Net Drawdown of Greenhouse Gases (CO₂, CH₄ and N₂O) by a Temperate Australian Seagrass Meadow

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Abstract
Seagrasses have some of the highest rates of carbon burial on the planet and have therefore been highlighted as ecosystems for nature-based climate change mitigation. However, information is still needed on the net radiative forcing benefit of seagrasses inclusive of their associated greenhouse gas (GHG) emissions. Here, we report simultaneous estimates of seagrass-associated carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) air–water emissions. Applying in situ sampling within a south-east Australian seagrass ecosystem, this study finds atmospheric GHG emissions from waters above seagrasses to range from −480 ± 15.96 to −16.2 ± 8.32 mg CO₂-equivalents m⁻² d⁻¹ (net uptake), with large temporal and spatial variability. Using a combination of gas specific mass balance equations, dissolved stable carbon isotope values (δ¹³C) and in situ time-series data, CO₂-e flux is estimated at −21.74 mg m⁻² d⁻¹. We find that the net release of CH₄ (0.44 µmol m⁻² h⁻¹) and net uptake of N₂O (−0.06 µmol m⁻² h⁻¹) effectively negated each other at 16.12 and −16.13 mg CO₂-e m⁻² d⁻¹, respectively. The results of this study indicate that temperate Australian seagrasses may function as net sinks of atmospheric CO₂-e. These results contribute towards filling key emission accounting gaps both in the Australian region, and through the simultaneous measurement of the three key greenhouse gas species.

Keywords Emissions · Carbon dioxide · Methane · Nitrous oxide · Diurnal · Seagrass · Primary producer · Aquatic

Introduction
Atmospheric concentrations of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O), have increased substantially since pre-industrial periods and are the three dominant greenhouse gases (GHGs) driving human-induced atmospheric warming (Solomon et al. 2007). Quantifying the global emissions of these gases is therefore of high priority as we move towards strategies for climate change mitigation. In the natural world, photosynthetic assimilation and storage of atmospheric CO₂ (i.e. biosequestration), and microbial breakdown of organic material with the subsequent release of GHGs through respiration (i.e. decay), are two of the most fundamental processes influencing the carbon cycle. Coastal vegetated ecosystems such as tidal marshes, mangroves and seagrasses have potential for inclusion into future carbon abatement programmes due to biosequestration rates up to 35 times higher than tropical rainforests (Mcleod et al. 2011), and limited carbon decay due to saline-aquatic redox conditions (Macreadie et al. 2019).

Despite covering only 0.1–0.2% of the ocean’s surface, seagrasses are responsible for an estimated 10–18% of total oceanic carbon burial (Duarte et al. 2005; Mcleod et al. 2011). Seagrasses are amongst the most productive coastal ecosystems with average net primary production equating to ~817 g carbon (C) m⁻² yr⁻¹ (Duarte and Cebrián 1996; Mateo et al. 2006; Fourqueuran et al. 2012), with sequestered carbon remaining in their anoxic sediments for millennia (Macreadie et al. 2015). However, a variety of human activities that induced degradation of seagrasses, such as the conversion of coastal areas for aquaculture, boat moorings and eutrophication, can increase organic matter respiration...
through the perturbation (and oxidation) of sediments and increased nutrient supply, leading to decreased sedimentary carbon and increased GHG release (Short and Wyllie-Echeverria 1996; Waycott et al. 2009; Pendleton et al. 2012; Macreadie et al. 2015). Alternatively, and commonly, used are continuous underway or discreet sampling spatial surveys that both inherently incorporate spatial GHG variance due to changes in sediment characteristics and seagrass cover etc., though do not integrate day-night fluctuations. Individually, these methods may not adequately constrain GHG variability, resulting in imprecise emission estimates (Gazeau et al. 2005; Call et al. 2015; Maher et al. 2015).

In order to establish seagrasses’ net radiative forcing benefit and therefore their potential use in natural climate change mitigation, it is crucial to accurately incorporate their baseline atmospheric GHG emissions. Common application of benthic chamber sampling methodologies has facilitated estimates of seagrass CO$_2$ and CH$_4$ flux rates between sediments and the water column (Maher and Eyre 2010; Barrón et al. 2014; Macreadie et al. 2014). However, the proportion of these seagrass sediment produced GHGs that pass through the water column to be emitted to the atmosphere is often unclear. For instance, aerobic microbially respired CO$_2$ can be recycled within photosynthetic cycles of the seagrass meadows, and anaerobically respired CH$_4$ is subject to methanotrophic oxidation in the water column. In addition, air–water GHG flux is a product of specific gas solubility in seawater (subject to temperature and salinity), air–water concentration gradients and gas exchange coefficients (Raymond and Cole 2001; Middelburg et al. 2002; Borges et al. 2004).

