Anonymous Referee #1

Summary:

Van Dam et al. present seagrass metabolic rate estimates from two sites within Florida Bay. They found net heterotrophy and evidence for carbonate dissolution with the seagrass meadows and discuss the various drivers and implications of their metabolic rate findings for seagrass buffering of seawater chemistry. There is need for more information about seagrass metabolism and its relationships with water chemistry, so the study is well-motivated. The authors have clearly done a lot of work and I commend them for their effort. However, I have significant concerns about the metabolic rate calculations that constitute the main results of the paper.

I would not be comfortable seeing this paper published until the concerns are sufficiently addressed because I believe that addressing the concerns may change the main results of the paper.

In the first part of the review, I discuss my primary criticism of the study. I provide some detailed comments that pertain to the various sections, figures, and tables in the second part of the review. There is a short list of typos at the end of the review.

Primary constructive criticism:

The metabolic rate estimates are based on the "slack water" approach which considers an isolated pool of water such that changes in water chemistry cannot be attributed to advection or dispersion. Yet the authors do not sufficiently justify their adoption of the slack water simplification. These areas are not tidally isolated (e.g. tide pools), and although they feature low currents (< 2 cm/s; section 2.4), we have no sense of the spatial variation in O2 and DIC that would help us assess how any advective fluxes would compare to fluxes from gas exchange and/or metabolism. In particular, as highlighted by Lowe and Falter (2015), it is difficult to have both a) weak enough currents to minimize advective fluxes and b) strong enough turbulence to sufficiently mix the water column (see reference below).

I want to try to convince you that ignoring small spatial gradients and weak currents could cause you to misinterpret your metabolic rate data by ignoring advective fluxes. As an example, let's consider a simple advection-reaction model of the TA mass balance at one of the sites (equivalent to Eq. 1 in the paper with a term included for advection):

dTA/dt = u * delta_TA/delta_x - (2 * NEC/ rho * h)
At steady state (dTA/dt = 0), we could simplify this to: (2 * NEC) / (rho * h * u) * delta_x = delta_TA

Assuming NEC = 5 mmol/m^2/hr (within the range of values presented in Fig. 4), rho = 1025 kg/m^3, u = 1 cm/s, h = 2m, and delta_x = 100m, we can solve for delta_TA and get: delta_TA = 13 umol/kg

In other words, just a 13 umol/kg gradient between upstream and downstream TA in a 100m long meadow with a velocity of 1 cm/s (below your instrumental detection limit) would generate an advective flux equivalent to your reported rates of NEC. This TA range is far below your reported ranges in daily TA variability (which may be confounding temporal and spatial variability from advection). I suspect that your metabolic rates are really some combination of metabolism and advection. In some cases ignoring advection may be causing you to underestimate metabolism and in other cases may be causing you to overestimate metabolism.

Without accounting for the role of advection in the TA, DIC, and O2 mass balances within the seagrass meadows, I am not confident in your conclusions about net heterotrophy and net dissolution.

Given that the authors have O2 and pH measurements from some of the other FCE- LTER sites, they should explore how their metabolic rate estimates might change if they considered spatial variation in the biogeochemical parameters and associated advective fluxes (even if currents were < 2cm/s). They could at least put some error bounds on their metabolic rate estimates this way. Such an exercise would be especially doable if you have information on current direction from your tilt meters, even if you don't have current magnitude.

Finally, the authors implicitly acknowledge the role of advection when they discuss TA:DIC export (Fig. 9). The concept of export implies entry and exit flow through a system (in this case, each seagrass meadow), otherwise there would be no export. So how does one rationalize slack water metabolic rates and export at the same time?

Lowe, Ryan J., and James L. Falter. "Oceanic forcing of coral reefs." Annual review of marine science 7 (2015): 43-66.

We appreciate Reviewer 1's thorough comments and constructive criticism of our manuscript. Their primary critique is well-founded and articulated, and we are thankful for the detailed argument that they have laid out in their review. Indeed, flow in seagrass systems is complex, producing vertical heterogeneities in water column physical and chemical properties. For example, flow is significantly reduced in the canopy, increasing the residence time of water in the canopy relative to the overlying water (Peterson et al, 2004). Shear between these two compartments (in and out of the canopy) drives vertical exchange across the canopy interface that partially or wholly homogenizes water chemistry. At a smaller scale, this turbulent mixing also helps to alleviate carbon limitation that may build up in the seagrass blade boundary layer (Koch 1994). Our NEP/NEC estimates were derived from concentrations measured near the surface. These measurements represent the cumulative effect of lateral DIC/TA fluxes (which we assume to be minor) and turbulent/diffusive exchange between the seagrass canopy and overlying water (which we assume are dominant). This is a partial motivation for why we chose surface-water rather than within-canopy measurements, because it integrates the seagrass metabolic signal from a larger footprint. Still, there is a potential for our slack water approach to be biased by lateral water exchanges, which we will try to address to the best of our ability here.

Unfortunately, we don't have any empirical data specifically addressing the spatial variability of carbonate chemistry at these sites, but we can build one line of evidence from the data that we do have. Our two sites are separated by a linear distance of approximately 4 km. Looking at figure 2, we can approximate the difference in nTA and nDIC between the sites to be at most 300 μmol/kg. Hence, we have an approximate spatial gradient of at most 75 μmol/kg/km (300 μmol/kg / 4km). This corresponds to at most 7.5 μmol/kg over a 100m stretch, which is about half of the 13 μmol/kg estimate that reviewer 1 derives in their comments. Furthermore, our seagrass meadows are much larger than 100m, in fact are typically a factor of ~5x greater (>0.5 km^2). Hence, the comparable TA gradient required to explain our metabolic fluxes would be appreciably greater, on the order of ~65 μmol/kg.

As further evidence, we are including the following figure which shows current speed and direction from the tilt current meters (TCMs). From this, it appears that flow was not unidirectional at these sites over the study period, but was instead variable in direction without a clear mode which might suggest tidal or wind-seiche. While we are reluctant to use these data in our manuscript because the water velocities were below the detection limit of the TCM, we hope they offer some support to our argument in this discussion forum. One prior study at a site just west of ours also reported generally low water current, especially within the seagrass canopy, despite a slightly greater tidal influence there (Hansen et al., 2017). Hence, we feel confident that water current at our site was indeed low. We also see no clear link between current direction and changes in TA/DIC, which should be apparent if there was a distinct TA/DIC source whose signature was being advected over our site. For example, there was a subtle decrease in salinity of ~0.5 on the morning of 11/27 at the high-density site (Fig. 2), but the indicated current speed and direction were apparently consistent during this time period (attached figure). Lastly, it is possible that small inputs of fresh water, either through surface or groundwater channels may have significant and nonlinear impacts on carbonate chemistry. However, the attached scatter plot of salinity vs depth indicates that the small changes in water level we observed did not coincide with any clear changes in salinity (i.e. freshwater input).

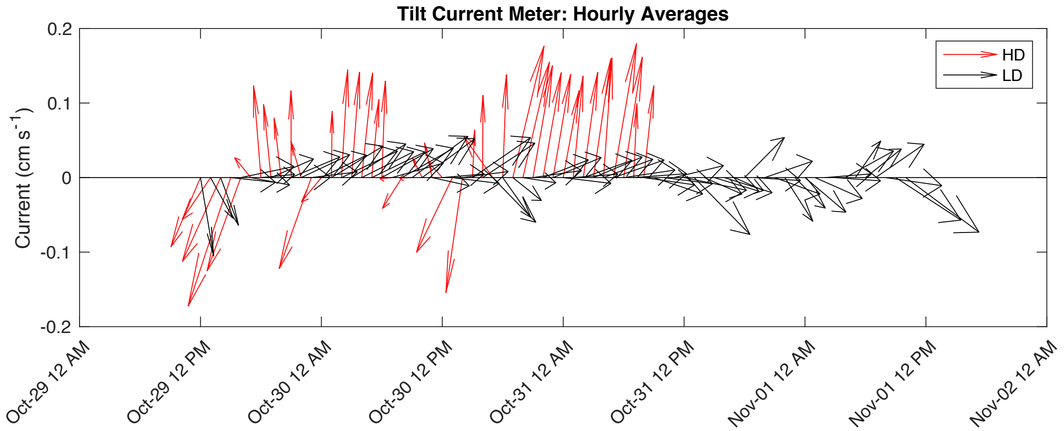
So, we strongly agree that the combination of spatial variability in carbonate chemistry and advection can cause TA/DIC variability that may impact the ability to estimate NEM/NEC. This would be especially problematic if we had collected water samples *within* the seagrass canopy where water chemistry is much more variable in space/time. However, if we consider all of these lines of evidence, along with the fact that our measurements were made above the canopy, we argue that lateral mixing over the study period was likely relatively low, and likely not sufficient to drive the diel variations we observed, which were generally 50-100 μmol/kg.

