Estuarine, Coastal and Shelf Science 187 (2017) 216–230

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Oxygen and carbon metabolism of *Zostera muelleri* across a depth gradient – Implications for resilience and blue carbon

Angus J.P. Ferguson [a, *], Renee Gruber [a, b], Jaimie Potts [a], Aaron Wright [a], David T. Welsh [c], Peter Scanes [a]

[a] NSW Office of Environment and Heritage, Sydney, Australia
[b] University of Western Australia, Perth, Australia
[c] Griffith University, Gold Coast, Australia

ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form
8 December 2016
Accepted 4 January 2017
Available online 7 January 2017

Keywords:
Blue carbon
Seagrass
Carbon budget
Metabolism
Depth gradient

ABSTRACT

There is growing interest in the role that seagrasses play as 'blue carbon' stores or sinks, and their potential to offset rising CO_2 levels in the atmosphere. This study measured primary aspects of the carbon balance (biomass, community metabolism, dissolved organic carbon [DOC] fluxes, seston trapping) across the depth gradient in a *Zostera muelleri* meadow during the seasonal biomass minimum and maximum. Over the annual estimation, the meadow was neither a sink nor source of carbon, with inputs of seston (~58% of total inputs) balanced by exports of wrack and DOC. The carbon sink represented by wrack export depends on the nature of the environment where the wrack accumulates; if it reaches subtidal sediments it will largely be remineralised over the annual cycle, whereas between 14 and 26% of the wrack may be preserved if the material is exported to terrestrial environments. The fate of DOC exuded by seagrasses is unknown due to a lack of knowledge about its composition and lability; however, a number of lines of evidence suggest that a large fraction of DOC is mineralised. The net community metabolism (NCM) of the meadow was balanced, indicating that photosynthetic O_2 production balanced community respiration and/or the reoxidation of reduced compounds (sulphur and iron) in the rhizosphere. We suggest that a balanced NCM may be the preferred state for *Zostera* spp. and may limit their occurrence in environments where plants cannot balance the respiratory demand exerted by seston inputs. There was a close coupling between metabolism and biomass, which in turn is forced by antecedent light over the preceding 120 days (the time integration window for antecedent light that best predicted biomass). Increased metabolism with depth and seasonal variation in light is accompanied by a decrease in the above ground:below ground biomass ratio (AGB:BGB). This trend is suggested to be a morphological adaptation that balances the competing requirements of maintaining a neutral plant carbon balance across enrichment and light gradients. Our results suggest that *Zostera muelleri* may be most important as a 'blue carbon' store (i.e. carbon stored as biomass standing stock), which is therefore vulnerable to degradation if seagrasses are lost.

1. Introduction

Seagrass meadows are important and iconic coastal systems that provide a range of ecosystem services including serving as a food source and habitat for other species; stabilising subtidal sediment; and constituting a store of carbon (Heck et al., 2003; Emmett Duffy, 2006; Waycott et al., 2009; Kennedy et al., 2010). Recent concern over rising atmospheric carbon dioxide levels due to climate change has prompted greater scientific focus on the 'blue carbon' sink potential represented by seagrasses (Duarte et al., 2010) (Fourqurean et al., 2012). Seagrass meadows store carbon in living biomass ('stores') and as buried refractory seagrass detritus ('sinks'), such as rhizome mats in *Posidonia* meadows (Duarte et al., 2011). Many seagrasses exist in sediments with low organic carbon (Ferguson et al., 2016), suggesting they may store carbon primarily in living biomass, therefore the significance of their role as 'blue carbon' stores depends on their continued survival (Lavery et al., 2013a). Seagrasses are under increasing pressure globally due to multiple stressors associated with human development (Orth et al., 2006), and global declines have already

* Corresponding author.
E-mail address: angus.ferguson@environment.nsw.gov.au (A.J.P. Ferguson).

http://dx.doi.org/10.1016/j.ecss.2017.01.005

resulted in substantial loss of carbon storage (McLeod et al., 2011).

Central to determining the carbon balance of seagrass meadows is a knowledge of the net community metabolism (NCM), defined as gross primary production (GPP) less community respiration (CR) over the diel period, which can also be expressed as a ratio of GPP to CR (P:R). Assessments of NCM are commonly based on the measurement of O_2 and/or CO_2 fluxes over a diel period (Duarte et al., 2010)). Net autotrophic systems (P:R > 1) fix more carbon than is released by respiration and provide the conditions for a potential carbon sink, as long as that carbon is buried either within the meadow or elsewhere. Balanced NCM (NCM = 0, P:R = 1), implies that all allochthonous and autochthonous carbon inputs are balanced by microbially mediated remineralisation of the labile organic matter (OM) plus burial of refractory material. In the absence of significant burial of OM within the meadow, a balanced NCM implies that it functions primarily as a store of carbon in living biomass. Net heterotrophic metabolism (NCM < 0, P:R < 1) implies an allochthonous carbon source to the meadow. However, the net import or export of carbon from the seagrass meadow complicates this simple assessment of carbon budgets based on NCM or P:R alone. If carbon is exported as 'wrack' (dead seagrass biomass) or exuded dissolved organic carbon (DOC), its fate is crucial to determining whether the meadow is a sink of carbon (Duarte and Cebrian, 1996). The import of organic matter (e.g. via trapped seston (Gacia et al., 2002); may contribute significantly to community metabolism and further complicate the assessment of the meadow carbon balance (Barron et al., 2004). Finally, there may also be temporal lags between O_2 production and consumption where OM undergoes anaerobic remineralisation and O_2 consumption is dominated by the re-oxidation of reduced compounds (e.g. sulphur) (Jφrgensen, 1982). Clearly, a comprehensive assessment of the role seagrass meadows play in the global carbon budget must consider rates of autochthonous and allochthonous inputs to the system, respiration and burial within the system, exports from the system and the fate of these exports (Macreadie et al., 2014). To date there are few examples of closed carbon budgets for seagrass meadows, with conclusions commonly being made despite the exclusion of one or more important terms.

Seagrass meadows commonly extend across wide depth gradients, with plants acclimating their morphology and physiology in order to optimise their growth across a range of light conditions (Collier et al., 2007). Seagrass productivity is commonly estimated ex situ or using fluorescence methods that do not include the natural benthic community, but *in situ* measures of NCM along a depth gradient are rare (Duarte et al., 2010; Maher and Eyre, 2011). Given the likely variation in processes such as net primary production and particle trapping across the depth gradient, extrapolation of results from a single depth is unlikely to realistically represent NCM of the meadow as a whole. Furthermore, variation in plant carbon balance across the depth (light) gradient is also a key feature determining depth limits and resilience to poor sediment conditions and periods of low light (Ralph et al., 2007).

Long-term persistence of seagrass meadows is critical to any evaluation of their continuing role in large-scale carbon budgets. Persistence is the result of an interaction between disturbance regimes and meadow resilience (Kilminster et al., 2015). The maintenance of a neutral plant carbon balance over seasonal timescales is critical in determining seagrass resilience at a given location (Dennison and Alberte, 1985). Seagrasses display a high degree of morphological plasticity across light and enrichment gradients (Perez et al., 1994; Lee and Dunton, 2000; Collier et al., 2007; Ralph et al., 2007; Clores and Santos Carandang, 2013, Ferguson et al., 2016), which is thought to be a strategy to balance the respiratory demands of non-photosynthetic organs (e.g. roots and rhizomes) with the oxygen production potential of its above ground biomass (Hemminga, 1998). The ratio of above ground:below ground biomass (AGB:BGB) therefore provides an important metric of functional morphological response to environmental drivers (Lee and Dunton, 2000). Despite the importance of this link, there have been few studies that simultaneously measure seagrass metabolism and biomass response t o environmental gradients. This knowledge gap impedes the development of robust seagrass resilience models that can be confidently applied across the range of environments colonised by seagrass. This, in turn, limits assessment of the potential future role of seagrass meadows in large scale carbon budgets.

This study measured key aspects of the carbon balance across the depth gradient of a seagrass (*Zostera muelleri*) meadow during the annual biomass minimum (early spring) and maximum (late summer). The aims were 1) to provide a comprehensive carbon budget for the meadow in order to determine whether it acted as a carbon store or sink, and 2) provide insights into seagrass community metabolism with respect to environmental gradients.

2. Methods

2.1. Study site

Lake Macquarie is an estuarine lagoon located on the east coast of Australia (Fig. 1). The region experiences a warm temperate climate, with most rainfall occurring during late summer to autumn. Water temperatures within the lake basin range from ~16 °C in winter to 26 °C in the summer (Table 1). A relatively small catchment area to lake volume ratio combined with efficient tidal flushing means that salinity within the lake is generally close to seawater, though large rainfall events can cause a reduction in surface salinities to <20. Tidal range is greatly attenuated by the constrained lake entrance and the tidal range within the lake basin is < 10 cm. Generally low nutrient concentrations within the lake indicate an oligotrophic system (Ferguson et al., 2016). This study was carried out in an extensive *Zostera muelleri* meadow (Swansea shoals) located just south of the lake's entrance (Fig. 1). Data were collected at 0.75 m, 1.5 m and 3 m water depth, corresponding to the upper, middle and lower limits of seagrass depth distribution in the meadow. Water quality data for the Swansea shoals site during the sample times (early September 2012, 'early spring'; and mid February 2013, 'late summer') are presented in Table 1. The sample times were close to the seasonal extremes of temperature, with similar salinity. Water clarity (as indicated by secchi depth) was lower during the late summer sample time, most likely due to higher phytoplankton biomass (indicated by chlorophyll-a concentration) at this time (Table 1).

2.2. Sediment properties and seagrass biomass

Samples for seagrass biomass measurements (n = 5) were taken by SCUBA divers using 250 mm I.D. cores at each depth during each sample time. Samples were stored on ice and processed within 2 days of collection. Processing involved carefully washing sediment and dead material from the sample and separating above ground biomass (AGB = leaves and shoots), and below ground biomass (BGB = roots and rhizomes). Samples were freeze dried and weighed, with biomass normalised to g DW m^{-2} using the surface area of the collection core. Samples for grain size and organic matter (n = 5) content were collected from each depth during summer by SCUBA divers using 90 mm I.D. cores at each depth during each season. Cores were gently extruded and the top 1 cm removed and frozen until analysis. Intact samples from 2 cores were combusted (550 °C for 4 h) to determine OM content. A pilot study conducted prior to sampling indicated that it was not