As such, estimates of seagrass atmospheric GHG emissions, based solely on benthic chamber methodologies, are subject to high variability and are unlikely to accurately capture seagrass-associated atmospheric emissions. Additionally, previous literature is largely constrained by region and seagrass species, whilst predominantly focusing on CO$_2$ flux, with fewer still incorporating CH$_4$ flux (Tokoro et al. 2014; Garcias-Bonet and Duarte 2017; Banerjee et al. 2018) and N$_2$O flux (Murray et al. 2015).

The application of in situ automated cavity ring-down spectroscopy has been shown as an effective method for the quantification of air–water gas flux in estuarine and mangrove systems (Maher, et al. 2013a, b; Maher et al. 2015; Reading et al. 2017). Seagrass meadows often span large areas and experience regionalised variation in environmental conditions that affect GHG dynamics, such as salinity (Bouillon, et al. 2007a, b; Touchette 2007), nutrient inputs (Smith et al. 1999; Duarte 2002), water turbulence (Raymond and Cole 2001) and anthropogenic disturbance (Macreadie et al. 2015). In addition, fluctuations in both light availability (and therefore photosynthesis) (Maher, et al. 2013a, b; Saderne et al. 2013) and tidal pumping of ground waters enriched in GHG solutes, dissolved organic matter and nutrients can influence seagrasses’ net autotrophic/heterotrophic balance over diel and tidal cycles (Santos et al. 2008; Bauer and Bianchi 2011; Gleeson et al. 2013). Automated fixed time-series measurements with high sampling resolution (i.e. minutes) are an effective way to incorporate temporal variation in emissions; however, they do not account for spatial changes in environmental conditions (Gazeau et al. 2005; Call et al. 2015; Macreadie et al. 2015).

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Here, we measure dissolved GHG concentrations from temperate seagrasses and model emission rates, with the aim to quantify the net GHG balance. To achieve this aim, we quantified CO$_2$, CH$_4$ and N$_2$O dissolved concentrations and diffusive fluxes in a temperate seagrass-dominated estuarine embayment in south-east Australia. We used a combination of fixed time-series measurements over a 44-h period and a continuous underway spatial survey throughout the embayment to assess temporal and spatial variability in GHG fluxes and concentrations (Fig. 1). Furthermore, we measured salinity, temperature, wind speed, tidal depth, dissolved oxygen and isotopic ($\delta^{13}C$) signatures of CO$_2$-C to help determine the major environmental conditions influencing seagrass emissions. This study presents the first use of in situ automated cavity ring-down spectroscopy within an Australian seagrass to determine simultaneous CO$_2$, CH$_4$ and N$_2$O atmospheric flux, including estimates of the cumulative function of temperate seagrasses as a source or sink of GHGs over the study period, providing data that can contribute to regional seagrass emission assessments.

**Materials and Methods**

To quantify the spatial and short-term temporal variability in GHG fluxes, we employed a combination of spatial survey and time-series sampling techniques. A time series was conducted for ~44 h within a seagrass meadow in Swan Bay, Victoria, Australia (~ 38.2701 N, 144.6349 E), and an underway survey was undertaken within Swan Bay between 11:00 and 17:00 (Fig. 1). The experiments were undertaken over a 4-day period from the 4th to 7th of December during the Austral summer. Swan Bay is located within Port Phillip Bay, has an area of ~ 26 km$^2$ and has extensive seagrass beds composed of *Zostera muelleri* and *Heterozostera tasmanica* species, which cover ~ 80% of the bay (Fig. 1). Swan Bay experiences a mean annual rainfall of 457 mm, with a mean maximum and mean minimum temperature of 20.4 and 9.4 °C, respectively.

During the time-series measurements, water temperature, depth, DO, pH and salinity were measured using a calibrated water quality sonde (Eureka Manta II) deployed within the seagrass meadow. Using a bilge pump, water was pumped from the location of the water quality sonde to an air–water equilibrator on the shore. The pump was fixed...
at a depth of 15 cm above the sediment surface. The headspace from the equilibrator was pumped in a closed loop to two cavity ring-down spectrometers (Picarro G2201-i and Picarro G2308) after passing through a desiccant (Drierite) to measure the partial pressure of CO₂ (±200 ppb), CH₄ (±80 ppb), N₂O (±10 ppb) and δ¹³C-CO₂ as described by Maher et al. (2013a, b). A 5-min rolling average was applied to the data, and to account for equilibration times of the various gases data was shifted by 10 min for CO₂ and N₂O, and 30 min for CH₄ (Webb et al. 2016). The same instrument setup was deployed for the underway surveys. The instruments were installed on a small (4 m) shallow draft aluminium boat. The pump and water quality sonde were fixed at a depth of ~30 cm, and a GPS (Garmin Map72) was used to record the track. Due to the time lag in gas equilibration times, the process results in a deconvolution of spatial positioning. However, due to the slow transit speed (~3 km/h) the effect is small.