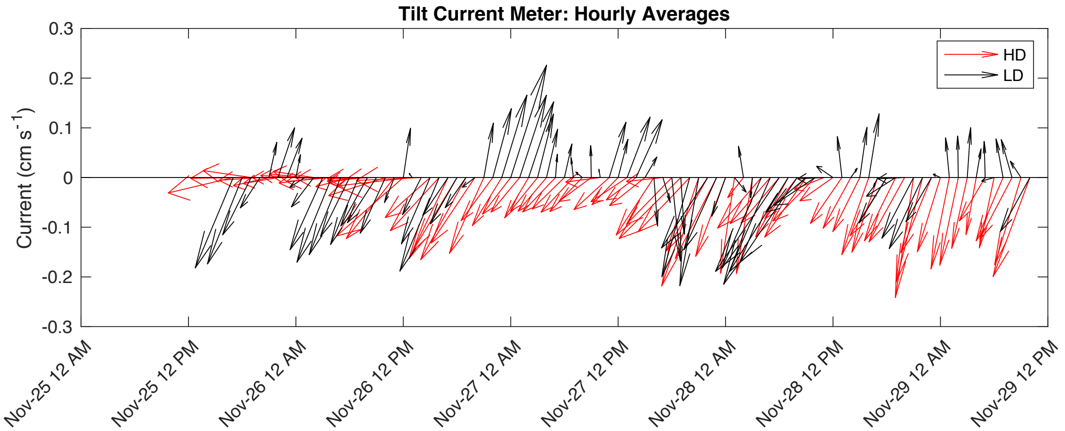
In light of Reviewer 1's concerns regarding the assumptions involved in the TA/DIC budget used for Figure 9, we have elected to remove section 4.3 (TA/DIC export) and figure 9 from the manuscript. Furthermore, we have made a concerted effort to more clearly state the assumptions and limitations of our 'slack water' approach throughout the manuscript.

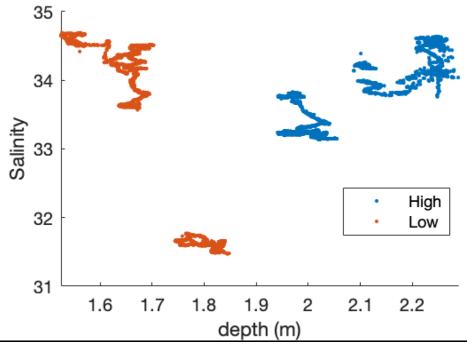
Peterson, C & Luettich, Jr, R & Micheli, F & Skilleter, G. (2004). Attenuation of water flow inside seagrass canopies of differing structure. Marine Ecology Progress Series. 268.

Hansen, J. C. R., & Reidenbach, M. A. (2017). Turbulent mixing and fluid transport within Florida Bay seagrass meadows. Advances in Water Resources, 108, 205–215. doi:10.1016/j.advwatres.2017.08.001

Koch, E.W. Marine Biology (1994) 118: 767. https://doi.org/10.1007/BF00347527
10.3354/meps268081.







Detailed comments:

Methods:

2.1: Move Table S1 to main text.

Table S1 moved to the main text as table 1

Define “primary sites” here since you reference this phrase. Don’t wait until 2.2 to define them.

This term is now introduced in the first sentence of 2.1

2.4: Why such low accuracy on the pH sensors? SeaFETs are capable of accuracy approaching 0.01 pH units or better.

This is the accuracy listed on the manufacturer’s website (https://www.seabird.com/seafet-v2-ocean-ph-sensor/product-details?id=54627921732). The precision is indeed much better than 0.05.

2.6: Why the poor precision on the DIC measurements? Please explain.

While TA was analyzed on a commercial instrument, we did not have such a machine for DIC determination. Instead, our DIC measurements were made on a home-made analyzer which consisted of a small impinger filled with 10% HCl, an N2 carrier gas, and a bench-top IRGA

(Licor 6262). There was uncertainty in sample injection, which was done manually, and peak area integration, which was done by the IRGA. While our precision was lower for DIC than for TA, it was still reasonably close to what is achieved by commercial units, which typically achieve ~2 μmol/kg accuracy (e.g. Apollo SciTech ASC3 [http://www.apolloscitech.com/dic.html]). While other instruments like the VINDTA 3C (http://www.marianda.com/index.php?site=products&subsite=vindta3c) claim ~1 μmol/kg precision, reported standard deviations of CRMs are generally higher for both TA and DIC, closer to 2-4 μmol/kg (McMahon et al., 2018; Lemay et al., 2018; Turk et al, 2016, etc…).

McMahon, A., I. R. Santos, K. G. Schulz, T. Cyronak, and D. T. Maher. 2018. Determining coral reef calcification and primary production using automated alkalinity, pH and p CO 2 measurements at high temporal resolution. Estuar. Coast. Shelf Sci. **209**: 80–88. doi:10.1016/j.ecss.2018.04.041

Lemay, J., H. Thomas, S. E. Craig, W. J. Burt, K. Fennel, and B. J. W. Greenan. 2018. Hurricane Arthur and its effect on the short-term variability of p CO 2 on the Scotian Shelf , NW Atlantic. Biogeosciences 2111–2123.

Turk, D., J. M. Bedard, W. J. Burt, and others. 2016. Estuarine , Coastal and Shelf Science Inorganic carbon in a high latitude estuary-fjord system in Canada ' s eastern Arctic. Estuar. Coast. Shelf Sci. 178: 137–147. doi:10.1016/j.ecss.2016.06.006

2.7:Your NEC model does not account for changes in TA due to organic production, despite your acknowledgement in the text and Fig. 3 that TA is influenced by organic matter production (see comment below about inconsistencies between delta_TA/delta_DIC ratios for organic production between your text and figure). You need to account for the other processes that influence TA in order to accurately calculate NEC.

Why are you using gas transfer velocity parameterizations designed for open ocean conditions when coastal parameterizations exist? See:

Ho, David T., et al. "Air-water gas exchange and CO2 flux in a mangrove-dominated estuary." Geophysical Research Letters 41.1 (2014): 108-113.

Ho, David T., et al. "Influence of current velocity and wind speed on air-water gas exchange in a mangrove estuary." Geophysical Research Letters 43.8 (2016): 3813-3821.

We chose to apply two separate parameterizations because together they constitute what might be considered a maximum range in *k*, within which we expect that the actual value lies. Because calculated NEP using these two (excessively) different parameterizations were very similar, we felt justified in reporting a single value from Ho 2006. While we are well aware of the Ho 2016 and Ho 2014 parameterizations, we elected not to use them because of the lack of quality water velocity data, and the fact that currents at our site (likely < 2 cm/s) were at least an order of magnitude lower than the velocities in the tidal river in Ho 2016 (20-40 cm/s). Likewise, Ho et al., 2014 reports average tidal velocities of ~35 cm/s, well outside the range at our site.

Results:

3.1

p. 7, L 17-18: The statement about lateral variations being insignificant because observed changes in SSS of < 1 is only correct if you knew that large spatial gradients in SSS existed and that they were correlated with TA, DIC, etc.

This is a very good point. We have removed 'lateral mixing' from the sentence, and have clarified that we were referring to sources of fresh water, not TA or DIC.

p. 7, L 22-23: Present O2 concentrations, not just percent of saturation (which is temperature and salinity dependent)

DO is now presented as a concentration rather than a percent saturation (Fig 2), and the text references have been corrected as well. The diel trends in DO remain apparent in the figure.

p. 7, L 28-29: t-tests assume independence between data sets, but your CO2 fluxes are likely to be linearly related (since the only difference is the estimated value of the gas piston velocity). I don't think t-tests are relevant since differences in gas flux should simply reflect differences in piston velocity.

