientific, Wilmington, USA).

Microbial community DNA was analysed by Automated Ribosomal Intergenic Spacer Analysis (ARISA). Approximately 25-40 ng of purified amplified PCR reaction was mixed with 0.4 µL of internal size standard (GS1200-Liz, Applied Biosystems) in deionised formamide and denatured at 94°C for 2 minutes. Denaturing capillary electrophoresis (on a 50cm capillary array) was carried out using an ABI 3130XL genetic analyzer (Applied Biosystems). ARISA profiles were analysed using GeneMapper v3.7 (Applied Biosystems), with peak height as a measure of community presence, and a peak threshold of 50 relative fluorescence units greater than the baseline.

Statistical analysis

Relationships between seagrass biomass, productivity, nutrient content, sediment enzyme expression and organic matter content were analysed using linear regression analysis. Prior to analysis, data were checked for normality and homogeneity of variances. The relationship between the sediment microbial community and measured environmental variables was investigated using a distance-based multivariate multiple regression model (distance linear based model [DistLM]) marginal tests with a Bray-Curtis distance matrix based on 9,999 permutations. Seagrass and sediment biogeochemistry data were analysed using R version 3.1.1 (R Core Team 2014) using the base program and the 'ggplot2' package for graphics (Wickham 2009). Variation in microbial community structure between samples was analysed in PRIMER v6.0 (Clarke and Gorley 2006) with the PERMANOVA+ Add-on (Anderson et al. 2008).

Results

Sediment biogeochemistry

Redox potential in sediments strongly decreased with the addition of organic matter (Fig. 1a, $R^2 = 0.86$, $p < 0.0001$, $n=15$), indicating more reducing conditions in sediments. Redox potential ranged from a maximum of -92.9 mV ($\pm 9.9 \text{ mV}$) in sediments with 0% OM to -375.4 mV ($\pm 14.2 \text{ mV}$) in sediments with 2.0% OM. Penetration resistance in sediments was significantly greater with the addition of organic matter (Fig. 1b, $R^2 = 0.93$, $p < 0.0001$, $n=18$), indicating a higher force needed for roots to penetrate into sediments. Resin extractable P tended to decrease in sediments as the concentration of OM increased, although this was not statistically significant at $p = 0.05$ ($R^2 = 0.15$, $p = 0.059$, $n=24$, data not shown).

Enzymes involved in C cycling showed variable responses to OM enrichment (Fig. 2). α -glucosidase expression was not significantly related to concentration of OM in sediments (Fig. 2a, $R^2=0.08$, $p=0.13$, $n=18$). β -glucosidase expression significantly increased as the concentration of OM in sediments increased, indicating the potential for increased cellulose degradation (Fig. 2b, $R^2 = 0.79$, $p < 0.0001$, $n=24$).

Enzymes involved in releasing phosphate from OM had higher expression in sediments with OM added, in comparison to the 0% OM treatment. Alkaline phosphatase expression also significantly increased as OM content in sediments increased (Fig. 2c, $R^2=0.45$, $p=0.0009$, $n=24$). Acid phosphatase expression significantly increased as sediment OM concentration increased (Fig. 2d, $R^2=0.53$, $p < 0.0001$, $n=24$).

Microbial community structure

The sediment microbial community composition was significantly altered upon enrichment with OM (PERMANOVA $p < 0.001$). Pairwise differences between treatments tended towards significance where differences in OM content were largest. For example, there were no significant pairwise differences between the 0%, 0.5%, 1%, and 1.5% OM treatments, whereas the 2.5% OM treatment was significantly different to all treatments except 1.5% OM, which showed overlap with communities from enriched and non-enriched treatments.

The other environmental data measured in the experiment only accounted for 16% of the total variation in the microbial community (Fig. 3). Changes in microbial communities were most strongly correlated to changes in sedimentary organic matter content (accounting for 12.2% of the variation), β -glucosidase expression (10.8% of total variation), and sediment redox potential (10.6% of total variation). All other variables accounted for $< 2\%$ of the

variation in the microbial community (i.e. over 50% of the microbial community variability is unexplained by our measured variables).