The dry molar fractions of each gas were converted to partial pressures using the procedures outlined in Pierrot et al. (2009). Briefly, water vapour pressure and the virial coefficients of N₂O were calculated according to Weiss and Price (1980), and the virial coefficients for CO₂ were calculated as described by Weiss (1974). Partial pressures were converted to concentrations and percentage saturations using the temperature and salinity-dependent solubility coefficients for each gas (CH₄, Wiesenburg and Guinasso (1979); CO₂, Weiss (1974); N₂O, Weiss and Price (1980)), and atmospheric concentrations of 1.8 ppm, 405 ppm and 328 ppb for CH₄, CO₂ and N₂O respectively.

Air-sea fluxes of each gas were calculated according to

\[ F = k \alpha (\Delta p_{gas}) \]

where \( k \) is the gas transfer velocity, \( \alpha \) is the solubility coefficient and \( \Delta p_{gas} \) is the difference in partial pressure of the gas between the water and atmosphere. As we do not have data on current speeds (although qualitative observations suggest this was low), we used the wind-based gas transfer parametrization of Wanninkhof (2014):

\[ k = 0.251 u^2 (Sc/660)^{-0.5} \]

where \( u \) is the windspeed measured at a height of 10 m (m s⁻¹) measured on site using a sonic anemometer (Airmar PB200 weather station), and Sc is the Schmidt number of the gas of interest. The Schmidt number was calculated as a function of temperature and salinity according to Wanninkhof (2014), assuming a linear dependence upon salinity.

A mass balance of each individual gas was constructed for a 24-h period of the time series, which accounted for sources and sinks, to determine the production or consumption of each seagrass associated gas:

\[ F_{seagrass} = \int_0^{24} Fatm - (\Delta volconc) \]

where \( F_{seagrass} \) is the water column flux from samples within the meadow (mmol m⁻² d⁻¹), \( Fatm \) is the air water flux (mmol m² h⁻¹), \( \Delta vol \) is the change in volume (m³ h⁻¹) calculated per m² by the change in depth and \( conc \) is the average concentration of the gas over the time interval of interest. Lateral exchange is accounted for by the \( \Delta volconc \) term; however, due to a lack of detailed carbonate chemistry data, there is uncertainty in our approach associated with DIC speciation.

We used the Keeling method to determine the time series δ¹³C signature of dissolved CO₂ source material from within the meadow (Keeling 1958). Keeling methodology is based on the conservation of mass mixing model.
between the background $\delta^{13}\text{C}$ (i.e. oceanic DIC which we assume is constant over the short timeframe of the experiment) and source $\delta^{13}\text{C}$ values, where the source $\delta^{13}\text{C}$ signature ($+95\%$ CI) can be determined as the $y$-intercept of a type II ordinary least squares regression (“lmodel2” function within “lmodel2” package, permutations = 1000) between $1/$concentration of measured CO$_2$ and the measured $\delta^{13}\text{C}$-CO$_2$ (Pataki et al. 2003; Maher et al. 2017). We assumed that the background concentration and $\delta^{13}\text{C}$-CO$_2$ of atmospheric CO$_2$ did not change during the 44-h period. All statistics were performed using R-statistics (V.3.5.3).

**Data Analyses**

To analyse conditions related to GHG dynamics within seagrass beds of a temperate Australian bay, carbon dioxide (CO$_2$), methane (CH$_4$) and nitrous oxide (N$_2$O) percent saturation (%sat) levels were separately run through a series of linear models fit with generalized least squares (“gls()” function within the “nlme” package) (Pinheiro et al. 2017). During spatial surveys, data was removed in small sections where the water sampling unit was exposed to the air (e.g. due to shallow depth). To achieve data normality and meet the assumption of homogeneity of residual variance, CH$_4$ was natural log transformed, and N$_2$O was square root transformed. To test the assumption of model non-collinearity, all pair-wise combinations of environmental variables were assessed using linear models (“lm()” function within “nlme” package): pH, dissolved oxygen (DO), salinity (PSS), tidal depth (m). Both tidal depth and pH were removed from the model due to a relationship with DO of 0.8 and 0.61 adjusted $R^2$, respectively. Weak collinearity between salinity and DO was also detected, though a variance inflation factor of 1 indicated that the interaction between these independent variables was negligible to the model (Miles 2014) (“vif()” function within the “car” package). To constrain both salinity and DO covariate residual non-normality, a power variance function was introduced to the model (“varCom()” and “varPower()” function from “nlme” package). Akaike’s information criterion (AIC) model selection was used to confirm the best model fit based on weighted structures, resulting in weights being applied to both DO and salinity. Final model structure was as follows: (Sqrt.GSi ~ Salinity + DO, weighted.variance = Salinity + DO), where GSi represents each Gas Species, CO$_2$, CH$_4$ and N$_2$O. Model validation was conducted using a plot of residual distribution, residuals over predicted values and a quantile-quantile plot (Q-Q plot, “qqnorm()” function in the “stats” package). Lastly, to obtain an $F$-statistic, an analysis-of-variance (“anova()” function, type II “marginal”, in “stats” package) was performed on the resulting model objects.

