Net heterotrophy and carbonate dissolution in two subtropical seagrass meadows

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10 **Abstract.** The net ecosystem productivity (NEP) of two contrasting seagrass meadows within one of the largest seagrass ecosystems in the world, Florida Bay, was assessed using direct measurements over consecutive diel cycles. We report significant differences between NEP determined by dissolved inorganic carbon (NEP_{DIC}) and by dissolved oxygen (NEP_{DO}), likely driven by differences in air-water gas exchange and contrasting responses to variations in light intensity. In this first direct determination of NEP_{DIC} in seagrasses, we found that both seagrass ecosystems were net heterotrophic, on average, 15 despite large differences in seagrass net aboveground primary productivity. Net ecosystem calcification (NEC) was also negative, indicating that both sites were net dissolving of carbonate minerals. We suggest that a combination of carbonate dissolution and respiration in sediments exceeded seagrass primary production and calcification, supporting our negative NEP and NEC measurements. Furthermore, a simple budget analysis indicates that these two seagrass meadows have contrasting impacts on pH buffering of adjacent systems, due to variations in the TA:DIC export ratio. The results of this study highlight 20 the need for better temporal resolution, as well as accurate carbonate chemistry accounting in future seagrass metabolism studies.

1 Introduction

Seagrass ecosystems are often net autotrophic, producing more organic matter than they consume (Duarte et al, 2005; Barrón et al., 2006; Duarte et al, 2010; Unsworth et al., 2012; Long et al., 2015a; Ganguly et al., 2017; Perez et al., 2018). In 25 terrestrial ecosystems, CO_2 uptake by photoautotrophs necessarily leads to an exchange of carbon from the atmosphere to the biosphere. However, such a net uptake of CO_2 by submerged seagrasses is attenuated as carbon produced or consumed by net ecosystem productivity (NEP) interacts with the carbonate buffering system and the processes of calcification and carbonate dissolution in the water and submerged sediments. The impact of seagrass carbonate chemistry on measurements of NEP is further obscured by physical processes at the air-water interface, which may cause temporal lags between NEP and air-water 30 CO_2 exchange.

Calcification is an important process in many tropical and subtropical seagrass ecosystems (Mazarrasa et al. 2015) and has the net effect of consuming total alkalinity (TA) and generating CO_2. Florida Bay is a well-studied seagrass-dominated ecosystem and is assumed to be net calcifying given the vast autochthonous sedimentary deposits of $CaCO_3$ that have accumulated in the bay in the last three millennia (Stockman et al., 1967; Bosence et al., 1985). While much of this $CaCO_3$

5   was produced by other photoautotrophic or non-photoautotrophic calcifiers (Frankovich and Zieman 1994), it is likely that some unknown fraction was also derived from calcification driven directly by the seagrasses (Enríquez et al., 2014). Existing measurements from Florida Bay show that net ecosystem calcification (NEC) can vary from positive to negative over diel cycles (Turk et al., 2015), and across gradients of seagrass productivity and substrate type (Yates and Halley 2006). The relative magnitudes of NEC and NEP in the context of the overall seagrass ecosystem carbon budget is unclear, and it is still uncertain

10   which component of the ecosystem dominates net calcification (seagrasses, benthic invertebrates, macroalgae, etc.). Early assessments of seagrass NEC in Florida Bay relied on species-specific calcification rates that were up-scaled to the community or ecosystem level. These studies indicate that epiphytic calcification can dominate NEC (Frankovich and Zieman 1994), and that the physical transport of carbonate mud within the bay is likely significant (Bosence 1989). The physical transport of carbonate mud is important because it can allow $CaCO_3$ formation and destruction to become spatially decoupled, such that

15   regions of net dissolution may exist within the larger context of a net calcifying Florida Bay. More recently, results from in-situ chambers have indicated that seagrass primary production can dominate short-term carbonate chemistry dynamics (Hendriks et al., 2014; Turk et al., 2015; Camp et al., 2016).

This biological CO_2 addition or removal causes non-linear changes in the marine carbonate system, further challenging direct measurements of seagrass ecosystem NEP. Hence, prior assessments of seagrass NEP were often made

20   using dissolved oxygen production (DO) as a proxy for CO_2 fixation, necessitating the assumption of a photosynthetic quotient (PQ) relating CO_2 fixation to DO production. The assumption of a PQ value is made problematic by the carbonate system reactions discussed earlier, which affect CO_2 but not DO. While is often assumed that PQ is approximately 1 (e.g., Duarte et al., 2010), prior measurements of $\Delta CO_2/\Delta DO$ in seagrass ecosystems show a wide range of values, from 0.3 to 6.8 (Ziegler and Benner 1998; Barrón et al., 2006; Turk et al., 2015). As a result, potential exists for a general disagreement between NEP

25   assessed using measurements of carbon, and those using its O_2 proxy (NEP$_{DO}$). Hence, we identify a need for simultaneous measurements of pH, O_2, pCO_2, TA and dissolved inorganic carbon (DIC) when assessing seagrass ecosystem NEP and NEC, which may explain the divergence between CO_2- and O_2-based methods.

In addition to the importance of primary production in seagrass beds as a source of energy to fuel coastal ecosystems, the net uptake of CO_2 from the overlying water could have other important impacts of the seascapes in which the seagrasses

30   occur. High primary production drives large diel variations in pH within seagrasses meadows (e.g. Hendriks et al., 2014; Turk et al., 2015; Camp et al., 2016; Challener et al., 2016), and it has been suggested that seagrass NEP may partially buffer coastal ocean acidification (OA) by consuming CO_2, thereby acting as refugia for calcifying organisms (Manzanello et al., 2012; Unsworth et al., 2012; Hendriks et al., 2014; Koweek et al., 2018; Pacella et al., 2018). They may also locally buffer OA by attenuating mangrove-derived lateral carbon fluxes (Buillon et al 2007). However, it remains unclear how NEP and NEC might

interactively affect carbonate system buffering in regions where primary producer biomass and NEP are limited by the availability of nutrients, like in the severely phosphorus-limited regions of Florida Bay (Fourqurean et al. 1992).

Prior studies of NEP_{DO} in Florida Bay have suggested net autotrophy (Long et al., 2015a), yet others were unable to infer long-term NEP_{DO} balance (Turk et al., 2015). Both of these estimates of NEP_{DO} necessarily ignore any anaerobic catabolic

5   biogeochemical processes that may cause NEP_{DIC} to decrease, but do not affect NEP_{DO}. Rates of denitrification (Eyre and Ferguson 2002) and sulfate reduction (Smith et al., 2004, Ruiz-Halpern et al., 2008) can be significant in seagrass soils, although rates may depend on specific seagrass morphology and physiological traits (Holmer et al., 2001). Additionally, despite the inferred net ecosystem autotrophy of seagrasses, pCO_2 is often found above (Millero et al., 2001) or near (Yates et al., 2007) equilibrium with the atmosphere throughout most of Florida Bay, suggesting the role of NEC or anaerobic catabolic

10   processes in generating excess CO_2.

In this study, we describe our direct measurements of NEP_{DIC}, NEP_{DO}, and NEC in two Florida Bay seagrass sites. We investigate variations in NEP and NEC across a seagrass productivity gradient, discuss potential differences between NEP_{DIC} and NEP_{DO}, and suggest potential drivers of NEP and NEC. We then assess the capacity of these two sites to potentially buffer adjacent ecosystems, and their potential impacts on coastal ocean acidification.