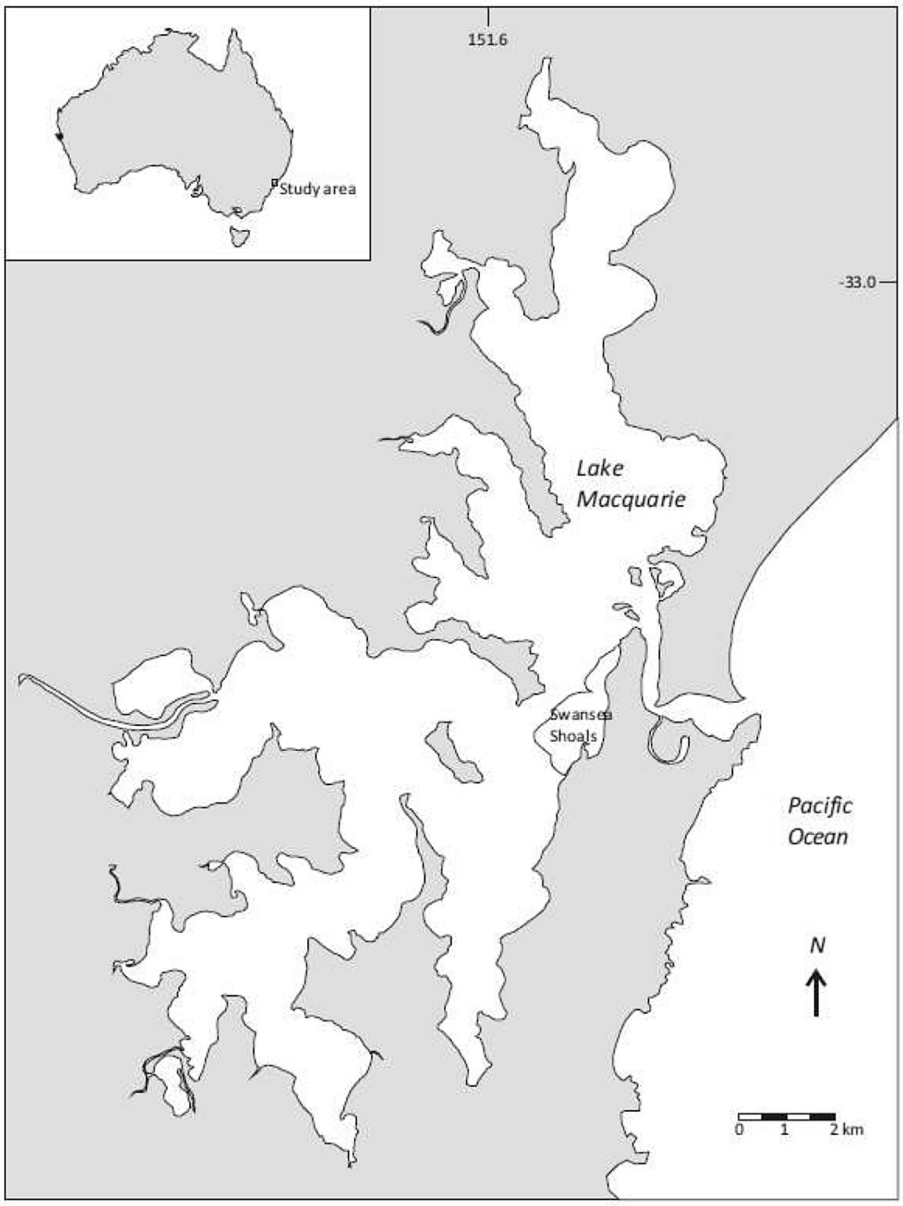

Fig. 1. Map of Lake Macquarie showing the Swansea shoals study area to the south of the lake entrance.

necessary to treat these sediments with hydrochloric acid to remove carbonates, which are lost on ignition and can misrepresent OM content.

2.3. Particulate trapping

In situ estimates of net sediment resuspension and deposition in seagrass beds and along depth gradients were made using sediment traps during the summer field study, and at three additional sites within Lake Macquarie during the summer and winter over the year preceding the seagrass metabolism experiments. A significant linear relationship was found between water column chlorophyll-a and seston trapping rates ($r^2 = 0.57$; $p < 0.001$). This empirical relationship was used to estimate the seston trapping rates for the early spring sample time (when seston trapping was not measured) based on chlorophyll-a measured at the time (Table 1). Traps were shallow circular dishes constructed of clear polycarbonate with an aspect ratio (height:diameter) of 0.25 (Flower, 1991). This design resulted in sediment resuspension from or deposition into the traps, allowing the net deposition of material within seagrass beds to be estimated. Duplicate traps were deployed at approximately 100 m intervals along four transects across the depth gradient of the seagrass meadow Prior to deployment, a known weight of pre-ashed (550 °C for 4 h) natural sediment was placed into the traps, which were then filled with seawater and sealed. This pre-filling with sediment gave the traps a

Table 1
Water quality and secchi depth at the study site during the sample times. The minimum and maximum values are for the period between Jan 2012 and Jan 2014.

	Temperature°C	Salinity	chl-a µg L^{-1}	TSS mg L^{-1}	Turbidity NTU	Secchi m
min	15.2	28.7	0.6	1.4	0.7	2.7
Sep-2012	17.3	34.3	0.5	2.4	1.1	5.1
Feb-2013	25.6	33.9	1.7	2.6	1.5	4.7
max	26.3	36.5	3.9	10.1	2.9	6.3

collection surface with natural roughness. Sediment traps ($n = 4$) were then fastened to stakes at each deployment location and positioned approximately 1 cm above the sediment surface. Aboveground seagrass biomass was not removed around traps.

The sediment traps were retrieved 4 days following deployment. Working carefully, a flexible plastic lid was used to seal the traps *in situ* so as not to disturb their contents during retrieval. Upon return to the laboratory, traps were refrigerated (4 °C) until processing occurred (<10 d). Trap contents were removed and dried (60 °C for >24 h) and weighed, before ashing (550 °C for 4 h) to derive total and organic matter accumulations. No seagrass leaf fragments greater than 1 mm diameter were observed in the trap contents.

2.4. Wrack decomposition

Wrack decomposition experiments were carried out in April 2013 (mid autumn) when water temperatures were approximately 17 °C. Rates of break-down of shed AGB ("wrack") were estimated for three experimental treatments: supratidal (deposited on the shoreline), submersed, and buried (subtidal). Fresh seagrass leaves were cleaned of epiphyte biomass and weighed (~20 g) into cylindrical containers (75 mm diameter × 50 mm high) with nylon mesh (1 mm) securing each end. Three replicate containers were exposed to each of the experimental treatments along each of three transects running perpendicular to the shoreline (i.e. n = 9 per treatment). A single container was retrieved from each treatment on each transect at 12 days and 32 days after deployment (n = 3 per treatment, per time). After retrieval, seagrass within the containers was rinsed with fresh water to remove sediment and fauna and oven dried (60 °C for >48 h) before weighing. Starting oven-dry weights in each container were estimated from the wet:dry weight ratios (0.31 ± 0.019 SD) derived from 50 samples prior to the experiment. The amount of material lost and rate of loss could then be calculated from the beginning of the experiment and from between the first and second collection periods.

2.5. Seagrass community metabolism

Community metabolism was measured using *in situ* benthic chambers at shallow (0.75 m), intermediate (1.5 m) and deep (3 m) sites within the Swansea Shoals during the annual biomass minimum (early spring, Sept 2012) and maximum (late summer, Feb 2013). Replicate chambers (n = 3) were deployed by divers at each depth during each sample time. Benthic chambers consisted of a flanged stainless steel base (diameter 450 mm 150 mm deep), and a detachable clear plexiglass top (diameter 450 mm, height 600 mm) fitted with a circulation pump and a Hobo U26 optical dissolved oxygen and temperature logger. The base was inserted into the sediment to the depth of the upper flange, taking care to avoid directly cutting rhizomes or trapping seagrass leaves. A pre-incubation period of 24 h was observed to allow any disturbance of sediment redox profiles associated with inserting bases to re-equilibrate with surrounding sediments. Incubations (24 h) commenced once the clear plexiglass tops were clipped onto the chamber bases in the afternoon of the following day. Incident light (above the water surface) and light at the seagrass canopy was logged at 15 min intervals at each site using Dataflow Odyssey light loggers. Loggers recording dissolved oxygen and temperature (Hobo U26) were also deployed outside the chambers just above the seagrass canopy at each depth in order to compare measurements made in the chambers with ambient water quality. These comparisons showed that the maximum and minimum concentrations of dissolved oxygen within the chambers over the 24 h incubation were within 85% of ambient concentrations, hence the containment effects of the chambers were deemed acceptable (Adams et al., 2015). Samples for dissolved organic and inorganic nitrogen were withdrawn via sample lines to each chamber at dusk, midnight, dawn, midday and 4 p.m. As the sample was withdrawn, an equal volume of ambient site water was drawn into the chamber via a small collapsible bag attached to the chamber (sample volume:chamber volume = 0.05%). Concentrations of t_1 and t_2 samples were corrected for dilution by replacement water drawn into the chamber during the preceding sample time. Nutrient samples were filtered immediately through 0.45 μm filters and stored at −20 °C until analysis.

2.6. Analytical methods

All nutrient analyses were carried out colourmetrically using a Lachat™ Flow Injection Analysis. Analytical accuracy for nutrient analysis was maintained using standard additions of certified laboratory standards in both Milli-Q and low nutrient seawater. Nitrite (NO_2^-) was determined using sulphanilamide, oxidised nitrogen (NO_x) was determined by cadmium reduction and nitrate (NO_3^-) was determined as the difference between NO_x and NO_2^-. Ammonium (NH_4^+) was determined using hypochlorite/phenolate (5.1%) and dissolved inorganic nitrogen (DIN) was determined as the sum of NO_x and NH_4^+. Total dissolved nitrogen (TDN) was determined by persulfate digestion and sulphanilamide (Valderrama, 1981) and dissolved organic nitrogen (DON) determined as TDN minus DIN.

2.7. Sediment oxygen demand

Oxygen consumption of sediments between roots and rhizomes was measured using *ex situ* sediment core incubations during each sample time. Replicate sediment cores (n = 5) were carefully collected by divers in polycarbonate tubes (500 mm × 90 mm diameter) at each depth, taking care not to include any root or rhizome material. Cores were capped and kept in the dark and at *in situ* temperature before being transferred to an incubator within 3 h of collection. Cores were stirred at a rate below the sediment resuspension threshold and flushed with site water at *in situ* temperature for 4 h to allow any disturbance of sediment redox profiles to re-equilibrate with surrounding sediments. Cores were then sealed and incubated for 4 h in the dark, with oxygen consumption rates determined from start − end point measurements using an optical dissolved oxygen probe (Hach RDO). All end point concentrations were within 15% of the initial concentrations, therefore containment effects were deemed acceptable.

2.8. Calculations

Seagrass community fluxes of oxygen and dissolved organic nitrogen (mmol O_2 m^{-2} h^{-1}) were calculated using the equation:

$$flux = \frac{\partial C}{\partial t} \times V \div SA \quad (1)$$

C is the solute concentration; V = volume of the overlying water within the chamber; SA = the surface area enclosed by the chamber.

Net primary productivity (i.e. positive O_2 flux) was only observed when light at the seagrass canopy exceeded 120 μE m^{-2} s^{-1} (referred to hereafter as the 'light-saturated period' or τ_{light}). The rate of community respiration (CR) was estimated by averaging O_2 flux rates for each hour (t) during the non light-saturated + dark periods:

$$CR = \frac{1}{\tau_{dark}} \int_{sunset\_sat}^{sunrise\_sat} O_2\ flux\ (t)\ dt \quad (2)$$

where τ_{dark} is the time elapsed (in hours) from the end of the light-saturated period in the afternoon ($sunset_{sat}$) to the beginning of the light-saturated period after the following dawn ($sunrise_{sat}$). Note that CR based on O_2 flux yields a negative value. The rate of net primary productivity (NPP in mmol O_2 m^{-2} h^{-1}) was estimated by averaging O_2 flux rates over the light-saturated period:

$$NPP = \frac{1}{\tau_{light}} \int_{sunrise\_sat}^{sunset\_sat} O_2\ flux\ (t)\ dt \quad (3)$$

where τ_{light} is the time elapsed (in hours) from the beginning of the light-saturated period after the dawn ($sunrise_{sat}$) to the end of the light-saturated period in the afternoon ($sunset_{sat}$). Gross primary productivity (GPP in mmol O_2 m^{-2} h^{-1}) was calculated as the sum of NPP and the absolute value of CR. Net community metabolism (NCM) and P:R were calculated as:

$$\mathrm{NCM} = \left(\mathrm{GPP} \times \tau_{\mathrm{light}}\right) + (\mathrm{CR} \times 24) \quad (4)$$

$$\mathrm{P:R} = \left(\mathrm{GPP} \times \tau_{\mathrm{light}}\right) \Big/ (-\mathrm{CR} \times 24) \quad (5)$$

Estimations of NCM and P:R were checked for consistency against concentration data from sunset on the first and second day of the incubation, which confirmed the use of light-saturated hours for scaling measurements to daily estimates.