**Results**

**Time Series**

Cumulative mass balance of GHGs over a 44 h period, incorporating changes in dissolved gas concentrations, atmospheric flux and water volume, showed a distinct diel pattern in CO$_2$ with net uptake of atmospheric CO$_2$ during the day and net release of CO$_2$ to the atmosphere during the night, with a net negative 24 h flux of $-20.57$ μmol m$^{-2}$ h$^{-1}$ (Fig. 2). Net CO$_2$ forcing was qualitatively associated with high windspeeds during the day and low windspeeds at night (Fig. 3). CH$_4$ maintained a constant net release.
to the atmosphere, with a 24 h release rate of 0.44 µmol m⁻² h⁻¹, whilst seagrasses were a net sink of N₂O over 24 h, at −0.06 µmol m⁻² h⁻¹ (Fig. 2). The estimated δ¹³C signature of the predominant CO₂-C source material, established through Keeling plots, was −13.71 (−13.75 to 13.67, 95% confidence interval).

Time-series sampling established distinct diel fluctuations in GHGs above seagrass beds, with mean CO₂ and CH₄ percent saturation (%sat) being 32 and 28% higher during the night, whilst N₂O was 11% higher during the day (Fig. 4, Table 1). Collinearity between these environmental variables, as assessed through linear models, showed that DO%sat was weakly positively correlated with salinity concentrations (P < 0.001, Coef = 37.2, adjusted R-squared = 0.11), whilst pH levels (P < 0.001, Coef = 210.82, adjusted R-squared = 0.61) and tidal depth (m) (P < 0.001, Coef = 0.39, adjusted R-squared = 0.8) showed stronger autocorrelation (Table 1). Tidal depth was only mildly correlated with salinity (P < 0.001, Coef = 41.55, adjusted R-squared = 0.03).

**Spatial Survey**

Across the survey area, N₂O percent saturation (%sat) showed the least variation with a range of 119.36–85.6%, whilst CO₂ ranged from 96.4 to 20.1%, and CH₄ had the largest variation with a range of 428.5–87.35%. CO₂%sat levels were highest at the opening of Swan Bay (Fig. 5a), whilst CH₄%sat was highest in areas only sparsely covered by seagrass towards the south-west Swan Bay (Figs. 5b and 1). Across the bay, salinity averaged 33.56 ± 0.07 with a range of 36.04–29.8. Akaike’s information criterion (AIC) determined that spatial survey CO₂, CH₄ and N₂O%sat were best modelled with both salinity and dissolved oxygen (DO). The variance inflation factor for all survey models was low, ranging from 1.004 to 1.006, indicating that the variance around the model was only minutely affected by collinearity between salinity and DO. Water column DO%sat was inversely related to CO₂%sat (f(1, 298) = 264.81, P < 0.001, Coef = −0.25) (Fig. 6a), positively related to N₂O%sat (f(1, 298) = 17.47, P < 0.001, Coef = 0.001) (Fig. 6e) and did not correlate with CH₄ (Fig. 6c). Salinity was not significantly related to CO₂ or CH₄%sat; however, it was negatively correlated with N₂O%sat (f(1, 298) = 32.96, P < 0.001, Coef = −0.06) (Fig. 6f). To note, the linear relationships between N₂O%sat and both DO%sat and salinity (PSS) retained moderate variance and were heavily influenced by data groupings (Fig. 6e–f), as such inference from these relationships should be treated carefully.