15   ## 2 Methods

2.1 Study Site

This study took place in one of the largest seagrass ecosystems in the world, Florida Bay (Figure 1), where we occupied two nearby sites which experience similar hydrologic and climatologic conditions yet differ substantially in community composition and productivity (Table S1). The choice of these sites allowed us to discern the effects of seagrass

20   community structure and productivity on NEP and NEC that are independent of environmental setting. Both sites were dominated by the seagrass *Thalassia testudinum* in a phosphorus limited region (Fourqurean et al., 1992), have similar water depths (~2m), and were approximately 0.5 - 1 km from land. However, these sites differed in important factors like seagrass abundance and aboveground net primary productivity, nutrient content, morphology, as well as sediment depth, soil carbon (organic and inorganic), soil nutrient content, and grain size (Table S1). The potential for submarine groundwater discharge at

25   these locations is low (Corbett et al., 1999). In addition to the two primary study sites, we collected time series data of DO and pH for an additional four Florida Coastal Everglades Long Term Ecological Research (FCE-LTER) sites in an effort to test whether the relationship between NEP_{DO} and NEP_{DIC} observed in this study can be extended over larger areas of Florida Bay.
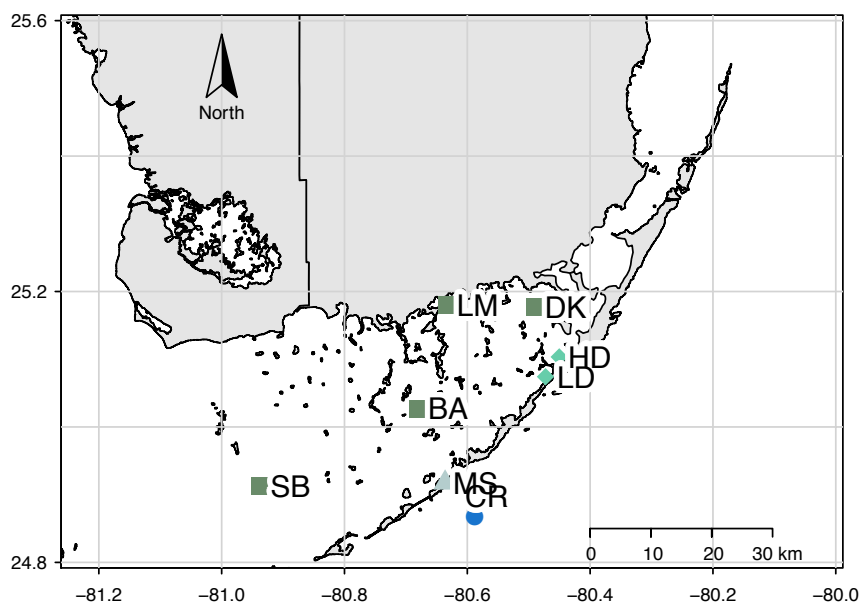
Figure 1. Site map, showing locations of the high- and low-density sites (HD and LD), meteorological stations used to derive U_{10} and pCO_2 data (MS and CR, respectively). Additional FCE-LTER sites used in this study are shown as the green squares: Sprigger Bank (SB), Bob Allen (BA), Little Madeira (LM), and Duck Key (DK).

2.2 Sampling Campaigns

We quantified NEP_{DO}, NEP_{DIC}, and NEC at our high density (HD) and low density (LD) sites by measuring diel excursions in DO, DIC and TA, and applying corrections to account for factors like air-water gas exchange and variations in water depth and light intensity. This is essentially a modification of the 'free-water' approach to assessing NEP (Nixon et al., 1976; Odum and Hoskin 1958), where the total inventory of DIC or O_2 is monitored over time. A benefit of this approach over traditional chamber-based metabolism methods is that the container effect is avoided, which is known to result in under-estimations of benthic respiration, due to a dampening of turbulent sediment-water exchange (Hopkinson and Smith, 2007). A weakness, however, is the reliance on modelled air-water gas exchange, which is subject to a high degree of uncertainty (Upstill-Goddard 2006). During two sampling campaigns in late 2018 (Oct. 28 - Nov. 01, and Nov. 25 – Nov. 29), measurements were made over consecutive diel cycles for a total of 8 days.

Samples were taken 3 times per day during the first campaign (dawn, noon, and dusk), and 4 times per day during the second campaign (dawn, late morning, early afternoon, and dusk). During the first sampling campaign, water samples were collected for the analysis of stable isotopic composition of DIC ($\delta^{13}C_{DIC}$), in an effort to constrain potential DIC sources. We applied Keeling plots to our isotopic data, where $1/n$DIC is plotted against $\delta^{13}C_{DIC}$. In this approach, the y-intercept (as $1/n$DIC approaches 0) indicates the $\delta^{13}C_{DIC}$ value as nDIC approaches infinite concentration (e.g., as $1/n$DIC approaches 0) and can be interpreted as an indicator of the $\delta^{13}C_{DIC}$ of the source of the DIC (Karlsson et al., 2007).

2.3 Discrete Measurements

At our primary study sites, water samples for total alkalinity (TA) and dissolved inorganic carbon (DIC) were collected with pre-rinsed borosilicate bottles at a depth of approximately 0.2 m. TA and DIC samples were preserved with a saturated solution of $HgCl_2$ and stored on ice until analysis (Dickson et al., 2007). Samples for $\delta^{13}C_{DIC}$ were taken at the same

5   depth, filtered to 0.45μm, and preserved with $HgCl_2$. Calcite saturation state ($\Omega_{calcite}$) was calculated in CO2Sys (Lewis and Wallace 1998) from measured TA, DIC, salinity and temperature, using the H_2CO_3 dissociation constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987).

2.4 Continuous Measurements

At each of our primary sites, we deployed a YSI EXO-2 water quality sonde which recorded water depth, sea surface

10   temperature (SST, °C), sea surface salinity (SSS), and dissolved oxygen (DO (mg L-1 and % saturation)) at an interval of 15 minutes. In-situ pH was measured at each site with an ion-sensitive field effect transistor sensor (Seabird SeaFET) at an interval of 5 minutes, with an initial accuracy of ± 0.05 pH on the Total scale. In order to assess the sensitivity of NEP and NEC to light availability, we recorded photosynthetically active radiation at the seagrass canopy (PAR; μEinstein m^{-2} s^{-1} [μE m^{-2} s^{-1}]) with a submerged Seabird ECO-PAR sensor equipped with an automatic wiper for the optics. We also deployed Lowell tilt

15   current meters (TCMs) at both of our primary sites to assess lateral transfer of water through the site, but the observed current speeds were below the minimum detectable speed for these instruments ($< \sim 2$ cm s^{-1}).

At the four FCE-LTER sites (Fig. 1), we measured DO and pH over a span of 4-7 days in September (BA, LM, and DK) and 8 days in December (SB), with an hourly sampling frequency using YSI EXO-2 sondes. These sites span broad gradients in phosphorus-limitation, seagrass productivity (Fourqurean et al. 1992), carbonate production (Yates and Halley

20   2006), DIC and TA concentrations (Millero et al., 2001), air-water CO_2 exchange (Yates and Halley 2006; DuFore 2012). We used these pH and DO data to calculate temporal excursions in DO (ΔDO) and hydrogen ion concentration ($\Delta[H^+]$) (mM hr^{-1}), which are proxies for NEP_{DO} and NEP_{DIC} respectively (Long et al., 2015b). Data from these FCE-LTER deployments was compared with data from the two primary sites to determine whether the results of this study were generalizable to the rest of Florida Bay.

25   ## 2.5 Benthic Chamber Fluxes

During the second sampling campaign, benthic chambers were deployed continuously over bare sediment at each site to measure sediment-water fluxes of TA and DIC driven by sediment processes. At the beginning of the experiment, acrylic chambers (~2.5L) were flushed with site water and placed on the sediment. Chamber incubations ran for a total of 4 days. At intervals ranging from 8-20 hr, ~150 mL samples were taken from the chambers using a syringe, and the chambers were re-

30   equilibrated with ambient site water. Fluxes were calculated based on the difference in concentration between the ambient water sample at the initial time of chamber placement, and the final concentration inside the chamber.

2.6 Sample Analysis

TA was analysed in triplicate 25 mL subsamples by automated Gran titration at a controlled temperature on an Apollo AS-ALK2, with an average precision (standard deviation of replicate measurements) of ±1.89 µmol kg^{-1} or 0.07% of the average measured TA. Samples for DIC were analysed by injecting 250 µL subsamples into an impinger filled with 10% HCl, converting all DIC to CO_2, which was subsequently transferred with a pure N_2 carrier gas to a LI-COR 6262 infrared gas analyser in integration mode. The average precision of this method was noticeably lower than that for TA, at ± 5.11 µmol kg^{-1} or 0.21%. Both TA and DIC measurements were converted to gravimetric units by multiplying the concentration (µM) by the calculated SSS and SST-derived seawater density using the Gibbs Seawater toolbox for Matlab (GSW; McDougall and Barker 2011) to derive units of µmol kg^{-1}.

Samples for $\delta^{13}C_{DIC}$ were analysed on a Thermo Gas Bench coupled to a Thermo Delta V Isotope Ratio Mass Spectrometer and reported in delta (δ) notation in units of per-mille (‰) relative to Vienna Pee Dee Belemnite. Precision for this measurement was ±0.4‰ based on replicate analyses of Certified Reference Material (Dickson et al. 2003).