We have removed the discussion of CO2 flux t-tests from section 3.

p. 9, L 6-9: When you plot nTA against nDIC, the slope is not nTA:nDIC, but delta_nTA/ delta_nDIC. Please be careful how you describe this in the text.

We have added a brief clarification on this point.

p. 9, L 9-10: When you only have two variables (nTA and nDIC), you can only resolve two processes (production and calcification). Right now, you are trying to resolve four processes (production, calcification, sulfate reduction, and denitrification) with only two variables. Your system is underdetermined.

We very much agree, and have added a sentence at the end of the paragraph reiterating this point.

3.2p. 9, L 21: I do not believe this section is well served by the inclusion of metabolic rate comparisons between this study and previous seagrass metabolism studies. Move the comparisons to the paper Discussion.

Yes, this discussion of metabolic rates in the context of previous studies is not suited for the results section. It has been moved to the discussion section 4.1.

p. 10, L 15-16: This is not the presentation of a statistical test result

These sentences were removed as per Reviewer 1's earlier comments.

Figures

Fig 2: I find this figure very difficult to follow. Multiple data series and and multiple variables along each subplot make it difficult to track what's going on where. Some axes are labeled and some are not. Please consider making additional plots, each with one variable, and labeling all axes. If there are too many resulting plots, you can put some in the supplement.

Fig. 2g,h: Point plots are difficult to track for understanding daily cycles. Recommend connecting points with a line.

We appreciate the advice, and have revised figure 2 to include axis titles for all sub-figures and have connected the points in figures g and h with lines.

Fig. 3: Where do you get the information that TA will decrease as DIC decreases? You reference the classical assumption of slight increases in TA with DIC uptake (p. 7, L 14 and also p. 17, L 16), but you have a positive line in Fig. 3 for TA/DIC relationships for organic production in Fig.

3 and the caption states "...., which generates 0.15 moles of TA for every mole of DIC respired." These two messages are inconsistent. Please clarify.

Reviewer 1 is correct, we should present a slope of -0.15 for the blue line in figure 3 representing TA uptake with productivity on NO_3. This has been corrected in the new figure.

Fig. 4: Same comment as for Fig. 2 about multiple data sets and multiple variables. It is unnecessarily confusing to try to interpret these graphs and impatient readers won't invest much time and energy into attempting to do so. Also, same comment about connecting points with lines as with Fig. 2g,h. Please also provide a figure legend.

We regret that this figure is difficult to follow, but we have tried a number of ways to plot these data and settled on the current display as the least bad representation. On a previous version of this figure, we tried to connect the points with lines, but it became far too busy and difficult to see. We also tried to use box and violin plots, but there simply aren't enough data points to make these plots work.

Fig. 6: Panels d) should be separated (split into a separate figure) from panels a-c) because they show fundamentally different relationships. Panels a-c) show relationships between metabolic rates and PAR. Panel d) shows relationships between oxygen and carbon fluxes during photosynthesis.

We appreciate Reviewer 1's advice, and have split Figure 6 into two separate figures. The in-text references have been revised accordingly.

Fig. 8: Units on x-axis are incorrect. 1/DIC is in units of kg/umol, not umol/kg

Units have been corrected in Figure 8.

Fig. 9: TA:DIC, not DIC:TA (check all labels)

Figure 9 has been removed from the manuscript.

Tables:

The information in Table S1 is key to understanding the differences between the high density and low density sites. At least an abridged version belongs in the main text.

Table S1 has been moved to the main text.

Typos:

p. 2, L 22: Missing "it" between "While" and "is"

p. 7, L 2: Missing a space between "k600" and "parameterizations"

Manzello et al. (2012) reference (not "Manzanello), also correct in-line citation (p. 16, L 23)

We thank Reviewer 1 for catching these mistakes, which we have now corrected.

Anonymous Referee #2

The study by Van Dam et al., aims at quantifying net primary production and calcification/dissolution rates of CaCO3 in Florida bay seagrass meadow. Although the methods used are correct, the study has a major flaw, and from my point of view, the manuscript in it's present form cannot be accepted. The authors are measuring benthic fluxes of TA, in seagrass and sediment, and consider that they are due to calcification or dissolution only (in seagrass, but not in sediment it seems). They therefore ignore all the other redox reactions producing of consuming TA, such as nitrification, denitrification, pyrite burial, sulfite burial, sulfate reduction etc. although those reactions are extremely important in seagrass beds, and indirectly controlled by the seagrass through sediment oxygenation and Corg addition. I strongly advise the authors to read Krumins et al., 2013 (biogeoscience) as well as Sippo et al., 2016 (global biogeochemical cycle). All the part regarding NEC is ill founded. The semi-quantitative arguments proposed by the authors tend to prove that the TA comes from dissolution (TA/DIC ratio and isotopes) are not convincing and only proves that part of the TA only come from this source. Measurements of fluxes of Ca2+, by titration, are necessary to quantify NEC. All parts regarding NEC should be removed, and only consider TA fluxes. This is a valuable and much needed data, the article should be rewritten to focus on this. NEC calculations could be proposed in discussion but it will need a very carefull and thorough discussions on sediment processes emitting TA.

Moreover, the study cover only two periods of ~5 days in October and November. This temporal coverage is not sufficient to obtain significant results. More campaigns in other seasons are needed.

We appreciate reviewer 2's constructive criticism, and have made a concerted effort to address their concerns regarding the role of anaerobic processes on NEC. Throughout the manuscript, we have added text reminding the reader when specific results may have been affected by anaerobic TA generation. We have also included extra text throughout that emphasizes the limited temporal scope of the study, and expressed the need for future studies using different approaches over longer time scales in order to confirm or refute our findings. We hope that these changes, along with those that have been made following reviewer 1 and 3's suggestions will be satisfactory for this reviewer.

Some specific comments: Introduction:

P2 : Please develop how calcification emits CO2.

This sentence was expanded to clarify how calcification generates CO2.

P2: 4-6: the experiment conducted by enriquez et al., consist in enclosing a piece of seagrass in a very small volume of water exposed to light. This is by no mean a proof that spontaneous CaCO3 can occur in the field. Besides, from my point of view, the observation of calcification within the tissues of seagrass they did remain to be confirmed.

We agree with the reviewer that more studies are required to confirm that CaCO3 formation occurs within seagrass tissues and have added phrasing to reiterate this point here.

P2: 34 - 35 : I do not understand that sentence

This sentence was revised to clarify that seagrasses can affect local pH trends by consuming DIC that was generated in adjacent mangroves.

P4: 20. I don't find Karlsson et al., 2017 in the references.

We apologize for the omission; this citation is now included in the reference list.

P5: 11. Did you sampled discrete sample for spectrophotometric pH used for the seafet data validation? See Bresnahan et al., 2014 for example

These SeaFET data were not used to calculate DIC/TA for metabolism assessments, and were simply presented to show the large diel cycles in pH. Our original intent was to estimate NEP/NEC at higher temporal resolution using sensor pH and pCO2 data, but because we were not confident in the pCO2 data, we could not do so.

P5: 25. Why using chamber for the bare sediment (and only for the sediment)

The intent here was to isolate the sediment source of TA/DIC by excluding seagrass aboveground biomass, thereby excluding any consumption or production by seagrass aboveground shoots themselves. We have edited this sentence to clarify the point.

P6: 1-10: Did you used "Dickson" CRM
Yes, and this is now explained in greater detail.

P6: 19: Please use the salinity normalization by Friis et al., 2003.

The water budget of Florida Bay is dominated by exchange with the ocean and evaporation and precipitation, which are approximately a factor of 10 greater than surface water inputs which may have a non-zero TA/DIC endmember (Nuttle et al., 2000). Therefore, we believe that the most appropriate approach is to normalize TA and DIC using a zero-salinity endmember, which represents the effect of precipitation and evaporation. Furthermore, the small freshwater input that does enter the northern bay through shark river slough has a highly variable TA concentration, and is located a great distance from our study sites.

P7: 10-14. Please precise the dissociation constants used and evaluate the propagation of error on the CO2 calculated, using the fct error in seacarb. Please therefore take this error in consideration in subsequent calculations.

We are confident that the largest source of error in our CO2 flux determination is derived from our parameterization of gas transfer, which is why we used two different equations to estimate k600. Furthermore, CO2 flux represents only a very small fraction (median=1.3%) of the estimated NEP rates. Therefore, we feel confident in presenting the results using a single H2CO3 dissociation constant.