**Methodical Comparison**

Across both time-series and spatial sampling, CO₂ percent saturation (%sat) ranged from 20 to 266, CH₄ ranged from 87 to 712% and N₂O from 56 to 119%. The mean percent saturation of GHGs were markedly different between survey and time-series methodology, with CO₂ and CH₄ being 192% and 112% higher in time-series
measurements, respectively, whilst N$_2$O was 22% higher in spatial surveys (Fig. 7a). The mean time-series CO$_2$ flux was $-0.46 \pm 0.18$ mmol m$^{-2}$ d$^{-1}$, 96% higher than that of spatial sampling, at $-11.48 \pm 0.28$ mmol m$^{-2}$ d$^{-1}$ (Fig. 7b). Time-series CH$_4$ flux estimates were also 125% higher than spatial surveys, at $10.29 \pm 0.17$ μmol m$^{-2}$ d$^{-1}$ and $4.56 \pm 0.19$ μmol m$^{-2}$ d$^{-1}$, respectively (Fig. 7b), whilst N$_2$O mean flux rates were 772% lower in time-series measurements compared to those of surveys, at $-1.07 \pm 0.02$ and $-0.12 \pm 0.02$ μmol m$^{-2}$ d$^{-1}$, respectively (Fig. 7b). Converting these GHG fluxes to CO$_2$-equivalents (CO$_2$-e) using the 20-year sustained global warming potentials of Neubauer and Megonigal (2015) equates to an average time series and spatial survey CO$_2$-e flux of $-16.2 \pm 8.32$ mg m$^{-2}$ d$^{-1}$ and $-480.94 \pm 15.96$ mg m$^{-2}$ d$^{-1}$, respectively.

**Discussion**

Through the capture of particulate organic matter and photosynthetic fixation of dissolved carbon dioxide (CO$_2$), seagrass ecosystems play a major role in oceanic carbon storage (Macreadie et al. 2019; Serrano et al. 2019). Recent literature has highlighted seagrasses’ potential in natural carbon-offset strategies; however, their net carbon balance inclusive of atmospheric greenhouse gas (GHG) emissions is yet to be fully established (Macreadie et al. 2019; Serrano et al. 2019). Using a combination of spatial survey and time-series sampling methodologies across a temperate Australian seagrass meadow, we present evidence that during our study, the seagrasses represented net sinks of CO$_2$-equivalent gases inclusive of CO$_2$, methane (CH$_4$) and nitrous oxide (N$_2$O).
Table 1 Greenhouse gas concentrations, atmospheric flux and associated environmental conditions in a temperate Australian seagrass meadow. TimeS represents single point time series, whilst Spatial survey represents continuous underway spatial sampling. Negative flux indicates uptake into the water column.

|                  | TimeS day (6am–6 pm) | TimeS night (6 pm–6am) | TimeS overall | Spatial survey |
|------------------|----------------------|------------------------|---------------|----------------|
| **CO₂ flux**     | Mean: −0.66 ± 0.37   | Range: 14.38           | Mean: 1.06 ± 0.16 | Range: −21.14 |
| (mmol m⁻² d⁻¹)   |                      |                        | Mean: 12.99    | Range: −18.08 |
|                  |                      |                        | Mean: −0.46 ± 0.18 | Range: −24.31 |
| **CO₂ (%sat)**   | 121.79 ± 1.52        | 189.62–61.38           | 160.56 ± 1.46  | 266.2–58.70   |
|                  |                      |                        | 140.93 ± 1.17  | 266.23–41.63  |
| **CH₄ flux**     | 13.26 ± 0.26         | 22.93–0.67             | 7.95 ± 0.24    | 44.87–0.01    |
| (μmol m⁻² d⁻¹)   |                      |                        | 10.29 ± 0.17   | 44.87–0.001   |
| **CH₄ (%sat)**   | 312.59 ± 2.3         | 434.31–202.2           | 401.03 ± 3.05  | 712.78–203.15 |
|                  |                      |                        | 357.73 ± 2.12  | 712.87–202.2  |
| **N₂O flux**     | −1.71 ± 0.03         | −0.23                  | −0.9 ± 0.02    | < −0.01       |
| (μmol m⁻² d⁻¹)   | −2.79                |                        | −2.65          | 1.07 ± 0.02   |
| **N₂O (%sat)**   | 84.99 ± 0.5          | 98.78–60.14            | 76.58 ± 0.24   | 97.01–56.02   |
|                  |                      |                        | 81.29 ± 0.25   | 106.09–56.02  |
| δ¹³C-CO₂         | −9.64 ± 0.08         | −6.2                   | −10.85 ± 0.05  | −6.09          |
| (−13°C)          | −12.71               |                        | −10.22 ± 0.04  | −4.93          |
| **Wind (m s⁻¹)** | 4.95 ± 0.08          | 7.78–1.11              | 2.98 ± 0.05    | 6.67–0         |
|                  |                      |                        | 3.86 ± 0.04    | 7.78–0         |
| **DO (%sat)**    | 121.9 ± 2.46         | 196.3–20.8              | 67.57 ± 1.21   | 184.5–16.9    |
| **Air temp**     | 17.09 ± 0.11         | 20.2–9.3               | 13.72 ± 0.07   | 18–8.7        |
| (°C)             |                      |                        | 14.98 ± 0.06   | 20.20–8.70    |
| **Salinity**     | 35.55 ± 0.03         | 36.42–33.62            | 35.92 ± 0.01   | 36.47–34.09   |
| (PSS)            |                      |                        | 35.7 ± 0.01    | 36.47–33.62   |
| **Tidal depth**  | 0.51 ± 0.01          | 0.68–0.25              | 0.28 ± 0.00    | 0.62–0.15     |
| (m)              |                      |                        | 0.37 ± 0.00    | 0.68–0.15     |
| **pH**           | 8.43 ± 0.01          | 8.8–7.08               | 8.36 ± 0.00    | 8.79–8.09     |
|                  |                      |                        | 8.41 ± 0.00    | 8.84–7.08     |