Seagrass biomass

Total seagrass seedling biomass did not significantly change with OM amendments (Fig. 4a, $R^2 = 0.003$, $p = 0.092$, $n=90$). Similarly, seedling leaf biomass did not change significantly with OM addition (Fig. 4b, $R^2 = 0.01$, $p = 0.302$, $n=90$). Organic matter enrichment did not change total leaf productivity (Fig. 4c, $R^2 = 0.01$, $p = 0.47$, $n=90$) or productivity in young leaves ($R^2 < 0.01$, $p = 0.9$, $n=90$, $n=19$). There were strong relationships in the biomass allocation between different leaves among OM treatments. Biomass was significantly greater in older leaves at lower OM concentrations (Fig. 4d, $R^2 = 0.32$, $p < 0.0001$, $n=90$), while biomass was significantly greater in newer leaves at higher OM concentrations ($R^2 = 0.13$, $p = 0.016$, $n=90$).

Rhizome biomass decreased significantly with sediment OM enrichment, although this relationship was weak (Fig. 5a, $R^2 = 0.059$, $p = 0.012$, $n=90$) and non-linear. Root biomass also decreased significantly with OM enrichment; again this relationship was weak and non-linear (Fig. 5b, $R^2 = 0.047$, $p = 0.023$, $n=90$). Total belowground biomass decreased significantly upon OM addition (Fig. 5c, $R^2 = 0.078$, $p = 0.008$, $n=90$). The decrease of root and rhizome biomass at high OM led to a significant increase in above:belowground biomass with OM enrichment ($R^2 = 0.046$, $p = 0.024$, $n=90$).

Seagrass nutrient concentrations

The nutrient concentration of seagrass leaves was variable with OM enrichment (Table 1). Carbon ($R^2 = 0.05$, $p = 0.78$, $n=18$), nitrogen ($R^2 = 0.15$, $p = 0.06$, $n=18$), and phosphorus ($R^2 = 0.13$, $p = 0.08$, $n=18$) concentrations in seagrass leaves did not significantly change with OM addition. However, the carbon:nitrogen (C:N) ratio of seagrass leaves significantly increased with increasing OM concentrations ($R^2 = 0.41$, $p = 0.0042$, $n=18$). Carbon:phosphorus (C:P) ratios of seagrass leaves tended to increase with elevated OM concentrations, though this relationship was weaker and not significant at the 0.05 level ($R^2 = 0.20$, $p = 0.062$, $n=18$). Nitrogen:phosphorus (N:P) ratios of seagrass leaves showed no significant relationship to OM concentrations ($R^2 = 0.02$, $p = 0.2768$, $n=18$).

Discussion

Seagrass detritus is a ubiquitous component of OM in seagrass sediments. Consequently, established seagrass meadows have been considered as hotspots for C burial over long temporal and spatial scales (Fourqurean et al. 2012), but the ecological importance of this OM over smaller temporal and spatial scales is largely unknown. Here, we show the significant impacts of seagrass-derived OM on the biogeochemistry and microbial communities within sediments, and on biomass partitioning in *P. australis* seedlings.

Elevated seagrass OM concentrations in sediments strongly increased the expression of hydrolysing enzymes in sediments, confirming our first hypothesis. The enzymes examined were those involved with C and P release from complex organic molecules (Fig. 2). We found that the addition of OM led to distinct changes in biogeochemical conditions in sediments between treatments, which could alter sediment nutrient availability. Resin P tended to decrease with OM enrichment, and increased production of phosphatase indicated a likely alteration in the physiological demands of the microbial community. For example, the addition of OM could have released the microbial community from C limitation, shifting demands onto other nutrients (e.g. P) (Haugwitz and Michelsen 2011). The immobilization of P and N into microbial biomass could then reduce the concentrations in porewaters, resulting in increased plant-microbial competition for nutrients. An increase in plant-microbe competition for nutrients after OM enrichment has been well established in agricultural systems (Lehmann et al. 2011; Quilliam et al. 2012). Alternatively, the greater aboveground biomass in OM enriched treatments may have led to a dilution of potential nutrient impacts, though the necessary pooling of samples for nutrient analysis prevent us from exploring in further detail. Regardless, the ecological role of seagrass detritus on sediments is poorly known, and has largely been considered as a refractory pool of C due to low turnover rates (McLeod et al. 2011). We suggest that in natural meadows, the deposition of detritus may increase competition for nutrients between seagrasses and microbes as meadows age and deposit more OM into sediments, shifting nutrient limitations as meadows develop. However, the extent of nutrient limitation would depend on other nutrient inputs, such as sedimentation of suspended materials, which are also enhanced by seagrass cover (Risgaard-Petersen and Dalsgaard 1998; Gacia and Duarte 2001). Clearly, the ecological role that seagrass detritus plays in coastal sediments must be considered when examining the biogeochemistry of seagrass meadows.