P7:11. Why not the latest Schmidt number calculations from Wanninkhof 2014? Please see Sippo et al, 2014.

We regret not using the updated Sc values from Wanninkhof 2014 in our analysis. However, re-doing the entire analysis with the 2014 values would require significant time, and would not appreciably change our CO2 flux estimates, which are most sensitive to variations in the gas transfer velocity (k600), rather than variations in Sc which are small. If reviewer 2 deems it necessary that we re-calculate all metabolism estimates with the updated CO2 and O2 Sc values from Wanninkhof 2014, we would of course be willing to do so.

P8: 7. Please express the hours in mean solar time. Fig 4, same.

Time is expressed in local time (EDT or EST) throughout the rest of the manuscript, so we elect to present time in the same format in this figure to avoid confusion.

P15: 9-12. I do not understand this section. The NEP(DIC) you calculate is a production rate of DIC, corrected for air-sea fluxes of CO2 and calcification (presumably), what is a proper way of doing. It is therefore including the DIC species HCO3- and CO32-, how can they escape the calculation?

This section is not intended to say that DIC is somehow ‘escaping’ the NEP calculation, rather that the large pool of DIC makes NEP calculated with DIC less sensitive to variations in gas transfer than NEP calculated with O2.

P15: 26. Seagrass themselves? See earlier comment on Enriquez et al., 2014.

Yes. While there is debate over the extent to which seagrass internal calcification occurs, we have mentioned this previously in the manuscript (as per this reviewer’s suggestion), and at this point, we also mention other calcifiers which likely contribute in some extent to our NEC estimates.

P15: 16. Your endvalues are far from 0 and close to the range for seagrass Corg. This does not reinforce the argument of TA coming from dissolution.

Indeed, the indicated y-intercept of the Keeling plot does suggest an endmember closer to seagrass Corg. However, the 95% confidence interval for the y-intercept is ~3-11 for the high-density site, and, ~2-16 for the low-density site. This factor, along with the extreme extrapolation involved, means that we cannot confidently say that the endmember is either decidedly “carbonate” or “seagrass OM”.

P16: 17. All your measurements are benthic TA fluxes. When it comes from bare sediment, it is a TA flux and when it comes from the seagrass, it is NEC.

We have revised the previous sentence to clarify our intended message that sediment-water TA/DIC fluxes may at times explain a large fraction of measured NEC.

P16: 20. Precisely, and denitrification and sulfate reduction emit TA and is NOT dissolution of CaCO3.

As per reviewer 2's comments, we have added a sentence expanding on the role of anaerobic processes on TA exchanges.

522. yes, exactly.

All the 4.3 section is dispensable.

As per all 3 reviewers suggestions, section 4.3 was significantly reduced in length and the budget was entirely removed. The remainder of section 4.3 received positive comments from the other reviewers, and we think that it brings up important points, so we elect to keep it in this revision.

Anonymous Referee #3

Received and published: 10 June 2019

General comments

Van Dam et al. present short-term carbonate chemistry variability from two seagrass meadows in Florida Bay. Assessments of net ecosystem productivity (NEP) and net ecosystem calcification (NEC) indicated net heterotrophy and CaCO3 dissolution during eight days in the fall season. Furthermore, the authors compare NEP inferred from dissolved inorganic carbon measurements and oxygen measurements, and discuss reasons for and implications of the observed discrepancy. The study is well-designed and very timely as there is a lack of knowledge on how seagrass systems modify sea- water carbonate chemistry on different temporal and spatial scales. However, although the carbonate chemistry methodology is appropriate, the interpretations and conclusions on TA fluxes and NEC would have benefited from additional measurements of e.g., Ca2+ and SO4+. Without constraining other biogeochemical processes that affect DIC and TA, it should be more clearly indicated that some of the conclusions are associated with uncertainty and are speculative. Provided that the issues raised here are properly addressed, I would be happy to recommend this manuscript for publication. Please see my comments below.

We thank Reviewer 3 for their thoughtful, thorough, and constructive remarks. After considering their comments, we have revised the manuscript in an attempt to more clearly state the extent to which our discussion of NEP and NEC is subject to uncertainty, both with respect to additional sources/sinks of TA, and lateral mixing.

Regarding the reviewer's comment about additional measurements of Ca and SO4, in fact we did collect samples for Ca2+, which were analyzed on an ion chromatograph. However, due to the ionic strength of these seawater samples, we had to dilute the samples by a factor of well over 100x. Because of the possible error in dilutions of this magnitude, we felt uncomfortable presenting those Ca measurements here. We strongly agree that Ca and SO4 measurements would have been highly valuable, and regret that we were unable to generate reliable data for the present study.

The Methods section needs improvement. Information is missing on how several variables were measured and what sample sizes were used. Moreover, there is no information on how error propagation was calculated for your flux measurements, which could affect your conclusions. In section 2.1 and 2.2, how do you define your High Density and Low Density sites? Is it based on seagrass shoot density? If so, some quantification of this density would be beneficial for the justification of your site categorization. Above- and belowground biomass and productivity are reported for the two sites in Table S1, but it is unclear if your site categorization is based on any of these variables. Please state this clearly in the Methods section.

The Results section contains speculations and comparisons to previous studies that would be more suitable in the Discussion section. For example, p. 9, line 7-10, line 21; p. 10, line 1-7, line 19-20.

The Discussion section is well-written and easy to follow. However, I am missing some discussion on residence time within your two sites. You state that current flows were low, but no information is provided on tidal regime, prevailing wind direction etc. You briefly state in section 2.4 that current speeds were low (<2 cm s-1), but it is unclear if this means that you treat your sites as closed systems. If not, your budget in Section 4.3 neglects lateral import of DIC and TA from upstream systems as the export flux calculations are based on several assumptions that cannot be resolved with discrete point measurements of only DIC and TA. Aside from this, Section 4.3 brings up very important and relevant considerations for seagrass carbon cycling.

Due to these comments, and those of Reviewer 1, we have elected to remove the budget that was presented in section 4.3. Reviewer 1 also had questions regarding the impact of advection on our metabolism estimates. For a more detailed discussion, please see our response to their comments.

Specific comments

Abstract and Introduction

p. 1, line 10: This is purely semantic but I do not agree that the two seagrass meadows are contrasting. They are the same species, similar physicochemical conditions, similar productivity and water depth (Table S1).

We agree that the main difference between these meadows is indeed limited to biomass and productivity, and have removed the word 'contrasting'.

p. 2, line 28: Seagrass beds and seagrass meadows are used interchangeably. Please use consistent terminology or if you treat these terms differently, please provide an explanation.

'Bed' has been replaced with 'meadow' throughout the manuscript.

Methods

p. 3, line 23-24: Does "aboveground net primary productivity" refer to the data on row three in Table S1? If so, can you really say that they differed with such high and overlapping standard deviations (2.05±0.90 vs. 1.42±1.25)? Were any statistical tests done to test these differences?

In light of the overlapping 95% confidence intervals for productivity (Table 1), we replaced productivity with biomass in this sentence. Primary productivity (as measured by biomass addition) can vary substantially over the short time scales (~1 week) and spatial scales (10s of meters) of studies like this.

p. 4, line 5: Information on how many of the variables presented in Table S1 were measured is missing. For example, how many samples were taken to assess above- and belowground biomass? If only one sample per site was taken, I would be careful to state that they differed in biomass. Similarly, how were sediment carbon and nutrient contents measured. Are the reported C:N:P ratios on mass or molar basis?

Table S1 has been updated to show the number of samples as well as the standard deviation for the analysis used in the main text. Additionally, section 2 now includes the methods as requested. We agree that analyses with only 1 sample are not to be considered for determining site differences, and have included appropriate discussion in the text.

p. 4, line 14-15: This is a bit confusing. Do these dates refer to the measurements of DOC, DIC, and TA for NEPDO, NEPDIC, and NEC or do they refer to air-water gas exchange? If the former, I suggest moving this last sentence up a bit or into the next paragraph where you describe the sampling campaigns.

We apologize for the confusion, and have tried to clarify over what intervals the sampling campaigns lasted.

p. 5, line 5: Is saturation state with respect to aragonite not relevant?