2.7 NEP and NEC Calculations

NEC, NEP_{DIC}, and NEP_{DO} were determined by integrating temporal excursions in salinity-normalized TA (nTA) and DIC (nDIC), and DO. We quantified the total TA or DIC inventory over time to determine NEC and NEP. First, NEC (mmol $CaCO_3$ m^{-2} hr^{-1}) was estimated using the alkalinity anomaly technique, which assumes that variations in TA are affected only by $CaCO_3$ precipitation and dissolution (1):

$$NEC = -0.5 \times \frac{\Delta n\text{TA}}{\Delta t} \times h\rho, \tag{1}$$

where ΔnTA was the difference in nTA (nTA = TA×SSS$_{Average}$/SSS), h the water depth, and ρ the seawater density. The -0.5 scalar was required because 2 moles of TA are required to form one mole of $CaCO_3$ production. Salinity normalized DIC (ΔnDIC) was calculated in the same manner as ΔnTA. The temporal excursion in nTA used for Eq. 1 was calculated between each sampling point shown in Fig. 2g and 2h, for a total of 28 individual measurements of NEC. SSS$_{Average}$ was determined for each sampling campaign at each site. By convention, NEC is positive when TA consumption occurs and $CaCO_3$ is inferred to have been precipitated. NEP_{DO} (eq 2; mmol O_2 m^{-2} hr^{-1}) and NEP_{DIC} (eq 3; mmol C m^{-2} hr^{-1}) were calculated in a similar manner, but with additional corrections for air-water gas exchange and DIC consumption by NEC:

$$NEP_{DO} = \frac{\Delta \text{DO}}{\Delta t} h\rho - O_2 \text{ Flux}, \tag{2}$$

$$NEP_{DIC} = \frac{\Delta n\text{DIC}}{\Delta t} h\rho - NEC - CO_2 \text{ Flux}, \tag{3}$$

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where O_2 and CO_2 fluxes (eq 4 and 5) were estimated with a bulk-transfer approach using two different formulations for the gas transfer velocity (k_{600}). These k_{600} parameterizations were intended to represent upper (Raymond and Cole (2001)) and lower (Ho et al., 2006) bounds for gas exchange, respectively. Wind data used to derive the k_{600} were taken from the NOAA meteorological station at Islamorada (DW1872; Fig 1) and normalized to a height of 10m above the sea surface under neutral
5   drag conditions (Large and Pond 1981).

$$O_2 \text{ Flux } = k_{600} * Sc * \big(O_2(water) - O_2(air)\big), \tag{4}$$

$$CO_2 \text{ Flux } = k_{600} * Sc * K * \big(pCO_2(water) - pCO_2(air)\big), \tag{5}$$

where $pCO_{2(water)}$ was the partial pressure of CO_2 (μatm), and O_2 was the measured DO concentration (mg L^{-1}). $pCO_{2(water)}$ was calculated from measured TA and DIC using CO2SYS as above. Atmospheric pCO_2 ($pCO_{2(air)}$) was taken from the nearby
10 Cheeca Rocks Mooring buoy operated by NOAA (Fig 1), while $O_{2(air)}$ was calculated from the measured DO (%) as . The gas solubility (K) and Schmidt numbers (Sc) were calculated from in-situ SSS and SST (Wanninkhof 1992; Weiss 1974). No attempt was made to refine NEC by accounting for the TA produced by ecosystem productivity, but preliminary calculations assuming TA increases with DIC consumption at a ratio of 17/106 (Middelburg 2019) indicated that this TA production was small compared to total NEC.

15 **3. Results**

3. Physico-chemical conditions

Variations in SSS were generally less than 1 over each deployment period at both sites, indicating that lateral mixing, evaporation, precipitation, and fresh groundwater inputs were likely minor during the study period (Fig 2c,d). SST ranged from 18.5 to 27.0 across both sampling campaigns, and was very similar between sites (Fig. 2c,d). SSS was also stable, between
20 33.15 and 34.63 at the high-density site, and 31.45 to 34.67 at the low-density site. Diurnal variations in PAR coincided with those in SST, as is typical for sun-lit shallow water (Fig 2a). Likewise, both DO and pH exhibited typical diel excursions. Peak DO saturation of 115 (High density) and 135% (Low density) occurred in the late afternoon, coinciding with maximum pH of approximately 8.05 (High density) and 8.35 (Low density) respectively. Average pH was 8.10 ± 0.06 at the high-density site, compared with 8.17 ± 0.06 at the low-density site. Calculated $pCO_{2(water)}$ at the high-density site (538.8 ± 123.5 μatm) was
25 generally greater than atmospheric equilibrium, while average $pCO_{2(water)}$ was less than $pCO_{2(air)}$ at the low-density site (390.3 ± 129.4) (Table S1). Calculated CO_2 flux was generally positive (from the water to the atmosphere) and small in magnitude, between 0.13 ± 0.62 and 0.38 ± 0.20 mmol C m^{-2} hr^{-1} at the high-density site (RC01 and Ho06 respectively), and 0.20 ± 0.40 and 0.067 ± 0.35 mmol C m^{-2} hr^{-1} the low-density site (Table S1). A paired t-test found a significant difference between CO_2 fluxes derived using the RC01 and Ho06 k_{600} parameterizations. However, this difference was small in magnitude compared

to NEP and NEC, so for the sake of simplicity, we only present results using the Ho06 parameterization in the main text of this manuscript. Results considering both parameterizations are given in the supporting information.
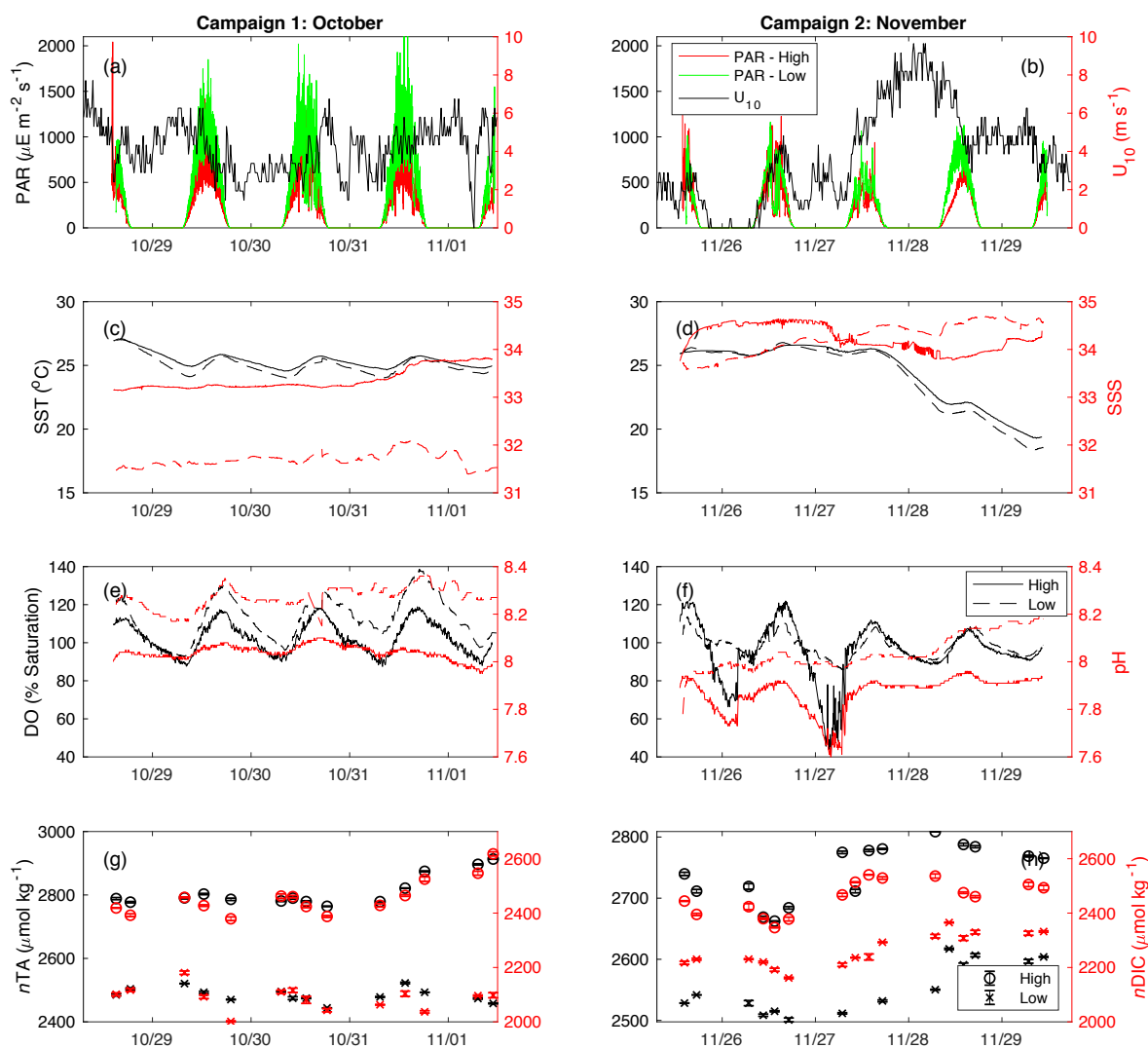

Figure 2. Time-series of a-b) PAR (μE m^{-2} s^{-1}) and U_{10} (m s^{-1}), c-d) SST and SSS, e-f) DO saturation (%), pH, and g-h) nDIC and nTA (μmol kg^{-1}). For plots b-c, the solid lines represent the high-density site, dashed lines are the low-density site.