Photosynthetic quotients (PQ = moles O_2:moles CO_2) can theoretically vary between 1 and 1.3 depending on factors such as the source of nitrogen, the amount of photorespiration occurring and physiological state of the plant (Zeigler and Benner, 1998). If nitrate is the sole source of nitrogen used the PQ would equal 1.3, due to reduction of nitrate to ammonium within the cell (Falkowski and Raven, 1997). At our site, ambient concentrations of nitrate and ammonium in the water column were at or below detection limit, and rhizosphere porewaters were dominated by ammonium with nitrate not detected (Gruber unpublished data). As such we have adopted a PQ of 1, which is consistent with studies assuming a PQ for seagrass systems in the absence of data e.g. (Herzka and Dunton, 1997; Duarte et al., 2010). The reported range for respiratory quotients is smaller RQ range 0.8–1.14: (Zeigler and Benner, 1998), hence we have adopted an RQ of 1 for simplicity and consistency with (Duarte et al., 2010).

Biomass-specific metabolism, below ground biomass-specific O_2 consumption ($BGBO_2$) and above ground biomass-specific GPP were calculated from the equations:

$$\mathrm{BGBO_2}\left(\mathrm{mmol\ O_2\ h^{-1}\ g\ DW^{-1}}\right) = \mathrm{CR}\left(\mathrm{mmol\ O_2\ m^{-2}\ h^{-1}}\right) \Big/ \mathrm{BGB}\left(\mathrm{g\ DW\ m^{-2}}\right) \quad (6)$$

$$\mathrm{GPP}\Big/\mathrm{AGB}\left(\mathrm{mmol\ O_2\ h^{-1}\ g\ DW^{-1}}\right) = \mathrm{GPP}\left(\mathrm{mmol\ O_2\ m^{-2}\ h^{-1}}\right)\Big/\mathrm{AGB}\left(\mathrm{g\ DW\ m^{-2}}\right) \quad (7)$$

2.9. Carbon budget

A carbon budget was constructed for the seagrass meadow accounting for depth dependent variation in metabolism (GPP and CR), seasonal biomass change, particulate trapping, and losses due to exudation of dissolved organic carbon and the shedding of wrack. The budget is predicated on the assumption that our measurements were made close to the annual minimum and maximum of biomass and metabolism for this site (Fig. 2; see also (Ferguson et al., 2016)). As such, the rates of seasonal biomass change were estimated as:

$$\Delta\mathrm{biomass}/\Delta\mathrm{t} = \left(\mathrm{biomass_{summer}} - \mathrm{biomass_{spring}}\right)/\mathrm{t} \quad (8)$$

The budget accounts for all inputs and losses during this period, assuming that it accounts for the total annual fluxes of biomass production and degradation. While it is likely that metabolic rates varied at the winter and summer solstices due to the effects of temperature and light, the average of measurements made close to the equinoxes represent reasonable estimates of the mean annual range. Losses of carbon due to exudation of dissolved organic carbon (DOC) by seagrass plants was estimated from mean dissolved organic nitrogen fluxes (±SE) assuming a DOC:DON of 42 ± 9 (Barron and Duarte, 2009). The error term for DOC loss in the budget was calculated from the measured variability in DON fluxes multiplied by the reported variability in DOC:DON fluxes (Barron and Duarte, 2009).

The upper limits to wrack production were estimated using two independent measures of GPP. Leaf extension measurements carried out at a nearby site during spring and summer (mean rate of AGB production 2.3 g m^{-2} d^{-1}; Gruber unpublished data) were converted to mmol carbon assuming a carbon content of 33% (Duarte, 1990). Maximum potential wrack production was assumed to equal the rate of gross AGB gain (from leaf extension estimates) minus the measured rates of AGB gain:

$$\mathrm{Wrack_{maximum1}} = \mathrm{AGB\ gross_{leaf\ extension}} - \mathrm{AGB\ gain_{biomass\ measurements}} \quad (9)$$

The second estimate of upper limits to wrack production was estimated by difference between GPP (estimated from the chamber incubations) and measured losses and stores:

$$\mathrm{Wrack_{maximum2}} = \mathrm{GPP_{oxygen\ flux}} - \mathrm{DOC_{DON\ flux}} - \mathrm{Total\ biomass\ gain_{biomass\ measurements}} \quad (10)$$

Both of these terms for the upper limit to wrack production include other unknown terms in the seagrass meadow budget (e.g. contributions from ephytic and benthic microalgae to GPP measured in the chambers, grazing of seagrass biomass, burial within the meadow). The predicted gain of BGB was estimated by subtracting losses due to DOC and allocation to AGB (based on leaf extension):

$$\mathrm{BGB_{predicted}} = \mathrm{GPP_{oxygen\ flux}} - \mathrm{DOC_{DON\ flux}} - \mathrm{AGB\ gross_{leaf\ extension}} \quad (11)$$

Budget errors were estimated from standard error of the mean for single terms (e.g. GPP) and where propagated errors due to the multiplication of two terms was likely (e.g. $z = x \times y$) from the equation:

$$\mathrm{Error_z} = \left[(\mathrm{error_x}/\mathrm{mean_x})^2 + (\mathrm{error_y}/\mathrm{mean_y})^2\right]^{0.5} \quad (12)$$

Where terms were added or subtracted, error was estimated from:

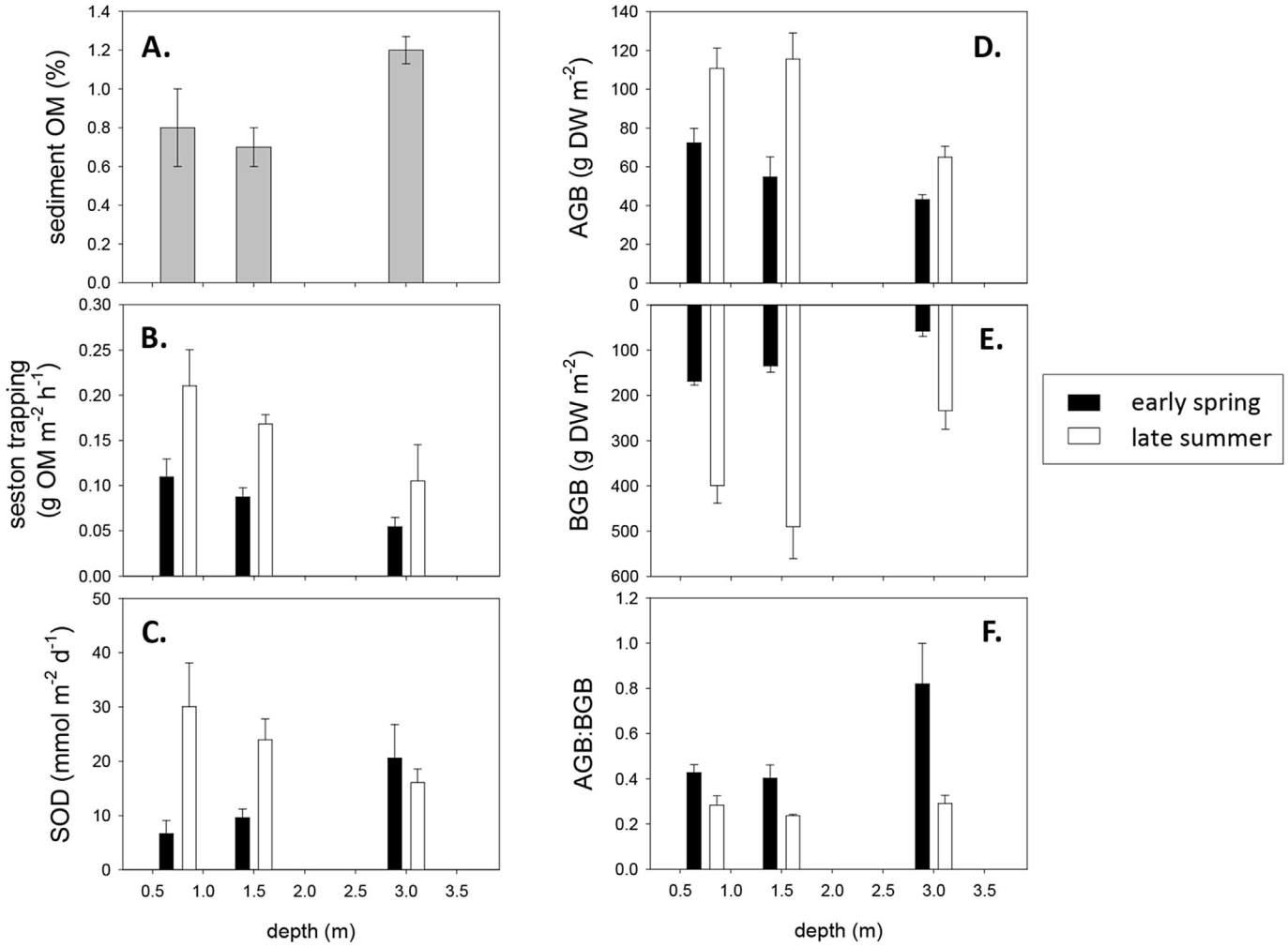

Fig. 2. A) Sediment organic matter (OM) content, **B)** particulate trapping rates; **C)** Sediment oxygen demand (SOD); **D)** seagrass above ground biomass (AGB); **E)** below ground biomass (BGB); and **F)** the AGB: BGB ratio. All plots show means ± SE.

$$\text{Error}_z = \left(\text{error}_x^2 + \text{error}_y^2\right)^{0.5} \quad (13)$$

2.9.1. Statistical analysis

Differences between depths and seasons were tested by 2 way ANOVA using the SPSS 13.0 software package. Heterogeneity of variances and normality of data were tested and data were transformed where necessary. If differences among depths were found, Bonferroni and Tamhane post hoc tests (SPSS v.10) were used to identify which means differed.

3. Results

3.1. Wrack decomposition

Decomposition of seagrass leaves (wrack) was significantly greater during the initial 12 days of incubations, with no differences in rates between the environments (Table 2). Decomposition rates slowed by an order of magnitude after 12 days, with slightly higher rates recorded in the submersed treatment. Assuming similar low rates for the rest of the year it is estimated that no wrack would remain in the submersed treatment, while there would be 14% and 26% remaining in the supratidal and buried submersed pools respectively.

3.2. Sediments and particulate trapping

Organic matter content of surface sediments was below 1% at the shallow and intermediate sites, and slightly higher at the deepest site (Fig. 2). Particulate trapping rates measured during the late summer sample time decreased with depth, with significantly lower rates at the 3 m depth ($p < 0.001$). Particulate trapping rates during the early spring sample time were estimated to be approximately half the late summer rates (based on water column chlorophyll-*a* concentrations). Sediment oxygen demand (SOD) displayed a significant increase with depth during early spring and

Table 2
Percentage loss of wrack under different environmental conditions (data from Gruber et al. in prep).