There were significant shifts in the sedimentary microbial community as OM concentrations increased, confirming our second hypothesis. Increased seagrass OM

concentrations may have benefitted microbial groups that were actively able to utilise the substrate by releasing enzymes (Coolen et al. 2011; Khodadad et al. 2011), or by altering environmental conditions (Allison and Martiny 2008). We observed here that seagrass OM addition resulted in more reduced sediments (Fig. 1a), which would favour microbes that require little or no oxygen, such as sulphate reducers (Rosselló-Mora et al. 1999). These bacterial taxa play an important role in OM remineralisation in marine sediments, and within the plant rhizosphere may even form mutualistic relationships with seagrasses, due to the capacity of many sulphate reducers to fix N, thereby making it available for uptake by the plant (Welsh 2000). It appears that 1.5% OM in sediments may represent a threshold concentration, after which a significant shift in the microbial community composition occurs. It is unknown whether such a threshold would also hold true *in situ*; especially given the capacity of large seagrass root systems to oxygenate sediments (Greve et al. 2003; Pedersen et al. 2004; Borum et al. 2005), therefore buffering environmental conditions and, presumably, microbial metabolic processes. Given that sulphide intrusion from sediments has been suggested as a contributor of seagrass decline (Borum et al. 2013; Holmer and Hasler-Sheetal 2014), the role of sulphate reducers in seagrass sediments warrants further investigation.

There were modest decreases in root and rhizome biomass in seedlings growing in enriched OM treatments (Fig. 5). *Posidonia australis* exhibits strong architectural and morphological plasticity in root growth under changing environmental conditions. For example, *P. australis* growing in summer at Oyster Harbour, Australia had more lateral roots than in winter (Hovey et al. 2011; Hovey et al. 2012). Drivers of root plasticity are well known in many terrestrial plants, but factors that influence root growth and morphology in seagrasses are poorly characterised, despite the importance of seagrass roots for nutrient uptake and anchorage (Alcoverro et al. 2000; Belzunce et al. 2008). Our study shows that *P. australis* seedlings demonstrate plasticity in root growth even at an early developmental stage (<6 months after settling) when exposed to variable OM concentrations, even if the mechanisms for this are still unknown. Signalling compounds such as hormones may play a role in seedling root development, though have shown variable results in previous experiments with *Posidonia spp.* (Balestri and Bertini 2003; Glasby et al. 2015).

Seagrass OM addition led to limited changes in aboveground seedling biomass and productivity (Fig. 4, Table 1). The use of nutritional reserves in seedlings or uptake of nutrients from seawater may have homogenised responses to increased OM, even where some treatments may confer an ecological advantage over a longer timescale. Though

productivity did not significantly change between treatments, seedlings allocated more biomass to old leaves when growing in low OM sediments, and to new leaves when growing in high OM sediments. This suggests changes in leaf senescence rates. Leaf senescence is a plastic trait that is highly dependent on environmental conditions such as nutrient availability (Guo and Gan 2005). By signalling a high availability of C relative to N in old leaves, sugar accumulation triggers leaf senescence, remobilizing N into new leaves and preventing loss of N from the plant (Wingler 2005). Nitrogen accumulation was lower in high OM treatments (Table 1), suggesting that a decrease in N availability driven by OM additions may alter leaf senescence rates.