Between the first and second sampling campaigns, average mid-day PAR (from 10:00 to 14:00) reaching the benthos at the low-density site fell by approximately 38%, from 916 ± 332 m^{-2} s^{-1} during the first sampling campaign to 567 ± 219 μE m^{-2} s^{-1} for the second sampling campaign. Similarly, average mid-day PAR at the high-density site fell by ~31%, from 627 ± 259 μE m^{-2} s^{-1} during the first sampling campaign, to 432 ± 211 μE m^{-2} s^{-1} for the second sampling campaign. After the passage of a large cold front and associated high wind speed on 11/28, SST fell by more than 5 °C. At the initial SSS, DIC, and TA,

the thermodynamic effect of this cooling was a nearly 0.1 increase in pH (CO2Sys), which was on the order of the typical diel range (Fig 2e). While this rapid pH increase (independent of DO%) was evident at the low-density site, no such change occurred at the high-density site (Fig 2f), indicating that biological factors outweighed the thermodynamic effect on pH there.

5 Across the study period, nTA at the high-density site was always greater than nTA at the low-density site, and nTA was generally higher than nDIC at both sites. Diel cycles were evident in both nDIC and nTA, coinciding with typical variations in net ecosystem production (consuming nDIC), calcification (consuming nTA). The average slope of nTA to nDIC was 0.64 and 0.41 for high- and low-density sites respectively (Fig 3), indicating that variations in TA and DIC were likely driven by a combination of ecosystem metabolism (expected slope of ~0), calcification (slope of 2), as well as SO_4^{2-} reduction (slope of 1) and denitrification (slope of 0.8), similar to other Florida seagrasses (Camp et al., 2016; Challener et al., 2016).
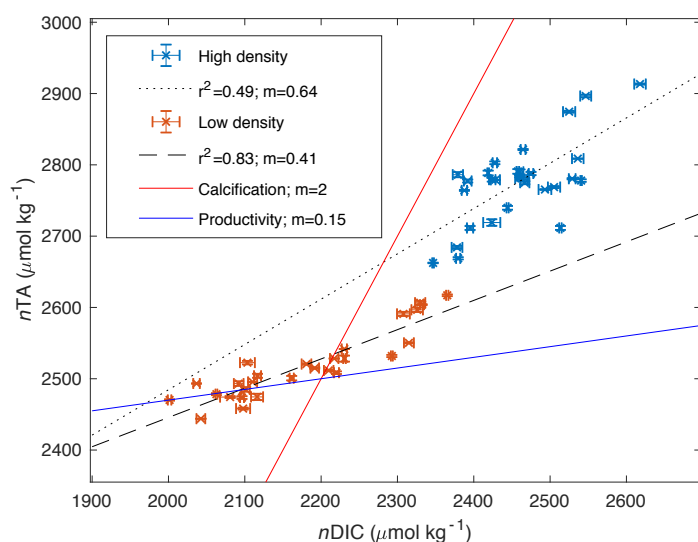
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Figure 3. Scatter plot of nDIC and nTA for both high-density (blue) and low-density (orange) sites, and associated slope (m) and correlation coefficient (R^2) of the linear regression. The red reference line indicates the expected relationship if calcification is dominant, consuming 2 moles of TA for every mole of DIC consumed to form $CaCO_3$. The blue reference line shows the approximate relationship expected for aerobic respiration/productivity, which generates approximately 0.15 moles 15 of TA for every mole of DIC respired.

3.2 NEP and NEC

At both sites, calculated NEP_{DO} and NEP_{DIC} followed a clear diel pattern, increasing between sunrise and early afternoon, and decreasing through sunset (Fig. 4). Night-time NEP_{DO} and NEP_{DIC} was nearly always negative (heterotrophic), while daytime values were larger and more variable, often exceeding ~15-20 mmol C m^{-2} h^{-1} in the late morning. Individual 20 measurements of NEP_{DIC} for the low- density site (-14.5 to 29.2 mmol C m^{-2} h^{-1}) and high-density site (-36.2 to 21.4 mmol C m^{-2} h^{-1}) were within the range of some previous studies, including NEP_{DO} from of Turk et al. 2015 (-6.2 ± 1.0 to 12.3 ± 1.0

mmol O_2 m^{-2} h^{-1}), Perez et al. 2018 (~ 23.8 mmol O_2 m^{-2} h^{-1}) and Long et al. 2015a (0.45-1.46 mmol O_2 m^{-2} h^{-1}). While NEC was also strongly negative (dissolving) at night, it was highly variable during the day, with no clear trend between sunrise and sunset (Fig 4). It is important to note that this approach does not account for any TA production by net SO_4^2 reduction and denitrification, and any such TA inputs may bias these estimates of NEC. However, our NEC estimates are at least an order of

5 magnitude larger than typical published measurements of seagrass SO_4^{2-} reduction (Holmer et al., 2003; Brodersen et al., 2019) and denitrification (Welsh et al., 2001) rates, suggesting that our NEC determinations were indeed largely driven by $CaCO_3$ precipitation and dissolution
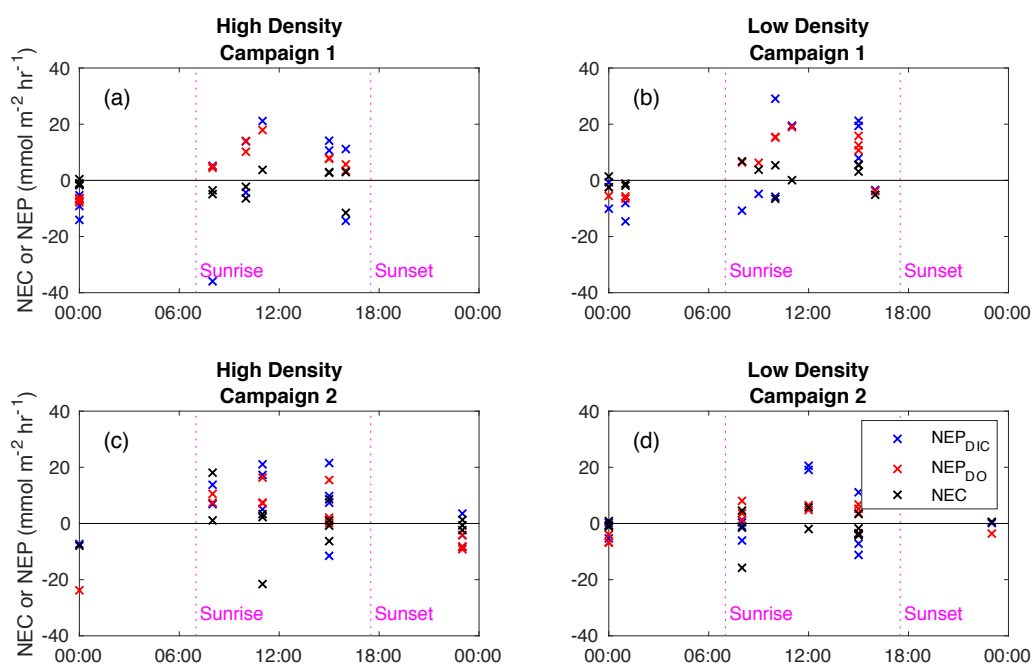

Figure 4. NEC (blue), NEP$_{DIC}$ (black) and NEP$_{DO}$ (red) plotted as a function of hour for the high-density site (a,c) and low-
10 density site (b,d), for sampling campaign 1(a,c) and 2 (b,d). The x-axis represents the midpoint time for each NEP or NEC calculation period.