Environment	% loss d^{-1} 0 -12 days	% loss d^{-1} 12–32 days	% remaining @ 365 days
Supratidal	5.23%	0.19%	14%
Submersed	4.62%	0.48%	0%
Buried	4.96%	0.11%	26%

a significant decrease with depth during late summer (Fig. 2). There were significant differences between early spring and late summer at the 0.75 m and 1.5 m depths ($p < 0.001$).

3.3. Seagrass biomass and light

Seagrass biomass in the meadow was dominated by below ground biomass (BGB) with mean values ranging from 58 g DW m^{-2} during the annual minimum in early spring to 490 g DW m^{-2} during late summer. Mean above ground biomass (AGB) ranged between 43 g DW m^{-2} during early spring to 116 g DW m^{-2} during late summer (Fig. 2). There were significant increases in biomass between early spring and late summer ($p < 0.001$), with a significant season × depth interaction reflected by greater seasonal variation at the intermediate and shallow sites and much smaller variation at the deep site. Light decreased across the depth gradient during each sample time, and was similar at each depth between seasons in terms of saturated hours and light quanta reaching the seagrass canopy, due in part to the lower water clarity during the late summer sample time (Table 1). There was however a significant difference in antecedent light for the two sample times (Table 3 and Fig. 3). Antecedent light history over the preceding 120 days was the best predictor of AGB during this study (Fig. 3). There were no relationships between light quanta measured during the incubations and either biomass or GPP.

3.4. Seagrass metabolism

Community respiration was positively related to seagrass biomass, ranging between −2.2 mmol m^{-2} h^{-1} during early spring and −9.9 mmol m^{-2} h^{-1} during late summer (Fig. 4). There was an overall significant increase in CR during late summer ($p < 0.001$), and significantly greater CR at the intermediate depth during both seasons ($p < 0.001$). Oxygen uptake at the beginning of the night was significantly higher than at dawn indicating enhanced CR at the end of the light period. Sediment O_2 demand (SOD) estimated from ex situ incubations accounted for between 7.5% and 36% of CR during early spring (mean 17%), and between 10% and 21% during late summer (mean 14%). There was a strong negative relationship between SOD and P:R within seasons (Fig. 5). Below ground biomass specific O_2 consumption increased significantly with depth during both seasons ($p < 0.001$), and were significantly were higher during early spring (mean = 0.78 mmol m^{-2} d^{-1} g DW^{-1}) compared to late summer (mean = 0.47 mmol m^{-2} d^{-1} g DW^{-1}; Fig. 4).

Light O_2 efflux was measured in all chambers during light-saturated hours indicating net primary productivity during the day. There was a consistent trend of lower O_2 efflux during the second half of the day post zenith (Fig. 6), most likely due to higher respiration rates during this period (Adams et al., 2015; Rheuban et al., 2014b). This decrease in light O_2 flux during the post zenith period was matched by significant increase in dissolved organic nitrogen efflux (Fig. 6). Gross primary productivity (GPP) was positively related to seagrass biomass (Fig. 7), and ranged between 3.1 mmol m^{-2} h^{-1} during spring and 27 mmol m^{-2} h^{-1} during summer (Fig. 4). There was a significant increase in GPP during late summer ($p < 0.001$), and GPP was significantly greater at the intermediate site compared with the other depths ($p < 0.001$). Biomass specific GPP increased between the shallow and intermediate depths ($p < 0.02$) with no significant difference between seasons (Fig. 4). In contrast, biomass-specific GPP was lowest at the deep site during early spring and increased significantly during late summer ($p < 0.001$) to the highest value recorded during the study (Fig. 4).

Net community metabolism tended to be heterotrophic at all depths during early spring (Fig. 4). During late summer, NCM was slightly autotrophic at the shallow and intermediate sites and moderately autotrophic at the deep site. The seasonal variation in NCM increased with depth, so that there was minimal difference at the shallow site between seasons, and a large shift from heterotrophy to autotrophy at the deep site. Community P:R tended to be net heterotrophic at low seagrass biomass and balanced at a biomass of 250 g DW m^{-2} and above (Fig. 7).

3.4.1. Carbon budget

The oxygen flux budget indicated that the Swansea Shoals seagrass meadow was balanced in terms of oxygen metabolism over the annual estimation (NCM = −0.9 mmol m^{-2} d^{-1} and P:R = 0.99; Table 4 and Fig. 8). Of the measured GPP, 20% was lost as DOC exudates, 5% and 30% incorporated into AGB and BGB standing stocks respectively, and 45% lost as wrack. The predicted potential increase in BGB (GPP minus DOC production and gross AGB production based on leaf extension) closely matched measured rates of increase in the BGB standing stock. Assuming all DOC and wrack is exported, GPP retained in the meadow only accounted for about 35% of the measured rates of community respiration, suggesting that other inputs of organic matter are required to sustain heterotrophic activity within the seagrass meadow. Accounting for mean depth integrated rates of seston deposition brought close agreement between net carbon inputs and community respiration ((GPP − DOC − wrack + OM deposition)/community respiration = 0.97). As such, seston deposition accounted for the majority of carbon retained in the system (~59%).

The export of wrack accounts for the greatest potential loss of carbon from the system, followed by DOC (Table 4). It is unclear what proportion of these components are retained and broken down within the system. The residual discrepancy between the oxygen and carbon flow budgets can be accounted for by assuming that 10% of the wrack produced is retained within the meadow, which brings close agreement between all measures (Table 4). Assuming that DOC and 90% of the wrack are exported, the ratio of carbon imports via seston deposition, to exports (via DOC and wrack) is close to unity (0.99), suggesting the meadow is most likely carbon neutral within the lake system.

Table 3
Light climate at the study site. The hours are the number of hours that light exceeded saturation (120 μE m^{-2} s^{-1}) at each depth. The first column of light values is the measured light at each depth during the incubations (mol m^{-2} d^{-1}), and the 120 day mean is the antecedent mean daily light (mol m^{-2} d^{-1}) at each depth averaged over the preceding 120 days (based on conversion of solar exposure data).

	September			February		
	hours	light	120day mean	hours	light	120day mean
incident	10.7	50.8	24.3	12.3	78.2	43.2
0.75 m	10.0	23.3	13.8	10.5	18.8	23.6
1.5 m	9.8	18.5	11.3	9.3	14.0	19.3
3.0 m	8.8	9.0	5.6	8.8	7.5	9.6

4. Discussion

4.1. Net community metabolism

The ranges of GPP, CR and NCM measured in *Zostera muelleri* meadows during this study and in other Australian systems (Eyre et al., 2011; Maher and Eyre, 2011) are similar to ranges reported for *Zostera marina* in 14 northern hemisphere temperate systems (Fig. 9 (Duarte et al., 2010);). Comparison of AGB-specific GPP revealed little difference between *Z. muelleri* and *Z. marina* (means 1.66 and 1.56 respectively), indicating similar productive potential.

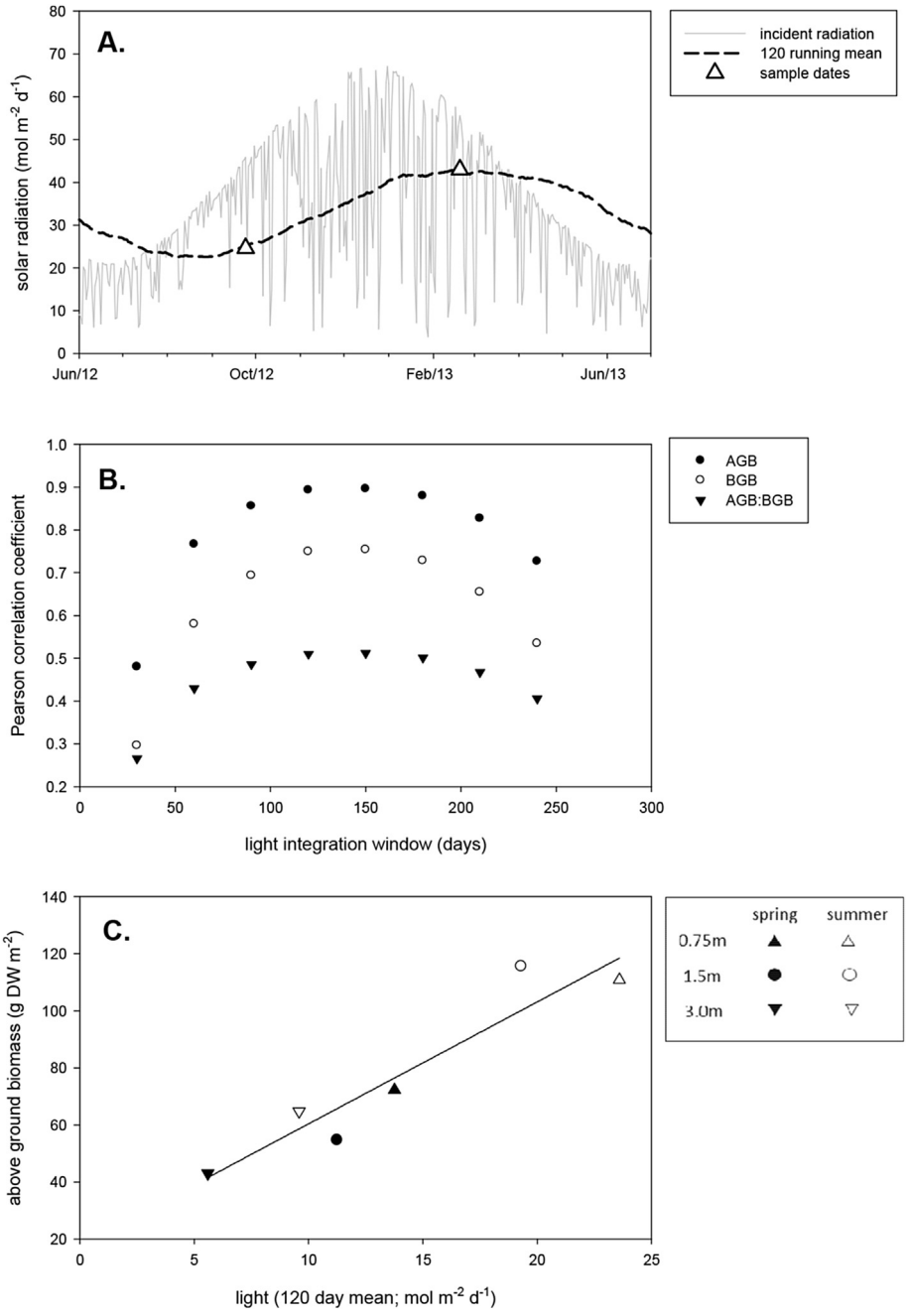

Fig. 3. A) Incident solar radiation at the study site, including the running 120 mean. B) Pearson correlation coefficients for the predictive power of different light integration windows for seagrass above ground biomass at the study site. C) The 120 day light integration window was the best predictor of above ground biomass.

Hence, the higher variation in GPP and CR reported for *Z. marina* most likely reflects the wider range of AGB (and hence metabolism) associated with measurements made across a wider range of enrichment states. Nutrient enriched habitats can support higher AGB (and hence metabolism) in *Z. muelleri* (Ferguson et al., 2016), and a similar trend is likely for *Z. marina* (Duarte, 1995). There are also likely to be variations in particulate trapping and organic matter content of the sediments at the *Z. marina* sites that may contribute to variability in measurements of CR. The narrower range of our metabolism estimates may therefore reflect the oligotrophic status of Swansea Shoals relative to some of the systems reported in Duarte et al. (2010). Our results are distinctly different from the reported values for *Zostera capricorni* (since reclassified as *Z. muelleri*) at another site within Lake Macquarie (Eyre and Ferguson, 2002). This site is located in a poorly flushed bay and is subject to relatively high catchment loads of suspended sediments and nutrients. As such, NCM at this site tends to be consistently net heterotrophic and seagrass presence is ephemeral (Ferguson pers. obs.)