We did not find significant evidence supporting the previous hypotheses that seagrass derived OM assists *P. australis* seedlings through sediment microbial activity (Statton et al. 2013), but there are other ecological advantages that higher seagrass OM concentrations in sediments may provide to seedlings. Though sediment penetration resistance was greatest in sediments with higher OM concentrations (Fig. 1b), the level of physical force required to remove seedlings from sediments was also greater in sediments with higher OM concentrations (J. Statton, pers. comm). Sediment OM enrichment increases secondary and tertiary branching of *P. australis* roots (Statton et al. 2013), which increase stability in sediments (Grossnickle 2005). As a result, the presence of OM may assist seedlings in early survival by limiting hydraulic removal; a major bottleneck to seedling survival (Kirkman 1999; Statton et al. 2012). An increase in anchorage potential in organic rich sediments would have implications for seagrass recovery following dieback events that can input large volumes of seagrass-derived OM into sediments (Walker et al. 2006; Fraser et al. 2014). This may also have restoration implications, as hydrodynamic scouring is a major problem in seagrass restoration projects (Statton et al. 2012). Choosing sites with higher seagrass OM in sediment or preconditioning sediments with seagrass OM prior to transplanting may provide means of increasing restoration success.

The addition of seagrass OM to sediments also changed other physical conditions that could have influenced seedling health. Sediments with higher OM concentrations had significantly more negative redox potentials (Fig. 1a), indicative of lower oxygen concentrations resulting from increased respiration rates. Increased OM concentrations in seagrass sediments also increase sulphide in porewater, which can contribute to seagrass dieback through sulphide intrusion (Borum et al. 2005). Seagrasses are particularly susceptible to sulphide intrusion during times of low oxygen concentrations. However, we found no decrease in seagrass health or survival in high OM treatments in this experiment.

The contribution of sedimentary organic matter to toxic sulphide intrusion may be overestimated due to the nature and concentrations of OM added in previous experiments. For example, experimental additions of OM are often in unique forms that would not be found in significant concentrations in natural seagrass sediments such as sugar (Pérez et al. 2007) or sodium acetate (Ruiz-Halpern et al. 2008), and therefore would not be ecologically representative of *in situ* conditions. Similarly, conclusions drawn from natural experiments have often been conducted in highly degraded systems such as fish farms with anthropogenically derived OM additions that again may not represent conditions that are typically found in healthy seagrass meadows (Delgado et al. 1999). The dominant source of OM in most natural seagrass meadows would be seagrass detritus, especially in meadows dominated by large, long-lived species such as *P. australis* (Fourqurean et al. 2012). Seagrass detritus is notoriously refractory in nature. The high difference in lability between the OM additions in this and other experiments would result in very different below ground impacts - with differences in decomposition rates, and microbial and environmental shifts resulting from the additions. This is evidenced by the lack of negative impacts on seedlings here and in similar experiments (Statton et al. 2013), and the often-abundant growth of seagrasses upon sediments naturally enriched with seagrass derived OM. For example, seagrasses form highly productive meadows in blue carbon hotspots like the Mediterranean Sea and Shark Bay, Western Australia, even where sedimentary sulphide concentrations are high (Cambridge et al. 2012). The ecological consequences of OM enrichment of seagrass sediments will be highly dependent on the types and quantities of OM added. Future experiments should focus on adding OM types and concentrations that are representative of natural conditions if ecologically relevant conclusions are desired.

Acknowledgements

We thank the support staff at the Department of Fisheries, WA for logistical support throughout the experiment. We also thank the CSIRO Marine and Atmospheric Research (CMAR) Environmental Genomics team in Hobart, Tasmania, for their help with microbial community analyses; in particular Dr Guy Abell. This project was funded through Australian Research Council Linkage Grants to GAK and JS (LP100200429 & LP130100155). BL was funded by an Indian Ocean Marine Research Centre (IOMRC) Postdoctoral Fellowship, supported by AIMS, CSIRO and the University of Western Australia (UWA). MF supported by Ph.D. funding from the School of Plant Biology, UWA. We thank 3 anonymous reviewers who provided thoughtful comments that greatly improved the manuscript.