In addition, we highlight the need for the incorporation of both spatial and temporal focused sampling designs when measuring seagrass emissions and discuss GHG environmental drivers. It is important to note, however, that due to the experiments being undertaken over a short period during the Austral summer period, our results may not be indicative of annual fluxes.

Through mass balance of in situ time-series sampling, seagrasses were shown to be a net sink of GHGs. The seagrass-associated fluxes of GHGs were estimated at 493.68 μmol CO₂ m⁻² d⁻¹, 10.47 μmol CH₄ m⁻² d⁻¹ and 1.46 μmol N₂O m⁻² d⁻¹, which at the 20-year sustained global warming potential of CO₂ equates to a net flux of 21.74 mg CO₂-e m⁻² d⁻¹. In addition, we found that the fluxes of CH₄ and N₂O negated each other at 16.12 and −16.13 mg CO₂-e m⁻² d⁻¹, respectively. Previous studies on CO₂ dynamics have shown that through photosynthetic production and the burial of organic carbon into their sediments, seagrasses act as net autotrophic coastal ecosystems (Gattuso et al. 1998; Gazeau et al. 2005). However, quantification of seagrass sediment gas flux has indicated that a portion of this sequestration may be offset by the release of radiatively potent CH₄ (Al-Haj and Fulweiler 2020; Alongi et al. 2008; Bahlmann et al. 2015; Barber and Carlson 1993; Debordre et al. 2010; Garcia-Bonet and Duarte 2017; Oremland 1975). Our data suggest that although temperate seagrasses are indeed a net source of CH₄, these emissions may be counterbalanced by the uptake of N₂O; however, as this result is from a single meadow in temperate Australia the finding should be used as a reference for further investigations.

The seagrass-associated CO₂ metabolism of −0.49 mmol CO₂ m⁻² d⁻¹ (uptake) within this study is two orders of magnitude lower than previous global estimates of seagrass net metabolism by Duarte et al. (2010), based on 155 sites at −99.45 ± 22 mmol CO₂ m⁻² d⁻¹, and more recent estimates of an Australian Zostera meadow at −99.9 mmol CO₂ m⁻² d⁻¹ by Maher et al. (2011). Whilst the net ecosystem metabolism (NEM = gross primary productivity − ecosystem respiration) of seagrasses varies as a result of a range of biotic and abiotic conditions including, faunal assemblage (Spivak et al. 2009; Kristensen et al. 2012), temperature (Staehr and Borum 2011), seagrass species (Duarte et al. 2010) and sediment quality (Udy and Dennison 1997; Terrados et al. 1999), such a large difference between previous literature and this study is notable. We suggest three reasons for this relationship:
1. Previous estimates of seagrass metabolism are predominantly based on the use of benthic chamber methodology (Duarte et al. 2010), where changes in the concentration of O$_2$ or CO$_2$ are directly linked to the sediment–water interface, whereas this study used an in situ open water sampling technique that inherently homogenizes with other dissolved gas sources, such as tidal pumping and microbial processes, that may affect the net balance of CO$_2$ flux estimates. Microbial breakdown and respiration of dissolved organic carbon associated with seagrass meadows have been shown as a significant term in net seagrass carbon budgets (Barrón et al. 2014; Maher and Eyre 2010) and may have offset the reductions in water column CO$_2$ from photosynthesis (Linto et al. 2014; Call et al. 2015).

2. The average solar irradiance during this sampling period was relatively low due to dense cloud cover at $372.98 \pm 9.97$ J m$^{-2}$ s$^{-1}$, whilst the comparable monthly average for this region is ~14.4% higher at 426.62 J m$^{-2}$ s$^{-1}$ (Australian Bureau of Meteorology). Variation in light conditions directly affects photosynthetic production in seagrass beds, with lower irradiance often resulting in lower rates of dissolved CO$_2$ uptake (Gacia et al. 2005; Touchette 2007).

3. A qualitatively large amount of seagrass wrack could be seen along the banks of the seagrass meadow (roughly 10 m from submerged sampling station), suggesting large concentrations of dead biomass in the water column. Microbial decay of leaf biomass is a major contributor to net ecosystem respiration within seagrass systems (Harrison and Mann 1975; Blum and Mills 1991; Mateo and Romero 1997; Liu et al. 2019), indicating a potential for large alternative CO$_2$ inputs and therefore potential offsets of mass-based estimates of net photosynthetic uptake.