It is notable that the NCM and P:R ratios for *Z. marina* and *Z. muelleri* were close to balanced with similar ranges (Fig. 9, see Rheuban et al., (2014b)). Both species occur in estuarine habitats and are therefore exposed to periodic inputs of seston that can contribute significantly to the CR of the meadow, as demonstrated by this study (Table 4) and others (Gacia et al., 2002; Greiner et al., 2016). Increased CR is usually associated with higher rates of

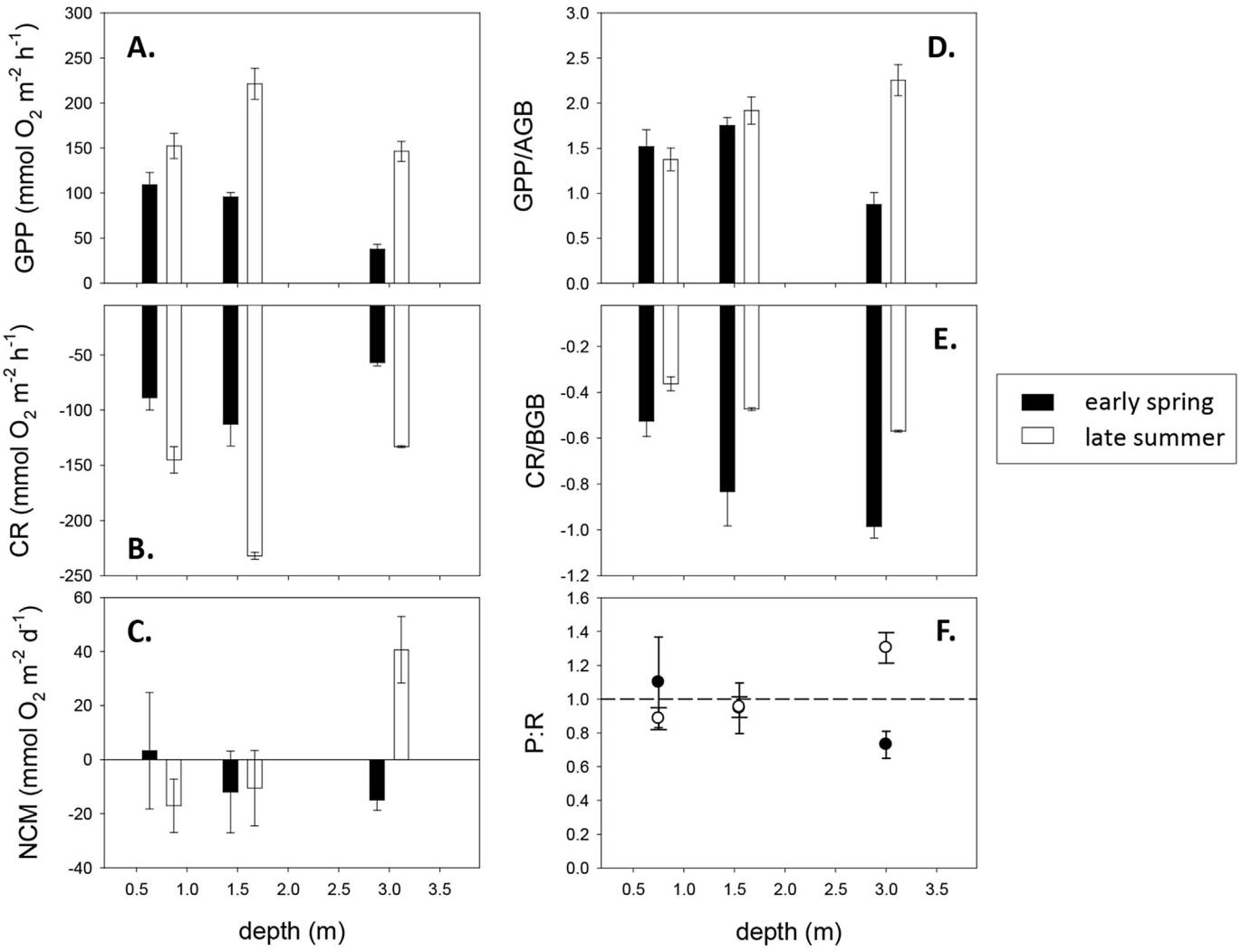

Fig. 4. A) Gross primary production (GPP); **B)** Community respiration (CR); **C)** Net community metabolism (NCM); **D)** and above ground biomass-specific GPP; **E)** Below ground biomass-specific CR; **F)** Community P:R. All plots show means ± SE.

sulphate reduction, which carries with it the risk of sulphide toxicity within the rhizosphere (Holmer et al., 2005). Therefore, the ability of seagrass to sustain itself within a given location must be directly proportional to the local rate of reoxidation and/or binding of reduced sulphur, since the annual burial of reduced sulphur in or adjacent to the rhizosphere would lead to an increasingly

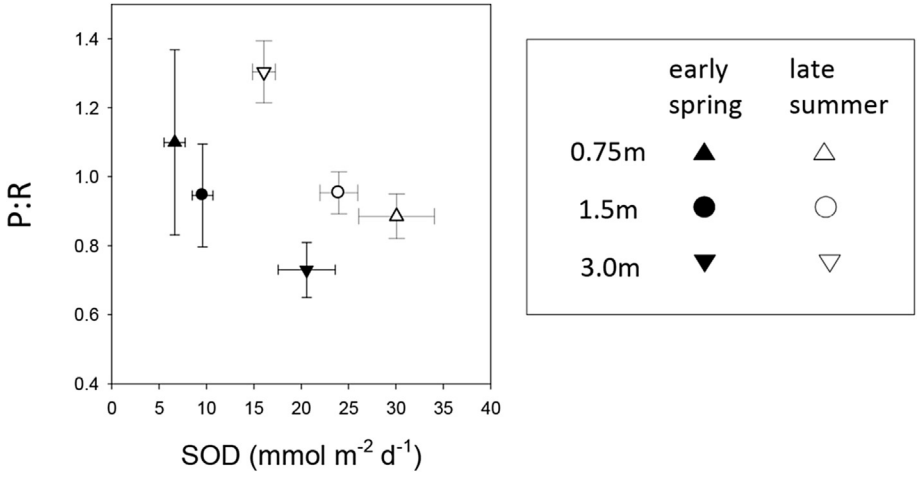

Fig. 5. The relationship between sediment oxygen demand and community P:R. Plots show means ± SE.

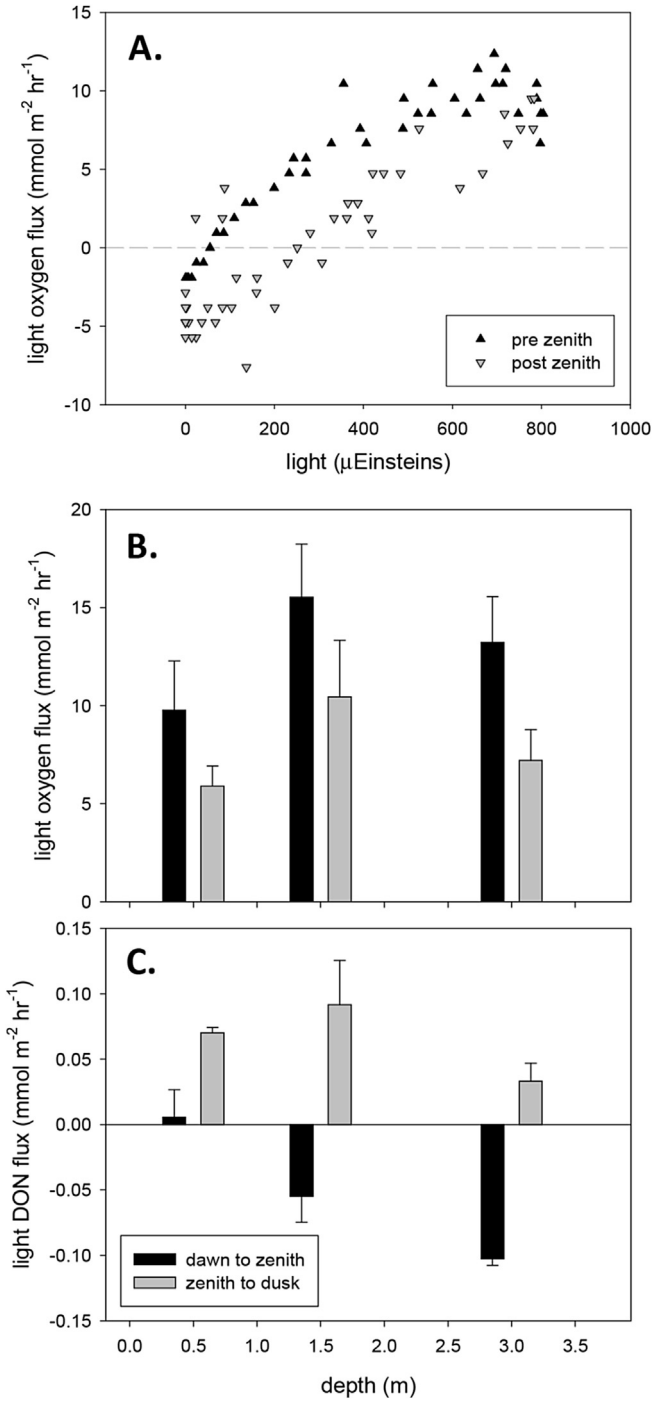

Fig. 6. A) Example of hysteresis in oxygen fluxes during the light, showing the effects of increased respiration during the post zenith period; B) Oxygen fluxes during the morning and afternoon across the depth gradient during summer; **C)** Fluxes of dissolved organic nitrogen during the morning and afternoon across the depth gradient during summer. Plots B and C show means ± SE.

intolerable environment for the long term maintenance of seagrass. Hence, on an annual basis, a balanced O_2 metabolism (i.e. NCM = 0) is required to ensure complete oxidation of reduced equivalents arising from anaerobic microbial activity throughout the year (Fig. 8). The cross latitude tendency for NCM = 0 in *Zostera* species (Fig. 9) suggests these seagrasses may be limited to estuarine environments where the balance between major drivers such as light and allochthonous OM supply (i.e. the trapping rate of seston) allow for a balanced sulphur cycle within the meadow. It is unclear why examples of net autotrophic *Zostera* communities are rare, however it is possible that *Zostera* spp. become severely nutrient limited in oligotrophic settings where seston inputs are low.

Relationships between seagrass productivity and NCM have been observed for seagrasses by Duarte et al. (2010) who suggested that seagrass communities in general appear to become net autotrophic once GPP exceeds 186 mmol O_2 m^{-2} d^{-1}. Our estimates for *Z. muelleri* show a slightly lower value of 150 mmol O_2 m^{-2} d^{-1}, but are in general agreement with this observation. The strong relationships between biomass and metabolism (GPP and CR) demonstrated in this study indicate that NCM may be also dependent on areal biomass. Our limited dataset for *Z. muelleri* suggested that NCM (P:R) increased as biomass increased, with net autotrophic metabolism occurring at a total biomass (AGB + BGB) of ~250 g DW m^{-2}, and mostly balanced metabolism beyond this point (Fig. 7).