References

- Abell G, Banks J, Ross DJ, et al (2011) Effects of estuarine sediment hypoxia on nitrogen fluxes and ammonia oxidizer gene transcription. *FEMS Microbiology Ecology* 75:111–122.
- Alcoverro T, Manzanera M, Romero Martinengo J (2000) Nutrient mass balance of the seagrass *Posidonia oceanica*: the importance of nutrient retranslocation. *Marine Ecology Progress Series* 194:13–21.
- Allison SD, Martiny JB (2008) Resistance, resilience, and redundancy in microbial communities. *P Natl Acad Sci Usa* 105:11512–11519.
- Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to software and statistical methods. Plymouth, UK
- Balestri E, Bertini S (2003) Growth and development of *Posidonia oceanica* seedlings treated with plant growth regulators: possible implications for meadow restoration. *Aquatic Botany* 76:291–297. doi: 10.1016/S0304-3770(03)00074-3
- Belzunce M, Navarro RM, Rapoport HF (2008) *Posidonia oceanica* seedling root structure and development. *Aquatic Botany* 88:203–210. doi: 10.1016/j.aquabot.2007.10.008
- Borum J, Pedersen O, Greve TM, et al (2005) The potential role of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. *Journal of Ecology* 93:148–158.
- Borum J, Raun AL, Hasler-Sheetal H, et al (2013) Eelgrass fairy rings: sulfide as inhibiting agent. *Marine Biology* 161:351–358. doi: 10.1007/s00227-013-2340-3
- Burns RG, DeForest JL, Marxsen J, et al (2013) Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology & Biochemistry* 58:216–234. doi: 10.1016/j.soilbio.2012.11.009
- Burns RG, Dick RP (2002) *Enzymes in the environment: activity, ecology and applications*. New York: Marcel Dekker
- Bünemann EK, Keller B, Hoop D, et al (2013) Increased availability of phosphorus after drying and rewetting of a grassland soil: processes and plant use. *Plant and Soil* 370:511–526. doi: 10.1007/s11104-013-1651-y
- Cambridge ML, Fraser MW, Holmer M, et al (2012) Hydrogen sulfide intrusion in seagrasses from Shark Bay, Western Australia. *Marine and Freshwater Research* 63:1027–1038.
- Cheesman AW, Turner BL, Reddy KR (2010) Interaction of Phosphorus Compounds with Anion-Exchange Membranes: Implications for Soil Analysis. *Soil Sci Soc Am J* 74:1607. doi: 10.2136/sssaj2009.0295
- Clarke KR, Gorley RN (2006) *User manual/tutorial*. PRIMER-E Ltd, Plymouth
- Coolen MJ, van de Giessen J, Zhu EY, Wuchter C (2011) Bioavailability of soil organic matter and microbial community dynamics upon permafrost thaw. *Environmental*

464 Microbiology 13:2299–2314.

465 Cooperband LR, Logan TJ (1994) Measuring in situ changes in labile soil phosphorus with
 466 anion-exchange membranes. *Soil Sci Soc Am J* 58:105–114.

467 Cottingham KL, Lennon JT, Brown BL (2005) Knowing when to draw the line: designing
 468 more informative ecological experiments. *Frontiers in Ecology and the Environment*
 469 3:145–152.

470 Danovaro R (1996) Detritus-Bacteria-Meiofauna interactions in a seagrass bed (*Posidonia*
 471 *oceanica*) of the NW Mediterranean.

472 Delgado O, Ruiz J, Pérez M, et al (1999) Effects of fish farming on seagrass (*Posidonia*
 473 *oceanica*) in a Mediterranean bay: seagrass decline after organic loading cessation.
 474 *Oceanol Acta* 22:109–117.

475 Dick RP (2011) *Methods of soil enzymology*. Soil Science Society of America, Madison,
 476 USA

477 Dornbush ME (2007) Grasses, litter, and their interaction affect microbial biomass and soil
 478 enzyme activity. *Soil Biology & Biochemistry* 39:2241–2249. doi:
 479 10.1016/j.soilbio.2007.03.018

480 Evrard V, Kiswara W, Bouma TJ, Middelburg JJ (2005) Nutrient dynamics of seagrass
 481 ecosystems: 15N evidence for the importance of particulate organic matter and root
 482 systems. *Marine Ecology Progress Series* 295:49–55. doi: 10.3354/meps295049

483 Fourqurean JW, Duarte CM, Kennedy H, et al (2012) Seagrass ecosystems as a globally
 484 significant carbon stock. *Nature Geoscience* 5:505–509. doi: 10.1038/ngeo1477

485 Fraser MW, Kendrick GA, Grierson PF, et al (2012) Nutrient status of seagrasses cannot be
 486 inferred from system-scale distribution of phosphorus in Shark Bay, Western Australia.
 487 *Marine and Freshwater Research* 63:1015–1026. doi: 10.1071/MF12026

488 Fraser MW, Kendrick GA, Statton J, et al (2014) Extreme climate events lower resilience of
 489 foundation seagrass at edge of biogeographical range. *Journal of Ecology* 102:1528–
 490 1536. doi: 10.1111/1365-2745.12300

491 Gacia E, Duarte CM (2001) Sediment retention by a Mediterranean *Posidonia oceanica*
 492 meadow: the balance between deposition and resuspension. *Estuarine, Coastal and Shelf*
 493 *Science* 52:505–514.