It certainly is relevant, but for simplicity, and because this was not a central point of our manuscript, we chose to present just one carbonate mineral saturation state. Prior studies have shown that the spatial distribution in $\Omega_{calcite\ and}$ $\Omega_{aragonite}$ look very similar, as does their relationship with salinity (Millero et al., 2001)

Frank J . Millero, William T Hiscock , Fen Huang, Mary Roche, J. Z. Z. 2001. Seasonal Variation of the Carbonate System in Florida Bay. Bull. Mar. Sci. **68**: 101–123.

p. 6, line 1-7: Information on the accuracy of your measurements of DIC and TA is missing. Did you verify your measurements against Certified Reference Material? If you did, please state batch number. The precision of ±5.11 µmol kg-1 is quite poor. Could you provide a possible explanation for this? Were the DIC samples sufficiently preserved (e.g., enough HgCl2)? Also, please add number of samples (n=) for your accuracy and precision assessments.

We have added additional information regarding TA/DIC analysis, including CRM batch number and additional corrections that were made based on CRM measurements. We acknowledge that the +/- 5.11 std dev for DIC is relatively high, but it is still within the upper range for commercial instruments, and we feel that it was sufficient for our purpose. Please see our response to a similar remark from Reviewer 1 for further information regarding TA/DIC analytical uncertainty.

p. 7, line 6-8: What is the unit of k600? cm hr-1? p. 7, line 10: End of sentence is missing.

Yes, we have clarified that we estimated k600 in units of cm/hr.

p. 7, line 17-20: This paragraph is a bit confusing as to what refers to the variation within each deployment and what refers to variation between each field campaign. I would not state that a salinity range from 31.45 to 34.67 is stable, but rather a substantial increase.

We revised this passage for clarification.

p. 7, line 23-24: You have already abbreviated your site names as HD and LD. Please be consistent with site terminology or remove the site abbreviation entirely (HD and LD) as there are already many other abbreviations throughout the manuscript.

We understand that these abbreviations were used inconsistently, and have now removed them from the main text of the manuscript. However, we choose to keep the HD and LD abbreviations in a few of the figures due to space considerations, and to avoid excessive text on the figures.

p. 7, line 23: Please provide DO concentrations instead of just percent.

DO is now presented as a concentration rather than a percent saturation.

p. 9, line 9: These referenced studies did not measure sulfate reduction or denitrification. Please add additional references to back up the statement.

That is very true, our intent was simply to say that prior studies have observed similar relationships between *n*TA and *n*DIC. We have revised the text to hopefully clarify this.

p. 10, line 5: Yes, but see Hines and Lyons 1982 and Holmer and Nielsen 1997.

We thank Reviewer 3 for directing us towards these references, which are now included in section 3.2.

p. 14, line 14-15: Although this is probably correct, I do not think that the observation of high benthic TA fluxes at the bare site necessarily means that sediment redox processes are not important for NEC. Furthermore, although sulfate reduction rates have been found to be higher in seagrass sediments, the oxygen release from seagrass roots can also lead to rapid re-oxidation of sulfide (consuming 1 mol TA).

We agree that this sentence was not well supported, and have removed it.

Hines ME, Lyons WB (1982) Biogeochemistry of nearshore Bermuda sediments. I. Sulfate reduction rates and nutrient generation. Mar Ecol-Prog Ser:87-94

Holmer M, Nielsen SL (1997) Sediment sulfur dynamics related to biomass-density patterns in Zostera marina (eelgrass) beds. Mar Ecol-Prog Ser 146:163-171

Discussion and Conclusion

p. 15, line 2: I suggest you include these productivity numbers in the Results section and also present the high variability (stdev of ±0.9 and ±1.25 μmol m-2 hr-1).

These data are now presented in the results section 3.2

p.15, line 5: Do you consider seagrass belowground productivity as part of the "sediment processes"?

Yes, we certainly do agree that seagrass belowground production is relevant, and have now indicated so in this passage within section 4.1.

p. 16, line 16-18: Were these benthic chambers placed at bare spots within each seagrass meadow or at an adjacent bare site? Porewater chemistry vary on small spatial scales and can be quite different between unvegetated sediments and within the rhizosphere (e.g., due to differences in bioturbation, Corg, O2 release from roots etc.) and if your chamber measurements and δ13C measurements are spatially decoupled I would not combine the two as aggregate evidence.

Chamber measurements were made at bare spots within a few meters of our two main sites. We have updated the methods section 2.5 to make this clear. We understand that soils are highly heterogeneous, but feel strongly that these sediment flux measurements can be considered spatially coupled with our water column chemistry measurements.

p. 16, line 19-21: Yes, but these processes (along with other redox processes) could also affect your NEC estimates. Your TA:DIC ratios are the result of a combination of these processes and without measuring any other reactants and products it is difficult to constrain their contribution to your TA flux. Additionally, organic alkalinity may be produced in the sediments which is not accounted for in TA (see e.g., Lukawska-Matuszewska, 2016).

We agree with the reviewer's point, and have added a sentence to this effect.

p. 16, line 21-24: Yes, indeed. Very well formulated.

p. 17, 2-3: I suggest that these reflections are included in the abstract as well.

We agree that these limitations need to be laid out more clearly in the abstract, and we have now done so.

p. 17, line 10: . . . or throughout the year.

We have now included this remark

p. 18, line 23-24: Very true, but Corg burial operates on much longer timescales than the diel (fall season) NEP and NEC measured in this study.

Agreed; we have revised this sentence to highlight the difference in time scale.

Lukawska-Matuszewska K (2016). Contribution of non-carbonate inorganic and organic alkalinity to total measured alkalinity in pore waters in marine sediments (Gulf of Gdansk, S-E Baltic Sea). Marine Chemistry 186:211-220

Figures

Figure 1 and 2: Please define in the Methods section or figure caption what U10 rep- resents, to help readers who are not familiar with wind speed terminology.

This abbreviation is now listed in section 2.7.

Figure 2: Please place panel letters (a-g) so that they do not interfere with data points.

Panel letters were moved so as to not interfere with data points.

Figure 2g-h: Please use same nTA y-axis range for both campaigns to allow for easier comparison. Following these time series would also be easier if you use lines to connect data points.

These axes were corrected

Figure 3: Why do you not include the slopes for sulfate reduction and denitrification as you mention these processes in p. 9, line 9-10?

We had included lines for sulfate reduction and denitrification in an earlier version of the manuscript, but chose to leave them out here because the figure became too crowded. If the reviewer thinks this would be an important addition, we would be happy to include the extra lines in the future.

Figure 7: This figure is quite confusing to me. The generalized pattern in PPR, [P] and TA is unclear. Does it refer to the sites on the map (e.g., PPR and [P] decreases eastward, TA is high in site BA but low in sites SB, HD and LD?). Please clarify in the figure caption.

We have attempted to clarify the meaning of the generalized pattern at the top of figure 7 (now figure 8). If it is still confusing, we can remove the extra graphics, which are not necessary.

Figure 8: I suggest you move the legend from the inset figure to the main figure and increase the font size. Also, try and increase the size of the dotted confidence interval lines as these are very difficult to see.

Figure 3 has been modified according to reviewer 3's suggestions.

Figure 9: Change "DIC:TA" to "TA:DIC".

This figure was removed

Technical corrections

We thank the reviewer for these technical corrections

p. 2, line 23: Insert "it" after "while"
p. 2, line 30: Change "seagrasses meadows" to "seagrass meadows".

p. 3, line 9-10: Is there a word missing in this sentence? E.g. [. . .], suggesting the "significant/important/negligible" role of NEC or anaerobic catabolic processes in generating excess CO2.

p. 3, line 11-14: Many "potential" in this paragraph. I suggest you remove "potential" from the sentence "discuss potential differences"

p. 5, line 10: Superscript "-1" in mg L-1 and % saturation)
p. 9, line 6: Missing an "and" before "calcification".
p. 10, line 10: Should it not be "[. . .] sampling campaign 1 (a,b) and 2 (c,d)"? p. 16, line 16: Change NEPDIC to NEPDIC.
p. 19, line 2: I do not think coastal Ocean is spelled with a capital O.
p. 19, line 29: Remove "of pH".

Net heterotrophy and carbonate dissolution in two subtropical seagrass meadows

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Abstract. The net ecosystem productivity (NEP) of two seagrass meadows within one of the largest seagrass ecosystems in the world, Florida Bay, was assessed using direct measurements over consecutive diel cycles during a short study in the Fall of 2018. 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, 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. However, given the limited spatial (two sites) and temporal (8 days) extent of this study, our results may not be representative of Florida Bay as a whole and may be season-specific. The results of this study highlight the need for better temporal resolution, as well as accurate carbonate chemistry accounting in future seagrass metabolism studies.