NCM within a seagrass meadow integrates the relative influences of both autotrophic production and heterotrophic respiration over various timescales (e.g. diurnal, seasonal and annual). While antecedent light history had a primary bearing over biomass (and hence GPP) during this study (Fig. 2), it was not directly related to NCM suggesting that other factors related to heterotrophic metabolism were important. Broadly, heterotrophic metabolism within the meadow includes contributions from plant respiration, and the microbial breakdown of trapped seston, wrack, BGB, and dissolved OM (Holmer and Nielsen, 1997; Barron and Duarte, 2009; Buapet et al., 2013). The rates of heterotrophic metabolism due to all of these contributions are likely to be highest within the rhizosphere, and lesser in the adjacent sediments. As such, the SOD rates measured in our core incubations may underestimate the actual rate of heterotrophic metabolism based on allochthonous OM, however rates are likely to be directly proportional to actual rates. They therefore give a conservative indication of the contribution heterotrophic breakdown of labile OM in the meadow sediments makes to CR and P:R, independent of plant respiration. The strong negative correlation between SOD and P:R within each sample time (Fig. 5) confirms a primary influence of heterotrophic breakdown on the NCM of the meadow. Further, a comparison of NCM and SOD indicated that SOD could account for all of the variation in NCM during summer.

4.2. Coupling of biomass and metabolism

There were clear relationships between biomass, GPP and CR during this study (Fig. 7), which allude to a tight coupling between morphology and metabolism at seasonal and spatial scales (Hume et al., 2011; Rheuban et al., 2014b). The relationship between biomass and metabolism is intuitive; however the better predictive power with the inclusion of non-photosynthetic organs (i.e. BGB) indicates that productive potential cannot be assessed from AGB alone. The close relationship between the AGB:BGB ratio and metabolism (Fig. 7) shows that there was a disproportionate increase in BGB as GPP and CR increase during summer. It is possible that a reduction in AGB:BGB ratio with increased GPP may be driven by nutrient limitation during the growth season in oligotrophic waters (Hemminga, 1998), whereby BGB increases in order to optimise assimilation of remineralised nutrients in the rhizosphere, and to benefit from mutualistic relationships with diazotrophic sulphate reducing bacteria which preferentially colonise the roots and rhizomes (Welsh et al., 1996; Nielsen et al., 2001). However, any increase in BGB must be balanced by photosynthetic O_2 production in order to maintain 1) a neutral carbon balance within the plant (Dennison and Alberte, 1985; Touchette and Burkholder, 2000); and 2) a balanced NCM necessary for

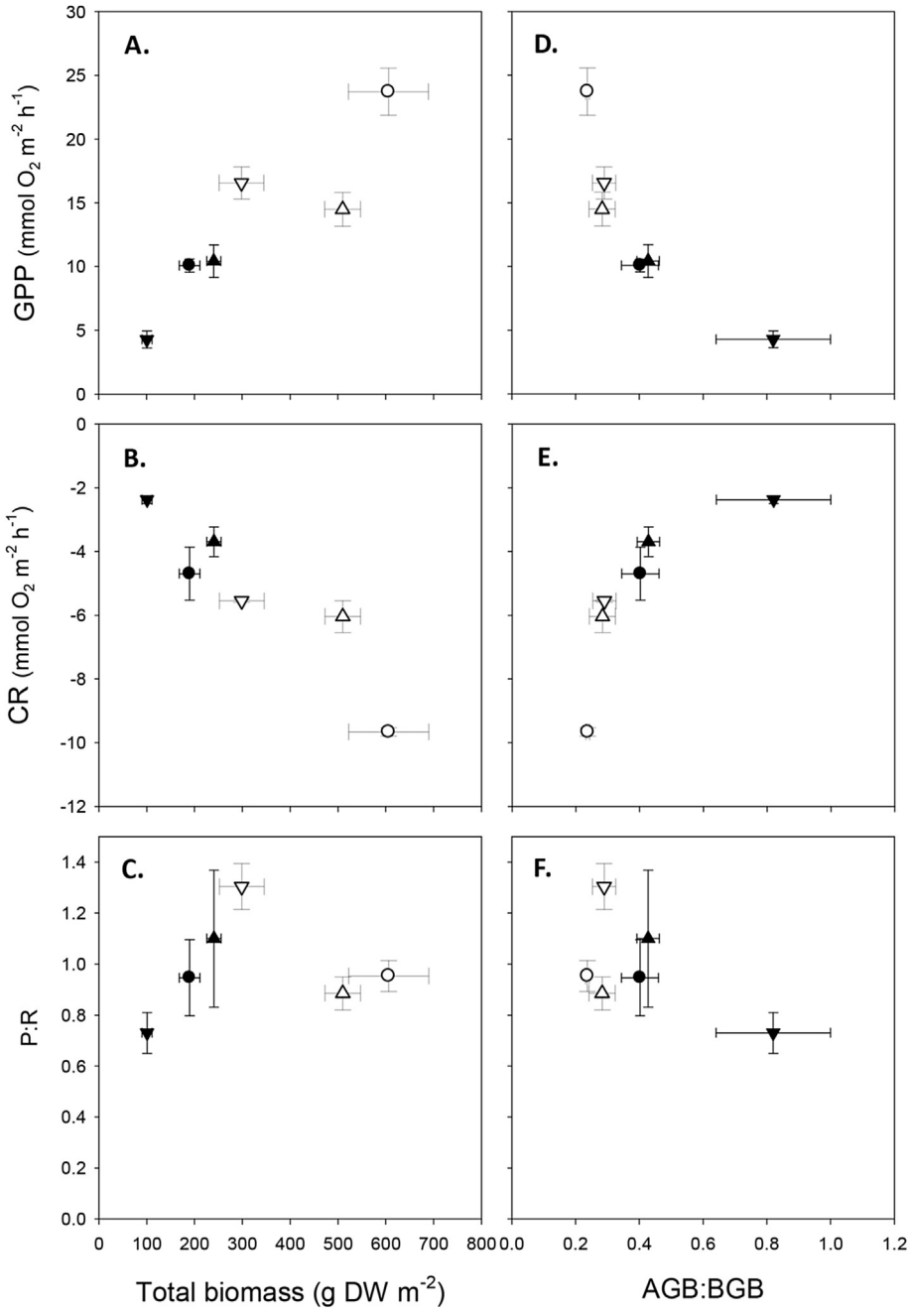

Fig. 7. The relationships between GPP, CR, P:R and total seagrass biomass (A–C), and the above ground: below ground biomass ratio (D–F).

minimising the potential risk of sulphide toxicity (Pedersen et al., 1998). We also recognise that the increase in temperature between the sample times would have contributed to higher CR rates in late summer, however this would have worked in concert with biomass increase.

The deepest site (located close to the depth limit of this meadow) provides insights to plant acclimation (in terms of morphology and metabolism) at the lower limits of light exposure. This site was characterised by significantly lower biomass, and smaller seasonal variation in biomass, relative to the shallower sites, suggesting limitations on AGB imposed by a need to reduce self-shading (Collier et al., 2007). However, seagrass at the deepest site also displayed the largest seasonal shift in AGB:BGB, biomass specific GPP, and community P:R relative to shallower sites (Fig. 4). We suggest that these trends are driven by the large seasonal variation in light climate at depth, and the needs of the seagrass to balance NCM over the annual cycle. Net heterotrophy at the deep site during early spring most likely arises from a combination of: 1) low antecedent light over the preceding winter; 2) low biomass; , and 3) low biomass-specific GPP, resulting in insufficient GPP to balance the SOD at this site. Similar periods of net heterotrophy during winter have been observed in seagrass communities that are estimated to be net autotrophic over the annual cycle (Frankignoulle and Bouquegneau, 1987). The increase AGB:BGB ratios during this period most likely allow plants to survive periods of negative NCM, however their continued survival in these settings is likely dependent on being able to significantly up-regulate photosynthetic potential in the growing season in order to

Table 4
Carbon budget for the Swansea Shoals seagrass meadow (mmol C m^{-2} d^{-1}). The method used to estimate each term is indicated, with relevant equations indicated within brackets. GPP_{day}.

depth	GPP_{day} [a] oxygen flux	CR_{day} [b] oxygen flux	NCM oxygen flux	P:R oxygen flux	DOC exudation DON flux	AGB gross gain leaf extension	ΔAGB measured (8)	ΔBGB measured (8)	ΔBGB predicted (11)	Wrack maximum1 (9)	Wrack maximum2 (10)	Wrack retained	Seston trapping measured	Carbon inputs/CR	Carbon imports/ exports
0.75 m	119.6	−116.9	2.7	1.0	23.9	63	5.8	34.9	32.4	57.4	55.0	5.5	99.0	1.2	1.3
1.5 m	162.2	−172.3	−10.1	0.9	32.4	63	9.2	53.6	66.5	54.0	66.9	6.7	79.2	0.9	0.9
3.0 m	100.0	−95.1	4.9	1.1	20.0	63	3.3	26.6	16.7	60.0	50.1	5.0	49.5	0.9	0.8
mean	127.2	−128.1	−0.9	0.99	25.4	63	6.1	38.4	38.5	57.1	57.3	5.7	75.9	1.00	0.99
error	11.0	3.4	21.6		5.1	6.2	1.8	9.1	11.1	17.9	11.3	1.5	25.0		
% of GPP_{day}					20%	50%	5%	30%		45%	45%	5%			

[a] GPP_{day} = GPP × light-saturated hours.
[b] CR_{day} = CR × 24.

balance NCM over the annual timescale. The large increase in biomass specific GPP during summer, coupled with little change in SOD allowed for the observed positive seasonal swing in P:R (Fig. 4). In keeping with the hypothesis that a balanced NCM is the preferred state for *Z. muelleri*, we suggest that these patterns may reflect a combination of morphological and physiological strategies allowing plants to survive at the lower limits of their depth range. If conditions are unfavourable during the growing season and do not allow a significant increase in productivity (e.g. due to extended periods of storms), this may result in the continuation of negative NCM and eventually to a net loss of seagrass from the lower depth limit (Gacia et al., 2005).

4.3. Carbon budget

The carbon flow and oxygen budgets provide further insights into carbon cycling in the meadow. All aspects of the carbon budget for the *Z. muelleri* meadow were closely balanced over the annual estimation (Table 4 and Fig. 8). Interestingly, allochthonous carbon inputs (seston) closely balanced autochthonous exports (DOC and wrack), suggesting that the meadow is neither a source nor sink of carbon with regards the lake system. Seston trapping accounted for ~60% of the OM available for heterotrophic breakdown within the meadow (Table 4), which is consistent with estimates of particulate trapping in seagrass meadows generally (Gacia et al., 2002; Greiner et al., 2016). Our method for measuring particulate trapping may include variable proportions of resuspended autochthonous material from within the meadow, however stable isotope signatures of trapped particulate material and sediments indicate the seagrass derived proportion to be generally <20% (Ferguson unpublished data). Regardless of the source of seston material, net inputs of autochthonous and allochthonous carbon were closely balanced by measured rates of CR suggesting little potential burial of organic carbon within the meadow. These results, combined with the broader trend of balanced P:R observed for *Z. marina* (Fig. 9) suggests that balanced community metabolism may be the preferable state for *Zostera* spp. in order to meet nutrient demands and maintain favourable redox conditions within the rhizosphere. As discussed above, we suggest that *Z. muelleri* may moderate (up or down regulate) biomass and productivity in order to balance the SOD due to inputs of seston to the meadow.