494 Glasby TM, Taylor SL, Housefield GP (2015) Factors influencing the growth of seagrass
 495 seedlings: A case study of *Posidonia australis*. *Aquatic Botany* 120:251–259. doi:
 496 <http://dx.doi.org/10.1016/j.aquabot.2014.09.003>

497 Greve TM, Borum J, Pedersen O (2003) Meristematic oxygen variability in eelgrass (*Zostera*
 498 *marina*). *Limnology and Oceanography* 48:210–216.

499 Grossnickle SC (2005) Importance of root growth in overcoming planting stress. *New Forests*
 500 30:273–294. doi: DOI 10.1007/s11056-004-8303-2

501 Guo Y, Gan S (2005) Leaf senescence: signals, execution, and regulation. Current topics in
502 developmental biology 71:83–112.

503 Haugwitz MS, Michelsen A (2011) Long-term addition of fertilizer, labile carbon, and
504 fungicide alters the biomass of plant functional groups in a subarctic-alpine community.
505 Plant Ecology 212:715–726. doi: 10.1007/s11258-010-9857-z

506 Hewson I, Fuhrman JA (2004) Richness and Diversity of Bacterioplankton Species along an
507 Estuarine Gradient in Moreton Bay, Australia. Applied and Environmental Microbiology
508 70:3425–3433. doi: 10.1128/AEM.70.6.3425-3433.2004

509 Holmer M, Hasler-Sheetal H (2014) Sulfide intrusion in seagrasses assessed by stable sulfur
510 isotopes—a synthesis of current results. Front Mar Sci. doi: 10.3389/fmars.2014.00064

511 Hovey RK, Cambridge ML, Kendrick GA (2011) Direct measurements of root growth and
512 productivity in the seagrasses *Posidonia australis* and *P. sinuosa*. Limnology and
513 Oceanography 56:394–402.

514 Hovey RK, Cambridge ML, Kendrick GA (2012) Season and sediment nutrient additions
515 affect root architecture in the temperate seagrasses *Posidonia australis* and *P. sinuosa*.
516 Marine Ecology Progress Series 446:23–30. doi: 10.3354/meps09483

517 Kendrick GA, Waycott M, Carruthers TJB, et al (2012) The Central Role of Dispersal in the
518 Maintenance and Persistence of Seagrass Populations. BioScience 62:56–65. doi:
519 10.1525/bio.2012.62.1.10

520 Kennedy H, Beggins J, Duarte CM, et al (2010) Seagrass sediments as a global carbon sink:
521 Isotopic constraints. Global Biogeochem Cycles 24:GB4026.

522 Khodadad CL, Zimmerman AR, Green SJ, et al (2011) Taxa-specific changes in soil
523 microbial community composition induced by pyrogenic carbon amendments. Soil
524 Biology & Biochemistry 43:385–392.

525 Kilminster KL, Walker DI, Thompson PA, Raven JA (2006) Limited nutritional benefit to
526 the seagrass *Halophila ovalis*, in culture, following sediment organic matter enrichment.
527 Estuarine, Coastal and Shelf Science 68:675–685.

528 Kirkman H (1999) Pilot Experiments on Planting Seedlings and Small Seagrass Propagules
529 in Western Australia. Marine Pollution Bulletin 37:460–467. doi: 10.1016/S0025-
530 326X(99)00146-0

531 Lehmann J, Rillig MC, Thies J, Masiello CA (2011) Biochar effects on soil biota—a review.
532 Soil Biology & Biochemistry 43:1812–1836.

533 López NI, Duarte CM, Vallespinós F, et al (1995) Bacterial activity in NW Mediterranean
534 seagrass (*Posidonia oceanica*) sediments. Journal of Experimental Marine Biology and
535 Ecology 187:39–49. doi: 10.1016/0022-0981(94)00170-I

536 McGlathery KJ, Berg P, Marino R (2001) Using porewater profiles to assess nutrient
537 availability in seagrass-vegetated carbonate sediments. Biogeochemistry 56:239–263.