        When discrete NEP and NEC rates were integrated over cumulative day and night hours, diel trends became more recognizable (Fig. 5a-b). Cumulative NEP$_{DIC}$ and NEP$_{DO}$ values were positive during the day (net autotrophic) and negative

15 (net heterotrophic) at night for both sites. There was a small difference in NEP$_{DIC}$ calculated using RC01 and Ho06 k$_{600}$ parameterizations (paired t-test, α=0.05, 0.13 ± 0.07 mmol m^{-2} h^{-1}). However, this difference was small for NEP$_{DIC}$ and non-existent for NEP$_{DO}$ (Figure S1), so we simply present the NEP$_{DO}$ and NEP$_{DIC}$ values derived using the Ho06 parameterization alone. Average NEC was less than NEP$_{DIC}$, such that the NEC: NEP$_{DIC}$ ratio was 0.54 and 0.31 for the high- and low-density sites respectively, well within the range of tropical seagrass ecosystems globally (Camp et al., 2016) and locally (Turk et al.,

20 2015). Although NEP$_{DIC}$ and NEP$_{DO}$ agreed in direction, NEP$_{DO}$ was significantly greater in magnitude than NEP$_{DIC}$ for all

time periods except at night for the low-density site (Fig 5a). Night-time NEC was not significantly different from zero because of the high variability in individual measurements (Fig 4). NEC was more consistently negative (net dissolving) at night (Fig. 4), causing cumulative NEC to be less than zero (Fig. 5). Night-time dissolution was greater at the high-density site than the low-density site.
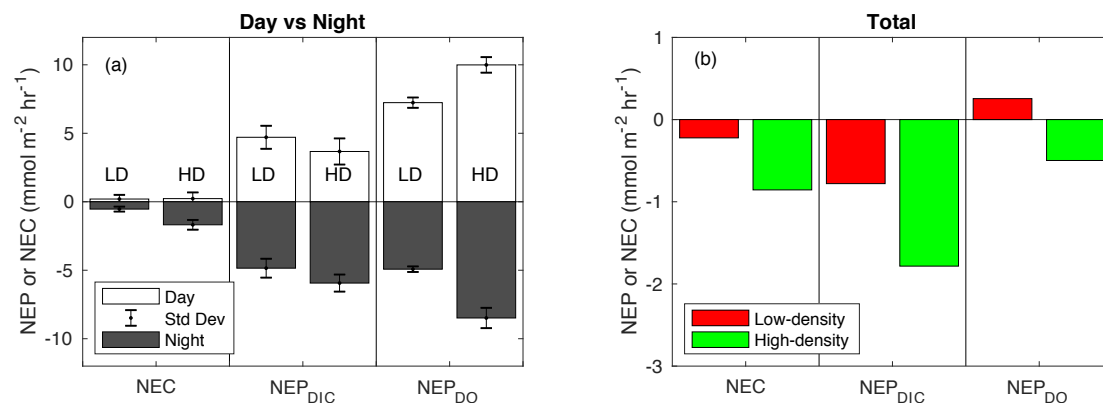
Figure 5. Average NEC, NEP_{DIC}, and NEP_{DO} (a) separated by day and night-time periods, and (b) expressed as a total for the entire study period. NEP values are shown for k_{600} of Ho et al., 2006.

NEP_{DIC} and NEC were negative (heterotrophic and dissolving) at the both sites over the entire study period (Fig 5b). In contrast, NEP_{DO} was small and positive at the low-density site, and small but negative at the high-density site. This difference between NEP_{DO} and NEP_{DIC} was significant when values were split by day and night (Fig. 5a). In fact, the linear relationship between NEP_{DO} and NEP_{DIC} in this study was not significantly different from 0 for the high-density site (p=0.095; r^2=0.11) and was significant but weak (p=0.001; R^2=0.35) for the low-density seagrass site (Fig 6d). While NEP_{DO} and NEP_{DIC} agreed in sign at night (dark blue points in Fig 6d), there was no such relationship for daytime NEP_{DO} and NEP_{DIC}. Correlations between net ecosystem processes and PAR were not strong (R^2<0.5) for NEP_{DIC} and NEP_{DO} and were very weak (R^2<0.05) for NEC (Fig. 6a-c).
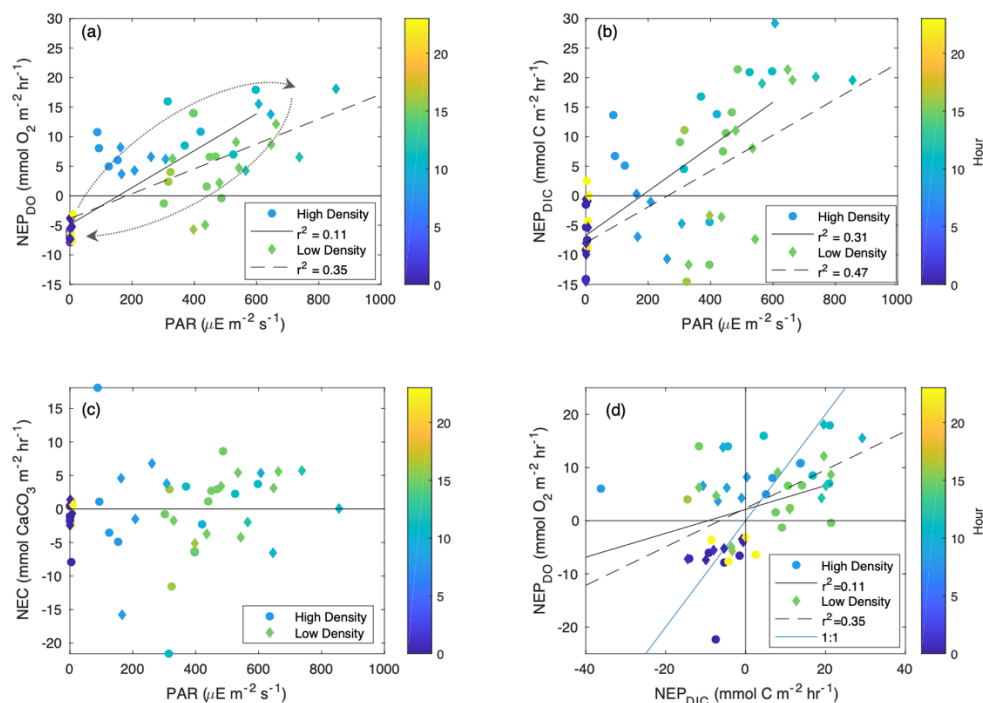
Figure 6. Scatter plots of (a) NEP_{DO} vs PAR, NEP_{DIC}, and NEC vs PAR (b-c), and NEP_{DO} vs NEP_{DIC} (d). Points are colored by the average hour for the respective time period over which NEP or NEC was calculated. The arrows in (a) are intended to highlight the hysteretic pattern between PAR and NEP_{DO}.

5

To address whether this disconnect between NEP_{DO} and NEP_{DIC} exists outside of the two primary sites (Fig. 7; HD and LD sites), we assembled pH and DO data from 4 additional sites across Florida Bay (Fig. 7: SB, BA, DK, and LM). Even though $\Delta[H^+]$ and ΔDO were correlated at our primary sites and one of the four LTER sites (LM), correlations were poor ($R^2 < 0.25$) at the remaining LTER sites. The LM site is heavily influenced by terrestrial inputs from the coastal Everglades and