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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 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 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) in excess of dissolved inorganic carbon (DIC), thereby decreasing pH 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 $CaCO_3$ 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), although the extent to which internal $CaCO_3$ formation occurs remains a debated topic. 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 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 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 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 it 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 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 meadows 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 occur. High primary production drives large diel variations in pH within seagrass 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 (Manzello et al., 2012; Unsworth et al., 2012; Hendriks et al., 2014; Koweek et al., 2018; Pacella et al., 2018). Seagrasses may also help to

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buffer local changes in pH by attenuating mangrove-derived fluxes of DIC (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).

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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 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, $p\text{CO}_2$ 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 important role of NEC or anaerobic catabolic 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 differences between NEP_{DIC} and NEP_{DO}, and suggest possible drivers of NEP and NEC.

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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 primary study sites which experience similar hydrologic and climatologic conditions yet differ substantially in community composition and biomass (Table 1). The choice of these sites allowed us to discern the effects of seagrass 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 above-ground biomass, nutrient content, morphology, as well as sediment depth, soil carbon (organic and inorganic), and soil nutrient content (Table 1). The potential for submarine groundwater discharge at 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.

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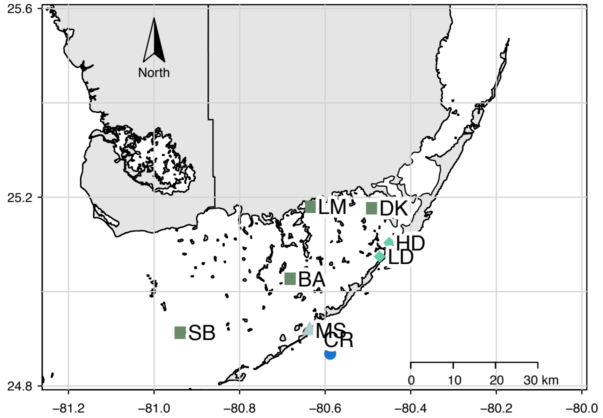

Figure 1. Site map, showing locations of the high- and low-density sites (HD and LD), meteorological stations used to derive U_{10} and pCO_2 data (MS and CR, respectively). Additional FCE-LTER sites used in this study are shown as the green squares: Sprigger Bank (SB), Bob Allen (BA), Little Madeira (LM), and Duck Key (DK).

2.2 Sampling Campaigns

We quantified NEP_{DO}, NEP_{DIC}, and NEC at our high density and low density 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). This approach has a number of weakness, however, related both to the reliance on modelled air-water gas exchange, which is subject to a high degree of uncertainty (Upstill-Goddard 2006), and the assumption that the system is closed and does not exchange water or material with adjacent systems. Both of these assumptions may be broken in shallow seagrass meadows, where tides are minimal but wind-driven seiche can be important. Furthermore, the physics governing air-water gas exchange in these systems are very poorly understood, and while it is assumed that wind-driven turbulence is the dominant driver, other factors like convection (MacIntyre et al., 2010; Podgrajsek et al., 2014), bottom-driven turbulence (Ho et al., 2016; Raymond and Cole 2001), surfactant activity (McKenna and McGillis 2004; Lee and Saylor 2010), and chemical enhancement may at times play an equal or greater role (Smith 1985; Wanninkhof 1992).

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During two sampling campaigns in late 2018, measurements were made over consecutive diel cycles for a total of 8 days. The first campaign lasted for ~4 days from Oct. 28 - Nov. 01, while the second campaign, also ~4 days, lasted from Nov. 25 – Nov. 29. 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 *n*DIC 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 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).

At each of our primary sites, small quadrats (n = 6, 10 cm × 20 cm) were randomly placed, at which aerial seagrass primary productivity (g m^{-2} d^{-1}) rates were determined using the leaf marking technique (Zieman et al. 1989). For this analysis, seagrass leaves were scraped of all epiphytes using a razor blade, rinsed, and dried at 65 °C until a constant weight. This dried seagrass material was then weighed as seagrass biomass. Dry samples were homogenized and ground to a fine powder using a motorized mortar and pestle in preparation for tissue elemental content analysis (C,N,P). Powdered samples were analyzed for total carbon (TC) and nitrogen content using a CHN analyzer (Thermo Flash EA, 1112 series). Phosphorus content was determined by a dry-oxidation, acid hydrolysis extraction followed by a colorimetric analysis of phosphate concentration of the extract (Fourqurean and Zieman 1992). Elemental ratio is reported as mole:mole. Surface soils were collected using a 60 mL manual piston core following previously described methods for determining soil carbon content (C_{org} and C_{inorg}) (Fourqurean et al. 2012b).

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 temperature (SST, °C), sea surface salinity (SSS), and dissolved oxygen (DO (mg L^{-1})) 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 current meters

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(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 (< ~ 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 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.

2.5 Benthic Chamber Fluxes

During the second sampling campaign, benthic chambers were deployed continuously over bare sediment at each of our primary sites to measure sediment-water fluxes of TA and DIC, excluding the effect of seagrass shoots. At the beginning of the experiment, acrylic chambers (~2.5L) were flushed with site water and placed at a naturally seagrass-free location on the sediment, within a few meters of each of our primary sites. 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-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.

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2.6 Sample Analysis

TA was analysed in at least triplicate (n = 3 to 5) 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. Samples were repeated injected (3-5 times) to improve the precision, which was still noticeably lower than that for TA, at ± 5.11 µmol kg^{-1} or 0.21%. During each TA and DIC run, a certified reference material (CRM) was repeatedly measured to quantify any drift or systematic bias with these analyses. The CRM used was purchased from Dr Andrew Dickson at the Marine Physical Laboratory in La Jolla, California, and was a part of batch #154. We used these CRM measurements to correct TA and DIC, assuming a linear drift between repeat CRM runs. The magnitude of this correction was on average 0.75% for DIC and 0.34% for TA. 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}.

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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 (*n*TA), DIC (*n*DIC), and DO. We quantified the total TA or DIC inventory over time to determine NEC and NEP, in what is an application of the 'slack water' approach. This approach requires a static water mass that is thoroughly mixed, and a water residence time that is sufficiently long to prevent lateral exchanges from affecting TA and DIC concentrations. This slack water approach is often applied to shallow coastal systems including tidally-inundated coral reef lagoons which are restricted from exchanges with the coastal ocean at low tide (Shaw et al., 2012; McMahon et al., 2018). While this approach may not be appropriate for coral reef lagoons at high tide due to excessive lateral mixing and vertical heterogeneities (McMahon et al., 2018), this region in Florida Bay is not subject to tidally-driven mixing to the same extent. 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):

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$$\mathrm{NEC} = -0.5 \times \frac{\Delta n\mathrm{TA}}{\Delta t} \times h\rho, \qquad (1)$$

where Δ*n*TA was the difference in *n*TA ($n\mathrm{TA} = \mathrm{TA}\times\mathrm{SSS}_{\mathrm{Average}}/\mathrm{SSS}$), *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 (Δ*n*DIC) was calculated in the same manner as Δ*n*TA. The temporal excursion in *n*TA 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. Because of this, other processes which act as sources or sinks of TA will necessarily impact calculated NEC. Such processes include denitrification, which is a net source of TA due to the consumption of HNO_3^-. Sulfate reduction also produces TA, but only if reduced sulfur is retained in the sediment and is not oxidized in oxygenated pore-water. 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:

$$\mathrm{NEP_{DO}} = \frac{\Delta\mathrm{DO}}{\Delta t} h\rho - \mathrm{O_2\ Flux}\ , \qquad (2)$$

$$\mathrm{NEP_{DIC}} = \frac{\Delta n\mathrm{DIC}}{\Delta t} h\rho - \mathrm{NEC} - \mathrm{CO_2\ Flux}, \qquad (3)$$

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}; cm hr^{-1}). These k_{600} parameterizations were intended to represent upper (Raymond and Cole (2001))

Formatted: Not Superscript/ Subscript 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 drag conditions (U_{10}; Large and Pond 1981).

$$\text{O}_2\ \text{Flux} = k_{600} * Sc * \left(\text{O}_2(water) - \text{O}_2(air)\right), \qquad (4)$$

$$\text{CO}_2\ \text{Flux} = k_{600} * Sc * K * \left(p\text{CO}_2(water) - p\text{CO}_2(air)\right), \qquad (5)$$

where $p\text{CO}_{2(\text{water})}$ was the partial pressure of CO_2 (µatm), and O_2 was the measured DO concentration (mg L^{-1}). $p\text{CO}_{2(\text{water})}$ was calculated from measured TA and DIC using CO2SYS as above. Atmospheric $p\text{CO}_2$ ($p\text{CO}_{2(\text{air})}$) was taken from the nearby Cheeca Rocks Mooring buoy operated by NOAA (Fig 1), while $\text{O}_{2(\text{air})}$ was calculated from the measured DO (%). 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.