538 Mcleod E, Chmura GL, Bouillon S, et al (2011) A blueprint for blue carbon: toward an

539 improved understanding of the role of vegetated coastal habitats in sequestering CO₂.
540 *Frontiers in Ecology and the Environment* 9:552–560. doi: 10.1890/110004

541 Murphy J, Riley JP (1962) A modified single solution method for the determination of
542 phosphate in natural waters. *Analytica chimica acta* 27:31–36.

543 Orth RJ, Harwell MC, Inglis GJ (2005) Ecology of seagrass seeds and seagrass dispersal
544 processes. In: Larkum AWD, Orth RJ, Duarte CM (eds) *Seagrasses: biology, ecology,*
545 *and conservation*. Springer, Dordrecht, The Netherlands, pp 111–133

546 Paul D, Skrzypek G, Forizs I (2007) Normalization of measured stable isotopic compositions
547 to isotope reference scales – a review. *Rapid Commun Mass Spectrom* 21:3006–3014.

548 Pedersen O, Binzer T, Borum J (2004) Sulphide intrusion in eelgrass (*Zostera marina* L.).
549 *Plant, Cell & Environment* 27:595–602. doi: 10.1111/j.1365-3040.2004.01173.x

550 Pérez M, Invers O, Ruiz JM, et al (2007) Physiological responses of the seagrass *Posidonia*
551 *oceanica* to elevated organic matter content in sediments: An experimental assessment.
552 *Journal of Experimental Marine Biology and Ecology* 344:149–160. doi:
553 doi:10.1016/j.jembe.2006.12.020

554 Quilliam RS, Marsden KA, Gertler C, Rousk J (2012) Nutrient dynamics, microbial growth
555 and weed emergence in biochar amended soil are influenced by time since application
556 and reapplication rate. *Agriculture* 158:192–199.

557 R Core Team (2014) R Foundation for Statistical Computing. Vienna, Austria.

558 Reed SC, Townsend AR, Taylor PG, Cleveland CC (2011) Phosphorus Cycling in Tropical
559 Forests Growing on Highly Weathered Soils. In: Bünemann EK, Oberson A, Frossard E
560 (eds) *Phosphorus in Action*. Springer-Verlag, Berlin, pp 339–369

561 Risgaard-Petersen N, Dalsgaard T (1998) Nitrogen balance of temperate eelgrass *Zostera*
562 *marina* bed. *Marine Ecology Progress Series* 174:281–291.

563 Rivers DO, Kendrick GA, Walker DI (2011) Microsites play an important role for seedling
564 survival in the seagrass *Amphibolis antarctica*. *Journal of Experimental Marine Biology*
565 *and Ecology* 401:29–35.

566 Rosselló-Mora R, Thamdrup B, Schäfer H, et al (1999) The response of the microbial
567 community of marine sediments to organic carbon input under anaerobic conditions.
568 *Systematic and applied microbiology* 22:237–248.

569 Ruiz-Halpern S, Macko SA, Fourqurean JW (2008) The effects of manipulation of
570 sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a
571 subtropical carbonate environment. *Biogeochemistry* 87:113–126. doi: 10.1007/s10533-
572 007-9162-7

573 Short F, Duarte C (2001) Methods for the measurement of seagrass growth and production.
574 In: Short FT, Short CA, Coles RG (eds). *Global seagrass research methods*, Elsevier,
575 Amsterdam, pp 155–182

576 Sinsabaugh RL, Follstad Shah JJ (2012) *Ecoenzymatic Stoichiometry and Ecological Theory*.

577 Annual Review of Ecology, Evolution and Systematics 43:313–343. doi:
 578 10.1146/annurev-ecolsys-071112-124414

579 Statton J, Cambridge ML, Dixon KW (2013) Aquaculture of *Posidonia australis* seedlings
 580 for seagrass restoration programs: effect of sediment type and organic enrichment on
 581 growth. Restoration Ecology 250–259. doi: 10.1111/j.1526-100X.2012.00873.x

582 Statton J, Dixon KW, Hovey RK, Kendrick GA (2012) A comparative assessment of
 583 approaches and outcomes for seagrass revegetation in Shark Bay and Florida Bay.
 584 Marine and Freshwater Research 63:984. doi: 10.1071/MF12032

585 Statton J, Kendrick GA, Dixon KW, Cambridge ML (2014) Inorganic nutrient supplements
 586 constrain restoration potential of seedlings of the seagrass, *Posidonia australis*.
 587 Restoration Ecology 22:196–203.