10   fringing mangroves, which likely contributed to the significant relationship between $\Delta[H^+]$ and ΔDO there ($R^2 = 0.48$).
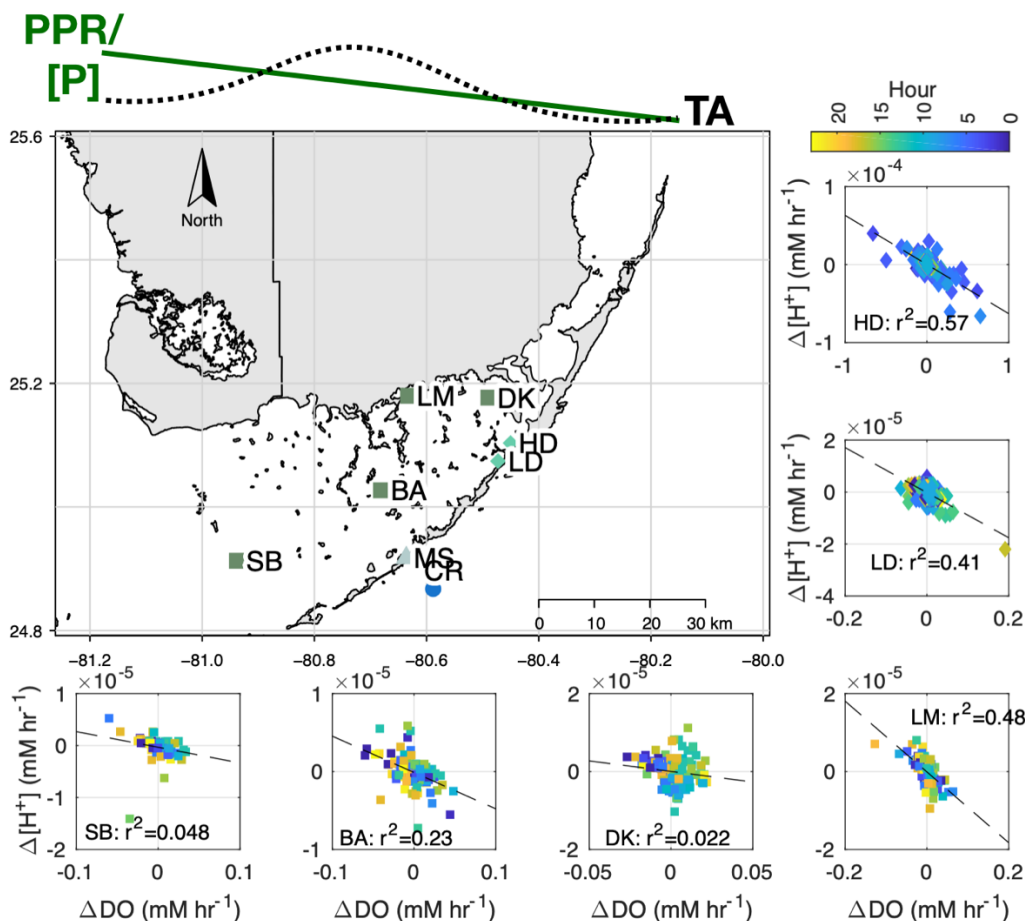
Figure 7. Map showing $\Delta[H^+]$ vs ΔDO relationship for sites associated with LTER (SB, BA, DK, LM) and the present study (high-density [HD] and low-density [LD]). Generalized pattern in seagrass primary productivity (PPR), phosphorus concentration ([P]), and TA are drawn above for reference. All LTER sites failed to meet the assumptions for a test of slope significance (gvlma package in R), so we simply report the R^2.

3.3 $\delta^{13}C_{DIC}$ and benthic flux of TA and DIC

While both sites were net dissolving (-NEC) over the study period (Fig. 5), the calculated calcite saturation state ($\Omega_{calcite}$, CO2Sys) was relatively high, at 5.83 ± 0.84 and 6.23 ± 1.15 at the high- and low-density sites, respectively (Table S1), indicating that dissolution of carbonates in the sediments was contributing to water column DIC. The uncertainty of this $\Omega_{calcite}$ calculation was ± 0.30, or approximately 5% of the average value. The 'Keeling plot' indicated source $\delta^{13}C_{DIC}$ values were -6.9 ± 3.7 and -8.8 ± 6.8 ‰ (95% confidence interval) for the high- and low-density sites respectively (Fig. 8).
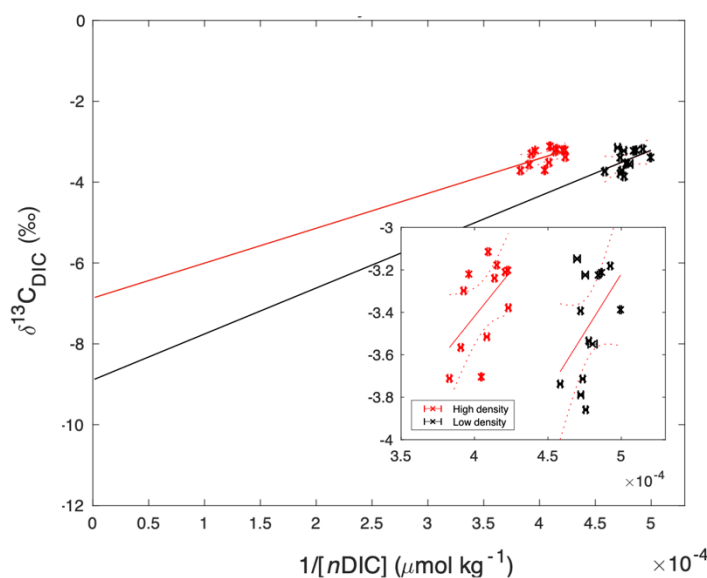
Figure 8. 'Keeling plot' of $1/[n\text{DIC}]$ versus $\delta^{13}C_{DIC}$, suggesting potential end-member isotopic values. These y-intercept $\delta^{13}C_{DIC}$ values were -6.9 ± 3.7 and -8.8 ± 6.8 ‰ (95% confidence interval) for the high- and low-density sites respectively. The inset figure is zoomed to the extent of collected data, while the large figure is scaled to demonstrate the extrapolation required in order to extend the data to the y-intercept.

Benthic chamber flux experiments (over bare sediment) during the second sampling campaign yielded average benthic DIC fluxes of 0.76 ± 0.7 and 1.26 ± 0.8 mmol m^{-2} h^{-1} at the low- and high-density sites, respectively. These benthic DIC fluxes could explain 109% ($0.76/-0.7 = 1.09$) of the average NEP_{DIC} at the low-density site, and 79% ($1.26/-1.6 = 0.79$) at the high-density site. Benthic TA fluxes were 0.24 ± 0.16 mmol m^{-2} h^{-1} at the low-density site but were highly variable and not significantly different from zero at the high-density site (0.16 ± 0.4 mmol m^{-2} h^{-1}). Benthic TA flux could explain 120% ($0.24/-0.2 = 1.2$) of cumulative NEC at the low-density site, but only 18% ($0.16/-0.9 = 0.18$) at the high-density site. Given the expectation that SO_4^2 reduction and denitrification are generally higher at greater seagrass density and productivity, the relatively high benthic TA fluxes at the low-density site indicate that our measurements of NEC were largely driven by $CaCO_3$ production and dissolution rather than TA production due to sediment redox processes.

4. Discussion

4.1 Drivers of NEP

Individual NEP_{DIC} measurements were variable and within the range of prior studies in Florida Bay (Long et al. 2015a; Turk et al. 2015; Perez et al. 2018), but over the entire study period, cumulative NEP_{DIC} was negative at both sites (Fig. 5b), indicating that heterotrophic conditions dominated in both seagrass meadows during these two sampling campaigns. CO_2

fluxes were positive at both sites, indicating a net release of CO_2 from the water to the atmosphere (Table S1). Seagrass aboveground primary productivity rates were between 1.5-2 μmol C m^{-2} h^{-1} at both sites (Table S1), approximately 3 orders of magnitude lower, and opposite in sign, than the measured NEP_{DIC}. This large difference provides further evidence that seagrass aboveground primary productivity is only a component of net ecosystem productivity, which was likely dominated

5   by sediment processes during our experiments. We found a clear disagreement between daytime NEP_{DO} and NEP_{DIC}, such that the linear relationship between NEP_{DO} and NEP_{DIC} was not significantly different from 0 for the high-density site (p=0.095; R^2=0.11) and was significant but very weak (p=0.001; R^2=0.35) for the low-density site (Fig 6d). Such a disagreement between NEP_{DO} and NEP_{DIC} has been observed recently in coral ecosystems (Perez et al., 2018). This discrepancy between NEP_{DO} and NEP_{DIC} may be related to the thermodynamics of CO_2 and O_2 dissolution, as the solubility of O_2 is much less than that of CO_2

10 (Weiss 1970; 1974). Any O_2 produced or consumed by NEP will rapidly exchange with the atmosphere, while most of the CO_2 generated by NEP will enter the carbonate buffering system and persist as HCO_3^- or CO_3^{2-} ions, rather than exchangeable CO_2. The standard deviation of O_2 fluxes was much larger than that of CO_2 fluxes, in part due to this effect. Furthermore, as the total pool of O_2 in the water column is far less than the total pool of CO_2 (i.e. DIC), the determination of NEP_{DO} is more sensitive to the parameterization of gas transfer than is NEP_{DIC}. This is highlighted in Fig S1, where the difference between

15 the two k_{600} parameterizations is much larger for NEP_{DO} than for NEP_{DIC}.