We estimate that wrack production may account for up to 45% of GPP, and while a small portion of wrack may be retained and broken down within the meadow, it is likely that a large fraction is

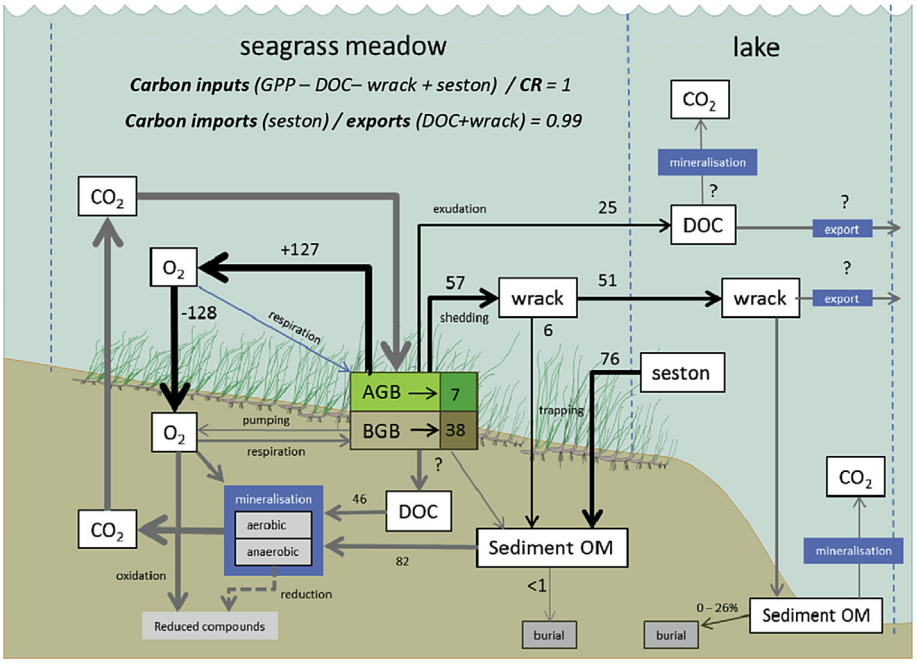

Fig. 8. Annual carbon and oxygen budget for the *Zostera muelleri* meadow. Black arrows indicate the pathways estimated in this study while grey arrows indicate likely important pathways. Numbers indicate daily fluxes (mmol C m^{-2} d^{-1}).

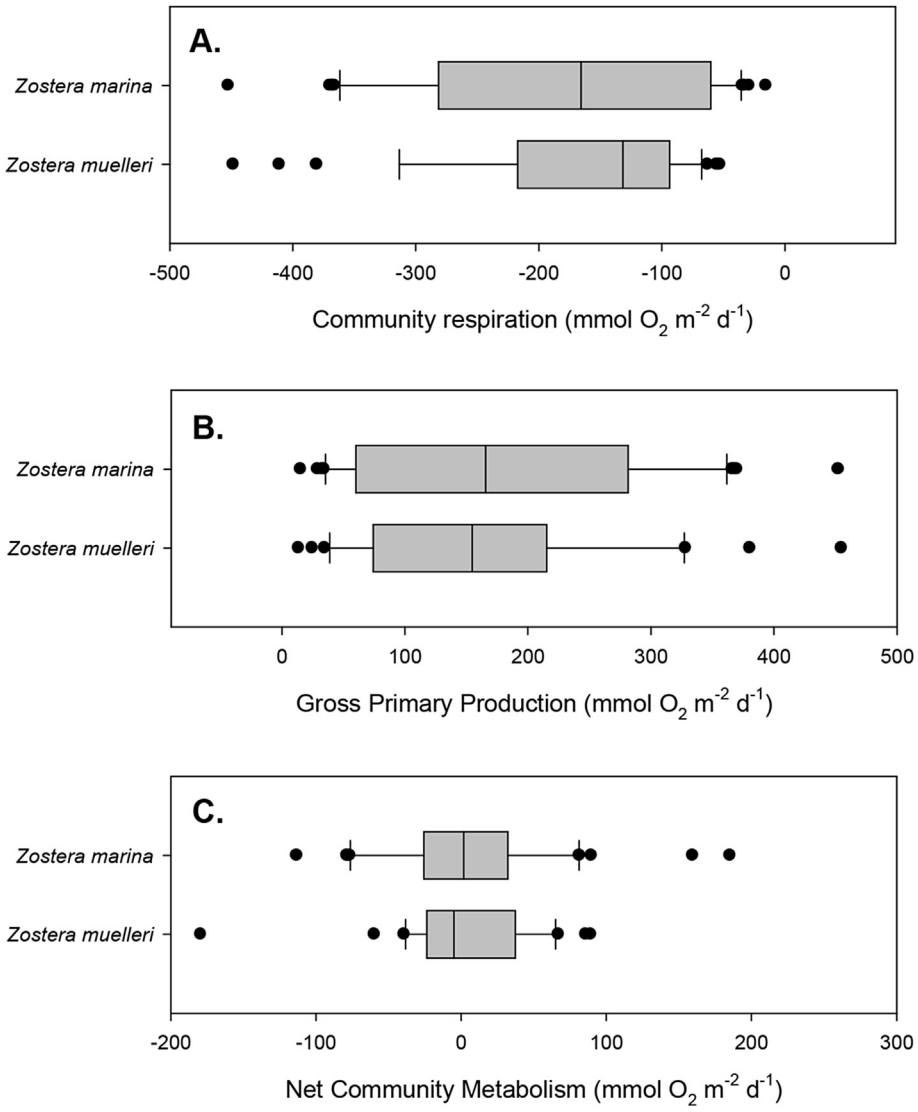

Fig. 9. Comparison of **A)** community respiration; **B)** gross primary production; and **C)** net community metabolism for *Zostera marina* (Duarte et al., 2010) and *Zostera muelleri* (this study). Data from Duarte et al. (2010) are from a range of studies, incorporating measurements from various depths within systems at a wide range of latitudes and trophic status.

exported carbon from the meadow (Table 4). It is unclear why such a large proportion of GPP should be shed and potentially exported in a nutrient poor environment, however this paradox is observed elsewhere in similarly oligotrophic meadows of other species (McMahon et al., 1997). It is possible that seagrasses can safely lose wrack at a rate roughly equal to seston trapping (e.g. Table 4), since this input replaces nutrients to the rhizosphere. This strategy may be advantageous in a system with low water column nutrient concentrations, where seagrasses must satisfy their nutrient requirements via the roots (e.g (Perez et al., 1994)). The fate of exported wrack depends on rates of decomposition relative to burial off site. Our estimates of wrack breakdown rates indicate an initial rapid loss as more labile fractions of the leaf material are either leached or mineralised (Lavery et al., 2013b), followed by a much slower rate of loss as more refractory compounds are mineralised. It is also possible that a small amount of fine material (i.e. <1 mm diameter)) was lost through the mesh of the experimental containers, which would serve to falsely increase the estimated rates of breakdown. Conversely, rates were measured at the lower end of the seasonal temperature scale, and as such are probably conservative estimates of the total wrack breakdown over the annual cycle, given the likely variation in decomposition with temperature. It is likely therefore that these errors would to some degree cancel each other, but they will not affect the results of our carbon budget. The differences in rates according to the environment in which the wrack is broken down (Table 2) indicate that there is minor potential for storage of carbon if wrack is washed up above the high spring tide level (and not removed through management action), or if wrack is buried. Estimates indicate that wrack remaining in suspension or deposited to the sediment surface (i.e., in an oxygenated environment) will fully decompose on an annual basis. In the case of our study site, it is likely that the bulk of wrack is lost to the deeper lake basin due to a combination of circulation patterns and shoreline modification. Once deposited to the lake basin, wrack may be either: 1) completely decomposed in oxic surface sediments; or 2) up to 26% of the material buried below the oxic sediment layer may be preserved.

Our estimates of DOC production are within the range of values reported for *Posidonia* meadows (Barron and Duarte, 2009), but substantially higher than for *Thalassia testudinum* ((Zeigler and Benner, 1999); Table 4). Similarly, we estimated that DOC exudates represented between 48% and 23% of NPP during spring and

summer respectively, which is similar to reported values for this fraction (Barron and Duarte, 2009). DOC production by the seagrass meadow is likely to arise from three main sources: photosynthate exudates from live leaves, leachate from senescing or recently dead leaves, and the hydrolysis of trapped seston (Zeigler et al., 2004). During our study there was a significant increase in DON flux during the second half of the photoperiod suggesting a dominance of phototrophic production (Fig. 6, Rheuban et al., 2014b). In addition, dark fluxes of DON tended to be greatly reduced or dominated by uptakes at some depths, suggesting a predominance of DOM consumption by heterotrophs. Exports of DOC from the seagrass meadow are mixed within the lake water and are most likely subject to mineralisation by bacterioplankton (Baines and Pace, 1991; Amon and Benner, 1996). The composition and lability of the DOC produced by the seagrass meadow is unknown, however observations in other seagrass systems suggest a tight coupling between DOC production and bacterioplankton productivity (Zeigler and Benner, 1999; Zeigler et al., 2004) suggesting the presence of a significant labile fraction. As such, we suggest that DOC export makes a relatively small contribution to blue carbon burial in this *Z. muelleri* meadow.

Overall, there is little evidence of significant carbon burial within this *Zostera muelleri* meadow, with sediment carbon contents suggesting that total burial rates are within the ranges for unvegetated estuarine sediments (Duarte et al., 2005). This is in contrast to reports for other seagrass species such as *Posidonia* spp., which appear to represent major hotspots of carbon burial based on NCM estimates (Duarte et al., 2010). These species tend to have biomass attributes such as higher C:N and lignin content which tend to make biomass more refractory (Enriquez et al., 1993). The maintenance of relic BGB observed in meadows of these species may benefit their long term survival in oligotrophic oceanic waters, where resilience depends on the persistence of existing biomass rather than seasonal recruitment (Kilminster et al., 2015). In contrast, *Zostera muelleri* appears to have evolved metabolic and morphological strategies to survive more opportunistically in more enriched estuarine settings (Ferguson et al., 2016). It is clear, therefore, that a global assessment of blue carbon burial by seagrasses must include a comparison of different species across their natural ranges. Further, a standardised approach to the construction of carbon budgets in seagrass meadows would greatly help any comparison of results between studies (Macreadie et al., 2014).

5. Conclusion

Our results suggest that standing stocks within *Zostera muelleri* meadows primarily represent a blue carbon store, and that the export of wrack and to a lesser extent DOC, are the most likely carbon sinks. The significance of the wrack sink depends on where this material accumulates since this influences the rate of breakdown, while the significance of the DOC sink is unknown, since little is known of the makeup and lability of these compounds. The maintenance and rehabilitation of *Z. muelleri* meadows is therefore critical to maintaining their role as a blue carbon store. Significant variation in processes among depths indicated that robust estimations of NCM and carbon budgets of meadows cannot be made at a single depth alone. Our study also provides new insights into the coupling between morphological plasticity and metabolism and its influence over the maintenance of *Zostera muelleri* meadows across seasonal and depth gradients. It appears that *Zostera muelleri* may be limited to environments where it can maintain not only a favourable plant carbon balance, but also a neutral NCM (i.e. balance total community respiration with photosynthetic O_2 production over the annual cycle). This understanding is critical in the development of better predictive models for seagrass resilience in response to environmental and anthropogenic stressors and hence it's potential as a long term carbon store.

Acknowledgements

We would like to acknowledge the extensive support provided by field staff including Peter Davies, Brendan Haine, Chris Baiada, without whose help this project would not have been possible.