Bay wide surveys showed large variations in CO$_2$ and CH$_4$ saturation, whilst N$_2$O saturation remained relatively homogenous (Fig. 5a–c and Table 1). Across medium to large spatial scales, variation in environmental conditions such as, topography, slope/depth, species composition and freshwater inputs (both from riverine and groundwater origins) can directly affect the production and chemical fate of GHGs in coastal systems (Gazeau et al. 2005; Allen et al. 2007; Maher et al. 2015). In accordance with our hypothesis, we found that both dissolved CO$_2$ and CH$_4$ were elevated in localised “hot spots” throughout the bay, whilst CO$_2$ and N$_2$O showed strongly inverse, and weak positive, correlations with dissolved oxygen, respectively (Fig. 6a, c). The photosynthetic productivity of seagrass meadows is reliant on a range of factors that include light availability, nutrient concentrations and substrate suitability, amongst others. In areas less suitable to seagrass establishment, a lack of photosynthetic productivity will lead to lower uptake of dissolved CO$_2$ and less oxygenated water. We found here that CO$_2$ concentrations were highest in the east of the bay, which is more influenced by mixing with oceanic waters (Fig. 5a).
Fig. 6 Generalized linear models of greenhouse gas relationships with dissolved oxygen and salinity across Swan Bay. Data was sampled through spatial surveys (one measurement per minute). %sat represents percent saturation, whilst PSS represents the practical salinity scale and * represents a significant correlation ($P < 0.05$). Data points are representative of CO$_2$; non-transformed, CH$_4$; natural log and N$_2$O; square root. Solid lines indicate the predicted model fit, and shaded areas represent 95% confidence interval.
High concentrations of CH$_4$ in the south-eastern area of the bay (Fig. 5b) were less easily explained, whereby no significant relationship with either oxygen or salinity was established, and both wind speed and pH remained relatively homogenous. Previous evaluation of coastal system sediment CH$_4$ flux has correlated higher CH$_4$ emissions with greater seagrass biomass (Bahlmann et al. 2015; Barber and Carlson 1993); however, in contrast to the literature we found the highest CH$_4$ concentrations above sediments with low sea-grass colonization (Figs. 5b and 1). The observed pattern in CH$_4$ may instead be due to a combination of organic matter supply from tidal inundation of the large adjacent tidal marsh (Fig. 1) and relatively anoxic sediments in the absence of seagrass O$_2$ production, facilitating a shift towards methanogenic microbial processes (Sansone and Martens 1981; Ollivier et al. 1994).

The concentrations of seagrass-associated dissolved GHGs were unsurprisingly shown to vary markedly over diel cycles. Both CO$_2$ and CH$_4$ percent saturation (%sat) in the water column were found to be 32 and 28% higher at night, whilst N$_2$O was 11% higher during the day (Fig. 4). The greater concentrations of dissolved CO$_2$ during the night are consistent with previous literature and our hypotheses that water column CO$_2$ is largely controlled by photosynthesis/respiration dynamics driven by light (Maher, et al. 2013a, b; Saderne et al. 2013). In addition, high oxygen concentrations facilitate nitrification in aquatic systems, where N$_2$O is produced during the metabolism of ammonium (NH$_4^+$) into nitrate (NO$_3^-$) (Elkins et al. 1978; Nishio et al. 1983). Higher N$_2$O concentrations during the day, in combination with the positive relationship between N$_2$O and dissolved oxygen saturation (DO%) from spatial surveys, indicate that oxygen-dependent aerobic nitrification is the predominant N$_2$O production pathway in temperate seagrasses (Xia et al. 2013; Johansson et al. 2011), although important to note is that throughout the entire diel cycle, N$_2$O was rarely above 100% atmospheric saturation (Fig. 4c). This suggests that similar to mangroves (Maher et al. 2016), seagrass systems may act as a sink of N$_2$O due to low nutrient concentrations and high rates of complete denitrification (Welsh et al. 2000; Eyre and Ferguson 2002; Eyre et al. 2016). Diel oscillations in autotrophic and heterotrophic respiration cycles within seagrass sediments may also lead to fluctuations in anaerobic methanogenesis and aerobic oxidation of dissolved methane (King et al. 1990; Maher, et al. 2013a, b). However, we did not find a significant relationship between DO and CH$_4$ as would be expected from respiration cycle-driven CH$_4$ production. Alternatively, CH$_4$ concentrations may be more tightly linked to fluctuations in tidal height that alter the exchange of sediment pore water rich in organic matter, with the water column, a process known as tidal pumping (Borges et al. 2003; Bouillon, et al. 2007a, b; Atkins et al. 2013; Maher, et al. 2013a, b; Macklin et al. 2014). Qualitative analyses of diel trends from this study show an inverse relationship between tidal height and CH$_4$%sat (Fig. 4b), a relationship that matches those of previous studies of tidal pumping in mangrove systems (Linto et al. 2014; Call et al. 2015); We therefore suggest that tidal processes were likely the predominant driver of CH$_4$ concentrations.