Further explanations for this discrepancy between NEP_{DO} and NEP_{DIC} can be related to differing responses of DO and DIC to variations in light availability. When PAR was plotted against NEP_{DO}, a clear pattern of hysteresis arose, with higher NEP_{DO} values during the morning hours than the afternoon at the same PAR intensity (shown by the arrows in Fig. 6a). Such a hysteretic pattern indicates that the response of NEP_{DO} to light is not uniform, and that photosynthetic efficiency may

20 vary with factors such as nutrient availability, history of carbon acquisition (carbon concentrating mechanisms) or temperature. Such a hysteretic pattern has been observed between PAR and NEC, but not for NEP, for a coral reef (Cyronak et al., 2013). This has important implications for the modeling of carbon processing in seagrass meadows, which generally assume a time-invariant relationship between light and photosynthesis (Zimmerman et al., 2015; Koweek et al., 2018).

4.2 Drivers of NEC

25 We found no relationship between PAR and NEC at our study sites, indicating that light-driven calcification by photoautotrophs (algal epiphytes, calcifying macroalgae and seagrasses themselves) does not dominate NEC, or that carbonate dissolution driven by respiration in the sediments dominated NEC. However, it is possible that the use of carbon concentrating mechanisms could cause calcification by photoautotrophs to become decoupled from direct irradiance. While not listed in Table S1, we did observe a variety of bivalves and tube-building polychaetes that may have contributed to the high NEC at

30 both sites. Furthermore, while $\Omega_{calcite}$ was always greater than 1, NEC was negative on average over the study period, indicating that the overall ecosystem was net dissolving. This co-occurrence of high $\Omega_{calcite}$ with overall net dissolving conditions (-NEC) can be reconciled by considering the seagrass ecosystem as a vertically de-coupled system, where positive NEC in the water

column is more than balanced by carbonate dissolution in the sediments. Such a relationship has been observed or inferred in seagrasses elsewhere (Millero 2001; Burdige and Zimmerman 2002; Burdige et al., 2010).

Our 'Keeling plot' approach indicated potential end-member $\delta^{13}C_{DIC}$ values that lie between the $\delta^{13}C$ of seagrass organic matter (~ -8 to -10 [Fourqurean et al., 2015; Röhr et al., 2018]) and sediment inorganic carbon (~0 ‰ [Deines 1980]),
5   indicating that both sediment organic matter respiration and carbonate dissolution were sources of DIC. It should be noted that this approach involves the extension of measurements to a theoretical $\delta^{13}C_{DIC}$ value at infinite DIC concentration, involving a substantial extrapolation (Fig. 8). Furthermore, this isotopic analysis implicitly assumes a closed system, which clearly is not the case in Florida Bay.

From these lines of evidence, we infer that OC remineralization in sediments, combined with carbonate dissolution
10   contributed to the net upward DIC and TA fluxes from the sediments, which appear to have driven the observed negative NEP (heterotrophy) and NEC (dissolution), respectively. Such net heterotrophy must be fuelled by C_{org} captured by the system, either from allochthonous sources or from autochthonous sources occurring at some time in the past. This study was conducted at two relatively deep-water sites during autumn with relatively low light levels and short days, so it is quite possible that there could be a different net annual signal when the bright summer months are included, highlighting the need for annually-resolved
15   measurements. However, the results of our benthic flux experiments support the isotopic evidence for the role of sediment OM remineralization in NEP and NEC at these sites. Benthic DIC fluxes were 79 and 109% of average NEPDIC at the high- and low-density sites, respectively. Likewise, benthic TA flux was 18-120% of cumulative NEC. Together, these benthic flux measurements, along with isotopic evidence, supports the role of sediment biogeochemical cycling in the overall carbon budget at these sites. Prior studies have shown high rates of denitrification (Eyre and Ferguson 2002) and SO_4^2 reduction (Holmer et
20   al., 2001; Smith et al., 2004) in seagrass soils, so it seems quite possible that these processes contributed to much of the inferred net ecosystem heterotrophy here. There is a clear need for more research exploring the linkages between sediment early diagenesis and water-column biogeochemistry over seagrasses. This is especially important, given the recent attention that seagrass systems have received lately, as potential 'buffering' mechanisms for coastal ocean acidification (Manzanello et al., 2012; Unsworth et al., 2012; Hendriks et al., 2014; Cyronak et al., 2018; Koweek et al., 2018; Pacella et al., 2018).

25   However, there is a geologic context for this observed negative NEC in the northeast region of Florida Bay. Florida Bay is geologically young, having formed during the retreat of the Holocene shoreline following the end of the last major glaciation approximately 4-5,000 years before present (Bosence et al., 1985). The sedimentary deposits that filled in this basin are dominated by calcareous mud formed by extensive Thalassia beds, and their associated epibionts and macroalgae (Bosence et al., 1985), and these autochthonous sources are sufficient to explain the observed sediment distributions (Stockman et al.,
30   1967). Early work suggests that calcareous sediments in Florida Bay can be separated into distinct zones of calcareous sediment formation, migration, and destruction, the last of which extends across NE Florida Bay, where this study took place (Wanless and Tagett et al., 1989). A limited sediment supply of ~0.01 mm yr^{-1} in this 'destructional' zone, compared to the rate of sea level rise, results in the presence of a thin veneer of sediment on the bottoms of the basins and narrow, erosional mud banks (Stockman et al., 1967). Our primary sites were in this "destructional zone", and our finding of negative NEC indicates that at

these sites (during the fall season), the "destructional" nature of this part of the bay may be partly explained by net carbonate dissolution. It is important to note the limited spatial and temporal scope of this study, and we caution that our findings of net negative NEP and NEC are likely not applicable to Florida Bay as a whole, or even to these sites across seasons. Indeed, prior studies have shown substantial seasonal and spatial variability in carbonate chemistry (Millero et al., 2001; Zhang and Fischer
5   2014) and seagrass primary productivity (Fourqurean et al., 2005).

Lastly, it is clear that sediments below seagrasses in Florida Bay have been accumulating autochthonous organic carbon (C_{org}) and carbonate sediments for over 3,000 years (Fourqurean et al. 2012b), suggesting that the ecosystem is producing more organic matter than it is consuming, and is storing more carbonates than it is dissolving. To reconcile our finding of net negative NEP and NEC with the knowledge that this system is a net producer of C_{org} and $CaCO_3$, we must infer that NEP and NEC are
10   not homogeneous throughout Florida Bay.

4.3 Regional Implications and Future Outlook

By combining our estimates of NEP, NEC and CO_2 flux, we can assemble a simple site-scale budget for DIC and TA (Fig. 9). At both sites, approximately 20% of the DIC generated by negative NEP was lost to the atmosphere as CO_2. We can infer that the remaining ~80% (or less, assuming some of the CO_2 produced by -NEP contributed to -NEC) of this metabolic
15   DIC was likely exported to adjacent areas, which in this case are more seagrass meadows. The budgets for these two sites differ for TA, where the lateral export of TA can be estimated simply as: $TA_{export}=2(NEC)+(NEP[17/106])$, assuming TA increases with NEP at a ratio of 17/106 (Middelburg 2019). As NEC and NEP both consume DIC in a 1:1 ratio, the export of DIC can be estimated simply as $DIC_{export} = -CO_2$ Flux + NEP + NEC. The inferred TA_{export} was much higher at the high-density site (1.99 mmol m^{-2} hr^{-1}), compared to the low-density site (0.56 mmol m^{-2} h^{-1}). Hence, the TA:DIC ratio of the export term
20   was greater for the high-density site at 0.90, compared with 0.60 for the low-density site. This TA:DIC ratio is implicitly related to the buffering capacity of the marine carbonate system and governs the sensitivity of pH to changes in DIC (Egleston et al., 2010). Export of low TA:DIC water will cause the pH of receiving systems to be more sensitive to NEP, while export of higher TA:DIC would effectively buffer variations in pH. Prior studies have suggested that seagrass meadows may have a limited capacity to buffer sensitive downstream systems like coral reefs (Manzanello et al 2012; Hendriks et al., 2014; Cyronak
25   et al., 2018; Pacella et al., 2018), although a recent modeling analysis indicated that this buffering capacity may depend on site biogeochemical and physical properties (Koweek et al., 2018). Our results show that the net impact of our studied seagrass ecosystems on pH buffering (related to TA:DIC export ratio) can vary in magnitude between sites that are otherwise quite similar. More work is needed to assess whether TA:DIC varies seasonally with changes in NEP and NEC.