References

Adams, M.P., Ferguson, A.J.P., Maxwell, P.S., Lawson, B.A.J., Samper-Villarreal, J., O'Brien, K.R., 2015. Light history-dependent respiration explains the hysteresis in the daily ecosystem metabolism of seagrass. Hydrobiologia 766, 75–88.
Amon, R.M.W., Benner, R., 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnol. Oceanogr. 41, 41–51.
Baines, S.B., Pace, M.L., 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: patterns across marine and freshwater systems. Limnol. Oceanogr. 36, 1078–1090.
Barron, C., Duarte, C.M., 2009. Dissolved organic matter release in a Posidonia oceanica meadow. Mar. Ecol. Prog. Ser. 374, 75–84.
Barron, C., Marba, N., Terrados, J., Kennedy, H., Duarte, C.M., 2004. Community metabolism and carbon budget along a gradient of seagrass (*Cymodocea nodosa*) colonization. Limnol. Oceanogr. 49, 1642–1651.
Buapet, P., Rasmusson, L.M., Gullstrom, M., Bjork, M., 2013. Photorespiration and carbon limitation determine productivity in temperate seagrasses. PLoS ONE 8, e83804, 83810.81371/journal.pone.0083804.
Clores, M.A., Santos Carandang, J., 2013. Chlorophyll content, productivities and biomass allocations of seagrasses in Talim Bay, Lian, Batangas, Philippines. Proc. Int. Acad. Ecol. Environ. Sci. 3, 247–256.
Collier, C.J., Lavery, P.S., Masini, R.J., Ralph, P.J., 2007. Morphological, growth and meadow characteristics of the seagrass Posidonia sinuosa along a depth-related gradient of light availability. Mar. Ecol. Prog. Ser. 337, 103–115.
Dennison, W.C., Alberte, R.S., 1985. Role of daily light period in the depth distribution of Zostera marina (eelgrass). Mar. Ecol. Prog. Ser. 25, 51–61.
Duarte, C.M., 1990. Seagrass nutrient content. Mar. Ecol. Prog. Ser. 67, 201–207.
Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41, 87–112.
Duarte, C.M., Cebrian, J., 1996. The fate of marine autotrophic production. Limnol. Oceanogr. 41, 1758–1766.
Duarte, C.M., Middelburg, J.J., Caraco, N., 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences J1-BG 2, 1–8.
Duarte, C.M., Kennedy, H., Marba, N., Hendriks, I., 2011. Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. Ocean Coast. Manag. 1–7. http://dx.doi.org/10.1016/j.ocecoaman.2011.09.001.
Duarte, C.M., Marba, N., Gacia, E., Fourqurean, J.W., Beggins, J., Barron, C., Apostolaki, E.T., 2010. Seagrass community metabolism: assessing the carbon sink capacity of seagrass meadows. Glob. Biogeochem. Cycles 24, GB4032.
Emmett Duffy, J., 2006. Biodiversity and the functioning of seagrass ecosystems. Mar. Ecol. Prog. Ser. 311, 233–250.
Enriquez, S., Duarte, C.M., Sand-Jensen, K., 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C: N:P content. Oceologia 94, 457–471.
Eyre, B.D., Ferguson, A.J.P., 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae- dominated warm-temperate Australian lagoons. Mar. Ecol. Prog. Ser. 229, 43–59.
Eyre, B.D., Ferguson, A.J.P., Webb, A., Maher, D., Oakes, J.M., 2011. Metabolism of different benthic habitats and their contribution to the carbon budget of a shallow oligotrophic sub-tropical coastal system (southern Moreton Bay, Australia). Biogeochemistry 102, 87–110.
Falkowski, P.G., Raven, J.A., 1997. Aquatic Photosynthesis. Blackwell Science, Malden, Massachusetts.
Ferguson, A.J.P., Gruber, R.K., Orr, M., Scanes, P., 2016. Morphological plasticity in Zostera muelleri across light, sediment, and nutrient gradients in Australian temperate coastal lakes. Mar. Ecol. Prog. Ser. 556, 91–104.
Flower, R.J., 1991. Field calibration and performance of sediment traps in a eutrophic holomictic lake. J. Paleolimnol. 5, 175–188.
Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marba, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., Serrano, O., 2012. Seagrass Ecosystems as a Globally Significant Carbon Stock, vol. 5, pp. 505–509.
Frankignoulle, M., Bouquegneau, J.M., 1987. Seasonal variation of the diel carbon budget of a marine macrophyte ecosystem. Mar. Ecol. Prog. Ser. 38, 197–199.
Gacia, E., Duarte, C.M., Middelburg, J.J., 2002. Carbon and nutrient deposition in a Mediterranean seagrass (*Posidonia oceanica*) meadow. Limnol. Oceanogr. 47, 23–32.
Gacia, E., Kennedy, H., Duarte, C.M., Terrados, J., Marba, N., Papadimitriou, S., Fortes, M., 2005. Light-dependence of the metabolic balance of a highly productive Philippine seagrass community. J. Exp. Mar. Biol. Ecol. 316, 55–67.
Greiner, J.T., Wilkinson, G.M., McGlathery, K.J., Emery, K.A., 2016. Sources of

sediment carbon sequestered in restored seagrass meadows. Mar. Ecol. Prog. Ser. 551, 95–105.
Heck, K.L.J., Hays, G., Orth, R.J., 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Mar. Ecol. Prog. Ser. 253.
Hemminga, M.A., 1998. The root/rhizome system of seagrasses: an asset and a burden. J. Sea Res. 39, 183–196.
Herzka, S.Z., Dunton, K.H., 1997. Seasonal photosynthetic patterns of the seagrass *Thalassia testudinum* in the western Gulf of Mexico. Mar. Ecol. Prog. Ser. 152, 103–117.
Holmer, M., Frederiksen, M.S., Møllegaard, H., 2005. Sulfur accumulation in eelgrass (Zostera marina) and effect of sulfur on eelgrass growth. Aquat. Bot. 81, 367–379.
Holmer, M., Nielsen, S.L., 1997. Sediment sulfur dynamics related to biomassdensity patterns in *Zostera marina* (eelgrass) beds. Mar. Ecol. Prog. Ser. 146, 163–171.
Hume, A.C., Berg, P., McGlathery, K.J., 2011. Dissolved oxygen fluxes and ecosystem metabolism in an eelgrass (Zostera marina) meadow measured with the eddy correlation technique. Limnol Oceanogr 56, 86–96.
Jφrgensen, B.B., 1982. Mineralization of organic matter in the sea bed - the role of sulfate reduction. Nature 296.
Kennedy, H., Beggins, J., Duarte, C.M., Fourqurean, J.W., Holmer, M., MarbÃ, Nr, Middelburg, J.J., 2010. Seagrass sediments as a global carbon sink: isotopic constraints. Glob. Biogeochem. Cycles 24, GB4026.
Kilminster, K., McMahon, K., Waycott, M., Kendrick, G.A., Scanes, P., McKenzie, L.J., O'Brien, K.R., Lyons, M., Ferguson, A.J.P., Maxwell, P., Glasby, T.M., Udy, J.W., 2015. Unravelling complexity in seagrass systems for management: Australia as a microcosm. Sci. Total Environ. http://dx.doi.org/10.1016/j.scitotenv.2015.1004.1061.
Lavery, P.S., M-Á, Mateo, Serrano, O., Rozaimi, M., 2013a. Variability in the carbon storage of seagrass habitats and its implications for global estimates of blue carbon ecosystem service. PLoS ONE 8, e73748.
Lavery, P.S., McMahon, K.M., Weyers, J., Boyce, M.C., Oldham, C.E., 2013b. Release of dissolved organic carbon from seagrass wrack and its implications for trophic connectivity. Mar. Ecol. Prog. Ser. 494, 121–133.
Lee, K.-S., Dunton, K.H., 2000. Effects of nitrogen enrichment on biomass allocation, growth, and leaf morphology of the seagrass *Thalassia testudinum*. Mar. Ecol. Prog. Ser. 196, 39–48.
Macreadie, P.I., Baird, M.E., Trevathan-Tackett, S.M., Larkum, A.W.D., Ralph, P.J., 2014. Quantifying and modelling the carbon sequestration capacity of seagrass meadows – a critical assessment. Mar. Pollut. Bull. 83, 430–439.
Maher, D., Eyre, B.D., 2011. Benthic carbon metabolism in southeast Australian estuaries: habitat importance, driving forces, and application of artificial neural network models. Mar. Ecol. Prog. Ser. 439, 97–115.
McLeod, E., Chmura, G.L., Bouillon, S., Salm, R., Björk, M., Duarte, C.M., Lovelock, C.E., Schlesinger, W.H., Silliman, B.R., 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. Front. Ecol. Environ. 9, 552–560.
McMahon, K., Young, E., Montgomery, S., Cosgrove, J., Wilshaw, J., Walker, D.I., 1997. Status of a shallow seagrass system, Geographe Bay, south-western Australia. J. R. Soc. West. Aust. 80, 255–262.
Nielsen, L.B., Finster, K., Welsh, D.T., Donelly, A., Herbert, R.A., de Wit, R., Lomstein, B.A., 2001. Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from Zostera noltii and Spartina maritima meadows. Environ. Microbiol. 3, 63–71.
Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck Jr., K.L., Hughes, A.R., Olyarnik, S., Williams, S.L., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Waycott, M., 2006. A global crisis for seagrass ecosystems. BioScience 56, 987–996.
Pedersen, O., Borum, J., Duarte, C.M., Fortes, M.D., 1998. Oxygen dynamics in the rhizosphere of *Cymodocea rotundata*. Mar. Ecol. Prog. Ser. 169, 283–288.
Perez, M., Duarte, C.M., Romero, J., Sand-Jensen, K., Alcoverro, T., 1994. Growth plasticity in *Cymodocea nodosa* stands: the importance of nutrient supply. Aquat. Bot. 47, 249–264.
Ralph, P.J., Durako, M.J., Enriquez, S., Collier, C.J., Doblin, M.A., 2007. Impact of light limitation on seagrasses. J. Exp. Mar. Biol. Ecol. 350, 176–193.
Rheuban, J.E., Berg, P., McGlathery, K.J., 2014b. Multiple timescale processes drive ecosystem metabolism in eelgrass (Zostera marina) meadows. Mar. Ecol. Prog. Ser. 507, 1–13.
Touchette, B.W., Burkholder, J.M., 2000. Overview of the physiological ecology of carbon metabolism in seagrasses. J. Exp. Mar. Biol. Ecol. 250, 169–205.
Valderrama, J.C., 1981. The simultaneous analysis of TP and TN in natural waters. Mar. Chem. 10, 109–122.
Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. 106, 12377–12381. http://dx.doi.org/10.1073/pnas.0905620106.
Welsh, D.T., Wellsbury, P., Bourgues, S., de Wit, R., Herbert, R.A., 1996. Relationship between porewater organic carbon content, sulphate reducstion and nitrogen fixation (acetylene reduction) in the rhizosphere of Zostera noltii. Hydrobiologia 329, 175–183.
Zeigler, S., Benner, R., 1998. Ecosystem metabolism in a subtropical seagrass-dominated lagoon. Mar. Ecol. Prog. Ser. 173, 1–12.
Zeigler, S., Benner, R., 1999. Dissolved organic carbon cycling in a subtropical seagrass-dominated lagoon. Mar. Ecol. Prog. Ser. 180, 149–160.
Zeigler, S., Kaiser, E., Benner, R., 2004. Dynamics of dissolved organic carbon, nitrogen and phosphorus in a seagrass meadow of Laguna Madre, Texas. Bull. Mar. Sci. 75, 391–407.