588 Walker DI, Kendrick GA, McComb AJ (2006) Decline and recovery of seagrass ecosystems -
 589 the dynamics of change. In: Larkum AWD, McComb AJ, Duarte CM (eds) Seagrasses:
 590 biology, ecology and conservation. Springer, Dordrecht, The Netherlands, pp 551–565

591 Welsh DT (2000) Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria
 592 interactions and significance to primary productivity. Ecology Letters 3:58–71.

593 Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York, Springer
 594 New York

595 Wingler A (2005) The role of sugars in integrating environmental signals during the
 596 regulation of leaf senescence. Journal of Experimental Botany 57:391–399. doi:
 597 10.1093/jxb/eri279

598
 599

Table 1 Carbon (C), nitrogen (N), and phosphorus content (% d.w), and carbon:nitrogen (C:N), carbon:phosphorus (C:P), and nitrogen:phosphorus (N:P) ratios of seagrass leaves growing in sediments with different concentrations of organic matter. Values are means $\pm$ standard error (in parentheses).

	<i>Sedimentary organic matter concentration (% dry weight)</i>					
	0%	0.5%	1%	1.5%	2%	2.5%
C (% d.w)	37.3 (0.3)	37.5 (0.1)	37.1 (0.3)	37.0 (0.2)	37.3 (0.5)	37.3 (0.2)
N (% d.w)	2.0 (0.1)	2.0 (0)	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)	1.9 (0.1)
P (% d.w.)	0.15 (0.01)	0.16 (0.01)	0.14 (0.01)	0.15 (0.002)	0.14 (0.004)	0.13 (0.02)
C:N	18.6 (0.3)	18.6 (0.2)	19.4 (0.7)	19.4 (0.5)	19.8 (0.1)	20.0 (0.4)
C:P	248 (17)	226 (16)	269 (12)	239 (4)	268 (7)	287 (33)
N:P	13.4 (1.1)	12.1 (0.9)	13.9 (0.1)	12.4 (0.4)	13.6 (0.30)	14.3 (1.4)

Figures

Fig. 1 Relationship between sediment organic matter % and (a) sediment redox potential (n=3) and (b) sediment penetration resistance (n=3). Black dots represent data points, dashed lines represent linear models fitted to the data, and the grey outline shows 95% confidence intervals. Note that no data was collected for redox potential in 2.5% OM treatments

617

618

619 **Fig. 2** Expression of the enzymes α -glucosidase (a, n=3), β -glucosidase (b, n=4), alkaline
620 phosphatase (c, n=4), and acid phosphatase (d, n=3) in sediments with *Posidonia australis*
621 seedlings ranging from 0% to 2.5% OM (d.w.). Enzyme activities are in μmol^{-1} substrate
622 consumed $\text{hr}^{-1} \text{ g}^{-1}$ dry sediment. Black dots represent data points, dashed lines represent linear
623 models (where significant) fitted to the data, and the grey outline shows 95% confidence
624 intervals

625

Fig. 3 Relationship between environmental data and changes in sediment microbial communities measured by ARISA. Numbers and shading denote organic matter treatment (0 – 2.5% OM). Plot represents a distance based redundancy analysis (dbRDA) ordination of ARISA trace data derived from Bray-Curtis distance matrix and with environmental variables chosen by stepwise selection. The axes plotted account for 16.4% of the total variation

Fig. 4 Total seedling biomass (a), total leaf biomass (b), leaf productivity (c), and leaf specific biomass (d) for *Posidonia australis* seedlings growing in sediments with organic matter concentrations ranging from 0% to 2.5% OM (d.w.). Values are means from 15 replicates, with bars showing $\pm$ 1 s.e.

Fig. 5 Total rhizome biomass (a), total root biomass (b), total belowground biomass (c), and aboveground: belowground biomass ratios (d) for *Posidonia australis* seedlings growing in sediments with organic matter concentrations ranging from 0% to 2.5% OM (d.w.). Values are means from 15 replicates, with bars showing ± 1 s.e.