Large differences in mean atmospheric GHG flux were observed between spatial and temporal sampling methodologies, though both methods estimated flux to be of net uptake into the water column. In coastal ecosystems, variation of environmental conditions, such as DO, salinity, temperature and turbulence, often occurs across both spatial and temporal scales (Smith et al. 1999; Raymond and Cole 2001; Duarte 2002; Bouillon, et al. 2007a, b; Touchette 2007). As photosynthetic production, microbial respiration and gas transfer dynamics are highly linked to these environmental conditions, accounting for both spatial and temporal variation in gas concentrations is important for accurate GHG emission estimates (Gazeau et al. 2005; Call et al. 2015; Maher et al. 2015). Here, net atmospheric GHG uptake
rates from bay-wide surveys were ~30 times greater than those from the time-series experiment, at $-480 \pm 15.96$ and $16.2 \pm 8.32$ mg CO$_2$e m$^{-2}$ d$^{-1}$ respectively, a disparity primarily attributed to much lower CO$_2$ uptake rates during the time-series sampling (Table 1). Variation in GHG flux between spatial and temporal sampling methodologies is in accordance with previous literature from mangrove and estuarine systems (Maher, et al. 2013a, b; Ho et al. 2014; Maher et al. 2015) and further highlights the need for the inclusion of both of these processes when estimating seagrass atmospheric emissions. Important to note, is that whilst the survey methodology from this study maintained a high sampling frequency (i.e. one sample per minute), it was both limited to the outer regions of the bay (Fig. 1) and did not incorporate diel or seasonal variations. The survey was carried out during daylight hours, amplifying the role of primary production on our estimates. Similarly, time-series methodology is constrained to a single point in the bay, whilst diel variation in GHG emissions is likely to vary across the bay. Therefore, the observed variations in flux between the methodologies can only be assessed qualitatively due to the lack of direct overlap, and a robust correlative equation for day-night GHG variations could not be established, though it is of high importance for more accurate future estimates of seagrass emissions based on spatial surveys alone.

The carbon isotopic signature (δ$^{13}$C) of water column dissolved CO$_2$ indicated that allochthonous terrestrial carbon was not a major contributor to microbial community respiration within Swan Bay. Seagrasses are effective ecosystems for the biosequestration of atmospheric carbon; however, they are also able to trap and bury allochthonous carbon from terrestrial run-off and other coastal ecosystems higher in elevation such as mangroves and tidal marsh (Agawin and Duarte 2002; Duarte et al. 2005; Kennedy et al. 2010; Deegan et al. 2012). In addition, a range of periphytic organisms establish themselves on seagrass blades and may contribute to sedimentary carbon concentrations (Walker and Woelkerling 1988; Gacia et al. 2002). Understanding the carbon source of microbial respiration within seagrasses allows for more targeted management of emissions (Gerald et al. 2019), for example, terrestrial carbon run-off may be reduced through better up-stream effluent management or increased fencing along waterways. Here, we find that the predominant CO$_2$-C source as established through Keeling plots (Keeling 1958; Pataki et al. 2003; Maher et al. 2017) was $-13.75$ ‰, an isotopic value that closely matches that of Zostera spp. and their associated periphyton ($-12.2$ $-13$ δ$^{13}$C) (Thayer et al. 1978). These results suggest that terrestrial C3 carbon inputs do not play a significant role in aquatic metabolism in the bay, and the GHG fluxes measured are likely indicative of in situ processes.

In conclusion, this study provides the first simultaneous in situ assessment of CO$_2$, CH$_4$ and N$_2$O atmospheric emissions from temperate Australian seagrasses. We find that the seagrasses studied were a net CO$_2$-e sink, due to CO$_2$ and N$_2$O uptake outweighing CH$_4$ release. We also note that seagrass atmospheric emissions were heavily linked to diel fluctuations in light availability and tidal pumping, and that omission of these variables when estimating seagrass emissions is likely to lead to inadequate estimates. Finally, we demonstrate large variation in CO$_2$ and CH$_4$ emissions at the habitat scale, highlighting the need for the inclusion of spatial parameters when upscaling seagrass emission estimates. As seagrasses maintain some of the highest rates of carbon sequestration and storage on the planet, they represent potential ecosystems for nature-based carbon offsetting. Further research into seagrasses overall carbon sink potential incorporating their associated GHG emissions is extremely important for future offset investments.

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