3. Results

3. Physico-chemical conditions

At each site, variations in SSS were generally less than 1 during each sampling campaign, indicating that precipitation and fresh groundwater inputs were likely minor sources of fresh water to these sites during the study period (Fig 2c,d). Across sampling campaigns, SSS was more variable, ranging from 33.15 to 34.63 at the high-density site, and from 31.45 to 34.67 at the low-density site. SST at both sites tracked each other closely, exhibiting diurnal variations of ~2 °C, and ranging from 18.5 to 27.0 across the entire study period (Fig. 2c,d). 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 concentration of 8.14 (High density) and 9.45 mg L^{-1} (Low density) occurred in the late afternoon, coinciding with maximum pH of approximately 8.17 (High density) and 8.29 (Low density) respectively. Average pH was 8.08 ± 0.05 at the high-density site, compared with 8.17 ± 0.05 at the low-density site. Calculated $p\text{CO}_{2(\text{water})}$ at the high-density site (538.8 ± 123.5 µatm) was generally greater than atmospheric equilibrium, while average $p\text{CO}_{2(\text{water})}$ was less than $p\text{CO}_{2(\text{air})}$ at the low-density site (390.3 ± 129.4) (Table 1). 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 1). There was a difference between CO_2 fluxes derived using the RC01 and Ho06 k_{600} parameterizations, but 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.

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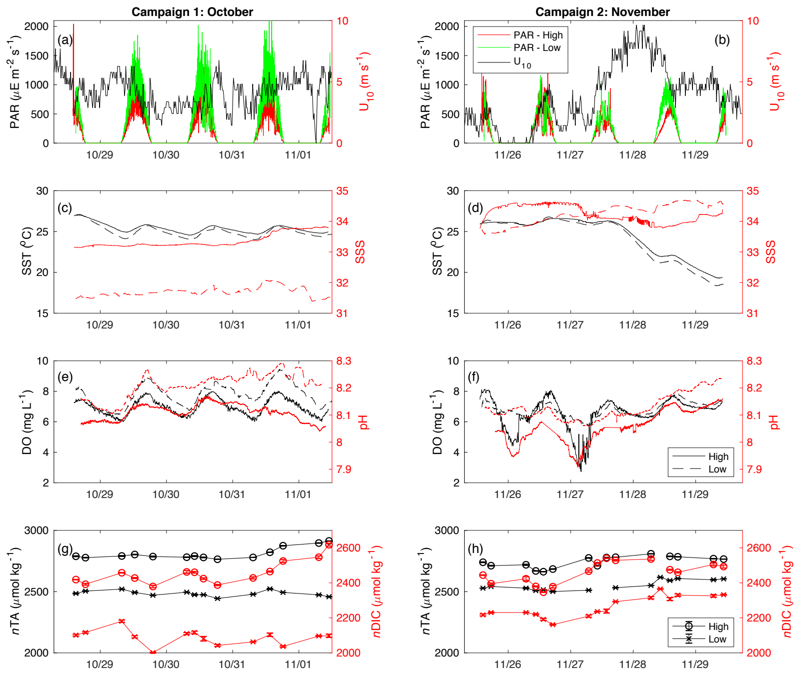
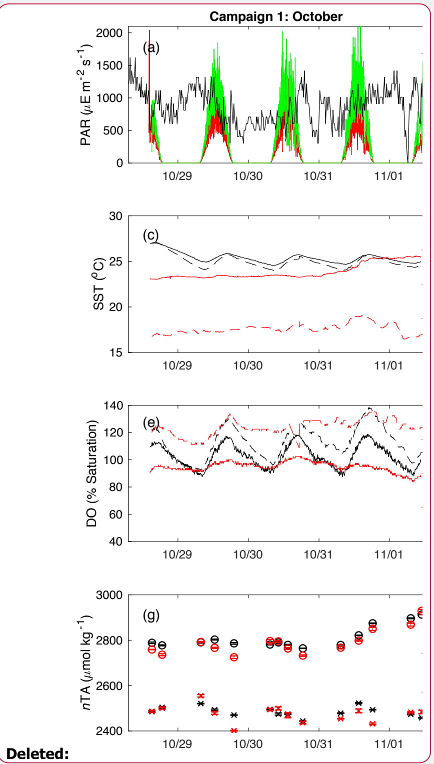


Figure 2. Time-series of a-b) PAR (µE m^{-2} s^{-1}) and U$_{10}$ (m s^{-1}), c-d) SST and SSS, e-f) DO (mg L^{-1}), SeaFET pH, and g-h) *n*DIC and *n*TA (µmol kg^{-1}). For plots b-c, the solid lines represent the high-density site, dashed lines are the low-density site.

Table 1. Table of physicochemical conditions (TA, DIC, Salinity), as well as seagrass and sediment chemical characteristics (average ± SD).

Water Depth (m)	TA (µmol kg^{-1})	DIC (µmol kg^{-1})	Salinity	Above-ground Biomass (g m^{-2})	Sediment Depth (cm)	C_{org} : C_{inorg}	Sediment C_{inorg} (% of dry weight)	Sediment C_{org} (% of dry weight)	Aerial Productivity (g m^{-2} d^{-1})	



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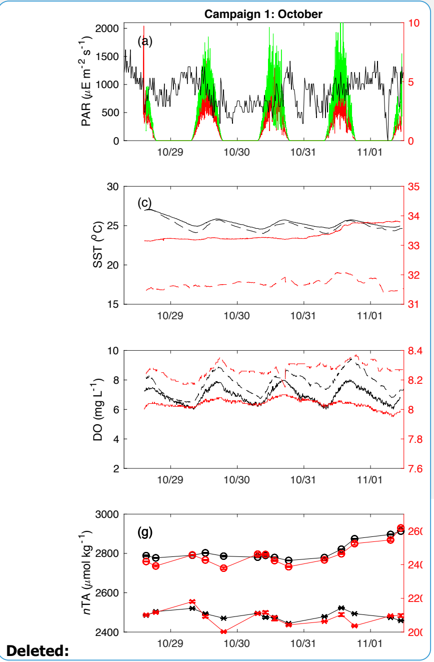

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High Density	Low Density		High Density	Low Density
		$\Omega_{calcite}$	5.83 ± 0.84	6.23 ± 1.15
0.59± 0.26 (n=6)	0.41±0.36 (n=6)	pCO$_2$ (µatm)	538.8 ± 123.5	390.3 ± 129.4
5.8	1.4	pH	8.10 ± 0.055	8.17 ± 0.062
7.9	10.1	CO$_2$ Flux– Ho06 (mmol m^{-2} hr^{-1})	0.38 ± 0.20	0.13 ± 0.62
0.74	0.14	O$_2$ Flux–Ho06 (mmol m^{-2} hr^{-1})	0.034 ± 1.2	0.75 ± 1.9
56±15 (n 10)	32±5 (n 10)	Seagrass N:P (mol:mol)	82.7	102.1
65.11± 17.66 (n=6)	15.09±14.46 (n=6)	Seagrass C:P (mol:mol)	1303.4	1892.7
33.8 ± 0.49	33.0 ± 1.3	Seagrass C:N (mol:mol)	15.8	18.5
2489.4 ± 74.7	2212.2 ± 134.0	Sediment N:P (mol:mol)	12.3	8.3
2810.6 ± 51.4	2550.5 ± 83.2	Sediment C:P (mol:mol)	321.8	1187.4
2.1	1.7	Sediment C:N	26.3	142.3

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.