Variations in TA and DIC exports also affects the carbonate system buffering of adjacent systems, further
30   complicating the relationship between NEP_{DO} and NEP_{DIC}. In Fig. 7, we show that correlations between $\Delta[H^+]$ and ΔDO at the LTER sites were generally poor and suggested that this may be partially due to variations in TA supply from adjacent seagrass systems. This seems quite likely, given the phosphorus-driven spatial gradient in seagrass primary production in Florida Bay

(Zieman et al., 1989; Fourqurean et al., 1992), and the realization that ecosystem production is linked with increased calcification (Frankovich and Zieman 1994; Enríquez and Schubert 2014; Perez et al., 2018). In addition, the mangroves that lie upstream of Florida Bay export water high in DIC and TA, and low in DO to Florida Bay (Ho et al., 2017), so that areas immediately affected by this runoff (like LTER site LM) will have a larger range in $\Delta[H^+]$ and ΔDO. Likewise, we can infer

5   that the relationship between NEP_{DO} and NEP_{DIC} is also altered by spatio-temporal variations in TA, although data are lacking in the present study to conclusively demonstrate this effect. Prior studies have shown that TA varies seasonally (Millero et al., 2001) and over diel cycles (present stud; Yates et al., 2007) in response to fluctuations in calcification (Yates and Halley 2006) and salinity (net water balance), offering some explanation for the poor across-site relationship between ΔDO and $\Delta[H^+]$. TA generated by calcite dissolution or anaerobic biogeochemical processes like denitrification and SO_4^{2-} reduction likely play an

10   important, yet currently unknown role. Anaerobic generation of TA through denitrification or SO_4^{2-} reduction in seagrass soils is an additional source not quantified here but should be addressed in the future. However, we can conclude that the observed lack of relationship between ΔDO and $\Delta[H^+]$ holds across the seagrass productivity gradient in Florida Bay, indicating that this discrepancy between NEP_{DO} and NEP_{DIC} may extend across broad regions of the subtropics. This may challenge the application of new in-situ approaches that rely on variations in pH and DO alone to infer rates of biogeochemical processes
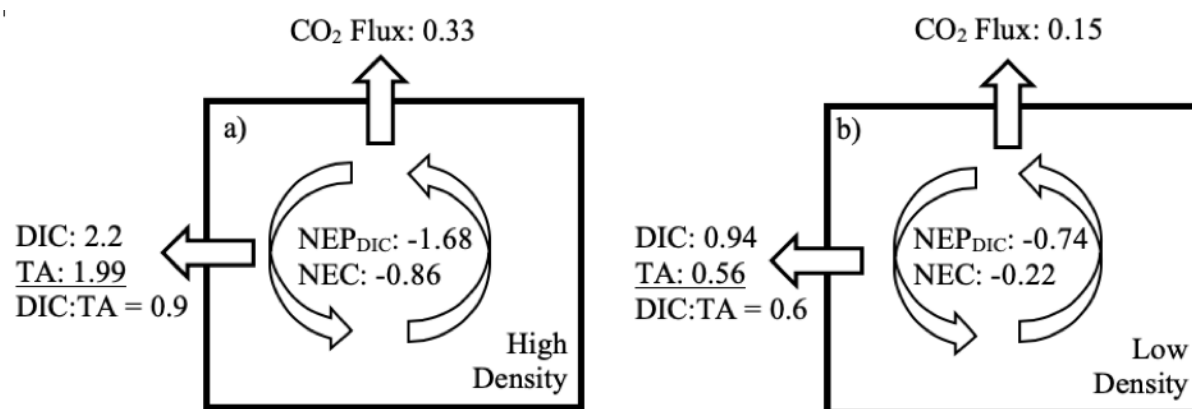
15   (e.g. Long et al., 2015b).

Figure 9. Simple carbon budget for both (a) high- and (b) low-density sites, including inferred lateral DIC and TA export (mmol m^{-2} h^{-1}). Values of NEP and CO_2 flux were averaged for RC01 and Ho06 k_{600} parameterizations, from which DIC lateral export estimates were derived.

20   Our results also suggest that the role of seagrass carbon cycling in larger, regional or global carbon cycles, may be much more complex than originally thought. Modern estimates of carbon uptake by seagrass ecosystems are based largely on measurements of C_{org} burial rates or changes in standing stock of C_{org} (Duarte et al., 2005; Fourqurean et al., 2012a; 2012b). While these C_{org} burial rates are valuable metrics, they do not consider the impact of seagrass NEP and NEC on air-water CO_2 exchange and lateral $CO_{2(water)}$ and TA export, which are also important components of the seagrass ecosystem carbon budget.

Indeed, it has been suggested that the dissolution of allochthonous carbonates in seagrass soils is an unrecognized sink of atmospheric CO_2 that exports TA to the coastal Ocean on scales significant to global CO_2 budgets (Saderne et al 2019). If we are to more accurately constrain the role of seagrass ecosystems in the global carbon cycle, we must begin to consider the net ecosystem carbon balance (NECB), which is the residual carbon produced or consumed after all sources and sinks have been

5   accounted for (Chapin et al., 2006). In aquatic systems, this will involve a precise measurement of the net ecosystem exchange (NEE) of CO_2 between the air and water. In the present study, we used a bulk-transfer equation (Eq 4 and 5) to estimate NEE, but new technologies such as eddy covariance and improved flux chambers mean that direct measurements of seagrass NEE are on the horizon. The combination of direct NEE measurements with rigorous assessments of NEP and NEC is one promising avenue through which NECB may be approached.

10   **5. Conclusion**

In this study, we present the first direct NEP_{DIC} measurements in a representative seagrass meadow by combining rigorous carbonate system analysis with a diel sampling approach. With this new framework, we found negative NEP_{DIC} and NEC at both sites, indicating that despite typical values of seagrass biomass and productivity (Table S1), both sites were net heterotrophic and net dissolving over the study period. Multiple lines of evidence point to sediment respiration and carbonate

15   dissolution (Fig. 8) as drivers of negative NEP and NEC. While our isotopic and benthic flux measurements were coarse, they support the role of aerobic and anaerobic remineralization (denitrification and SO_4^2 reduction [Holmer et al., 2001; Eyre and Ferguson 2002; Smith et al., 2004]) coupled with carbonate dissolution (Jensen et al 1998, Burdige and Zimmerman 2002, Jensen et al 2009) as under-recognized components of total ecosystem NEP and NEC. Further studies should refine our estimates of benthic DIC and TA fluxes from seagrass sediments (with benthic chambers [present study], underwater eddy

20   covariance [Long et al., 2015b; Yamamoto et al., 2015], or pore-water modeling), and compare these values to other component fluxes of NEP (seagrass primary production, CO_2 flux, etc).

A key finding of this study was the divergence between NEP_{DO} and NEP_{DIC}, which we attribute to the following factors 1) carbonate system buffering, which retains NEP-generated CO_2 in the water as DIC, 2) more rapid gas transfer, combined with a larger exchangeable pool for O_2 than for CO_2, and 3) a clear time-variant response of NEP_{DO} to irradiance

25   (Fig 6a). While DO-based approaches offer many advantages in cost and temporal coverage, we suggest that future studies should first constrain the underlying carbonate chemistry, and asses the relationship between NEP_{DIC} and NEP_{DO}.

Lastly, we used a simple budget to show that these seagrass meadows had contrasting impacts on the pH buffering of downstream ecosystems. Export ratios of TA:DIC varied across both of our sites, indicating that systems downstream of the low-density site may experience less pH buffering of pH, relative to the high-density site, where TA:DIC was greater.

30   Unfortunately, given the very limited sampling period of just 8 days, it is impossible to extend the results of this study to longer time scales. More research is needed to assess the role of seasonal to annual scale variability in NEP and NEC on coastal ocean acidification trends. The use of new techniques, such as eddy covariance and improved autonomous instruments for pH, pCO_2, and TA, should allow future studies to build on this work and fill in our understanding of carbonate chemistry dynamics over

longer, annual time scales. In particular, these new approaches should be targeted at constraining NEE (air-water CO_2 exchange), in conjunction with direct and rigorous measurements of NEP and NEC. The combination of these approaches will allow for the first direct assessments of seagrass NECB, a critical next step in the valuation of seagrasses in the context of the global carbon cycle.

5 **Data Availability**

All datasets generated during this project are published on the data sharing repository Figshare (https://doi.org/10.6084/m9.figshare.7707029.v1). Further requests for data or methods sharing can be directed towards the corresponding author.

Supplement

10 The supporting information related to this study will be published online.

Author Contributions

BRV designed the research methodology and formal analysis for this study, while field and lab work was carried out by BRV and CL. Isotopic analysis of DIC was conducted by CO. The original draft of this manuscript was prepared by BRV, while further review and editing was conducted by JF, CO, and CL. Funding for this study was acquired by JF.

15 **Competing Interests**

The authors declare no conflicts if interest

Acknowledgements

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