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Across the study period, *n*TA at the high-density site was always greater than *n*TA at the low-density site, and *n*TA was generally higher than *n*DIC at both sites. Diel cycles were evident in both *n*DIC and *n*TA, coinciding with typical variations in net ecosystem production (consuming *n*DIC), and calcification (consuming *n*TA). The average slope between *n*TA and *n*DIC (Δ*n*TA:Δ*n*DIC) 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), as has been suggested for other Florida seagrasses (Camp et al., 2016; Challener et al., 2016). However, in this underdetermined case in which all of the aforementioned processes are occurring, the application of a simple *n*TA vs *n*DIC plot cannot reveal the relative importance of these factors.

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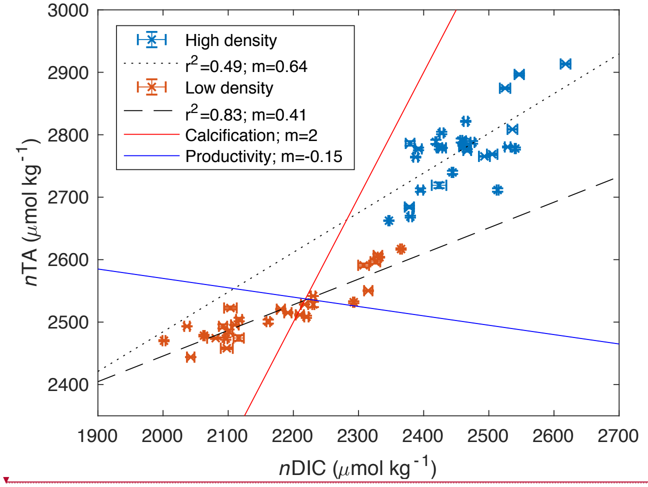

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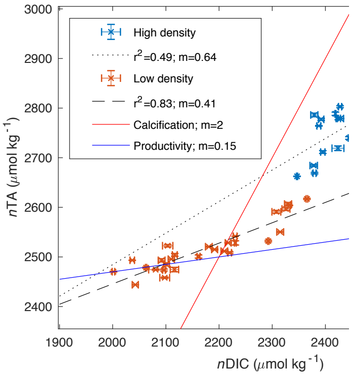

Figure 3. Scatter plot of *n*DIC and *n*TA for both high-density (blue) and low-density (orange) sites, and associated slope (m) and correlation coefficient (R^2) of the linear regression. The red reference line indicates the expected relationship if calcification is dominant, consuming 2 moles of TA for every mole of DIC consumed to form $CaCO_3$. The blue reference line shows the approximate relationship expected for aerobic respiration/productivity, which consumes approximately 0.15 moles of TA for every mole of DIC respired.

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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. 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 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. Still, other studies have found relatively high rates of SO_4^{2-} reduction in seagrass sediments (Hines and Lyons 2007), especially those with high seagrass shoot density (Holmer and Nielsen, 1997), so we express caution in the interpretation of our NEC results.

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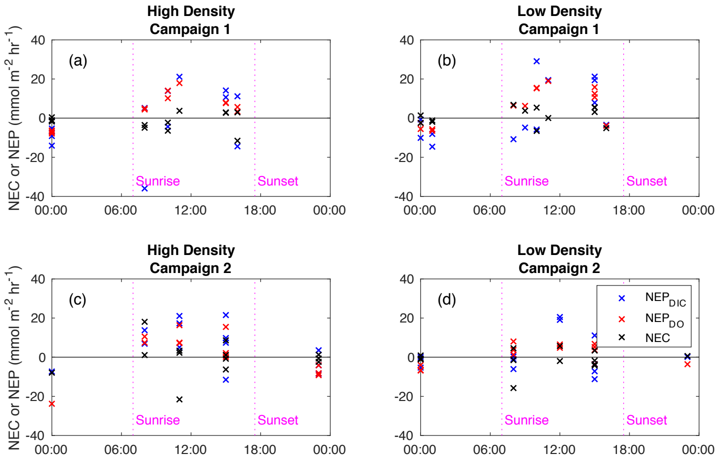

Figure 4. NEC (blue), NEP_{DIC} (black) and NEP_{DO} (red) plotted as a function of hour for the high-density site (a,c) and low-density site (b,d), for sampling campaign 1(a,b) and 2 (c,d). The x-axis represents the midpoint time for each NEP or NEC calculation period.

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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 (net heterotrophic) at night for both sites. Individual 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 very large compared with seagrass aboveground primary productivity, which was between 1.5-2 µmol C m^{-2} h^{-1} at both sites (Table 1). 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., 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.

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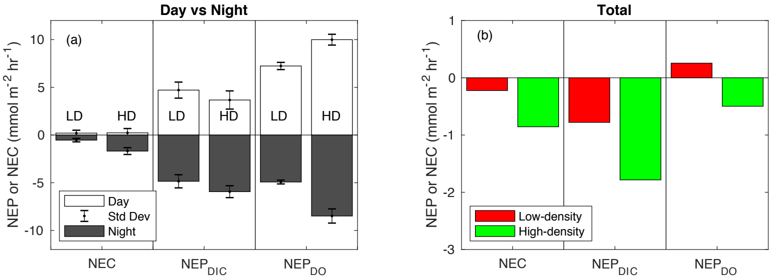

Figure 5. Average NEC, NEP$_{DIC}$, and NEP$_{DO}$ (a) separated by day and night-time periods, and (b) expressed as a total for the entire study period. NEP values are shown for k$_{600}$ of Ho et al., 2006.

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 7). While NEP$_{DO}$ and NEP$_{DIC}$ agreed in sign at night (dark blue points in Fig 7), 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).

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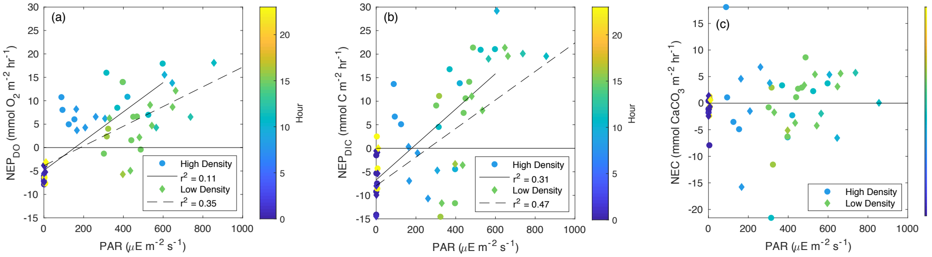


Figure 6. Scatter plots of (a) NEP$_{DO}$ vs PAR, NEP$_{DIC}$, and NEC vs PAR (b-c). Points are colored by the average hour for the respective time period over which NEP or NEC was calculated. The arrows in (a) are intended to highlight the hysteretic pattern between PAR and NEP$_{DO}$.

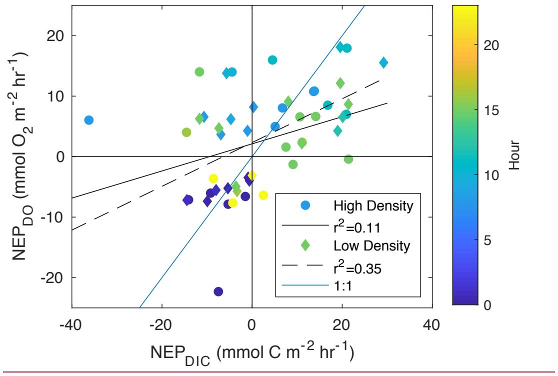
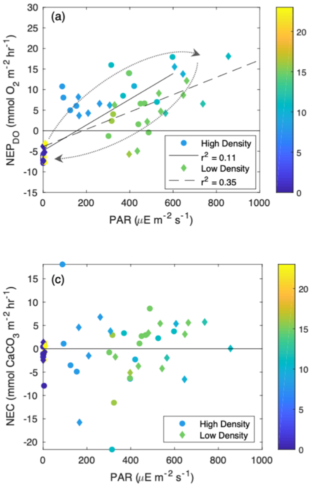


Figure 7. Scatter plots of NEP$_{DO}$ vs NEP$_{DIC}$.

To address whether this disconnect between NEP$_{DO}$ and NEP$_{DIC}$ exists outside of the two primary sites (Fig. 8; High- and Low-Density sites), we assembled pH and DO data from 4 additional sites across Florida Bay (Fig. 8; 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 fringing mangroves, which likely contributed to the significant relationship between $\Delta[H^+]$ and ΔDO there ($R^2 = 0.48$).



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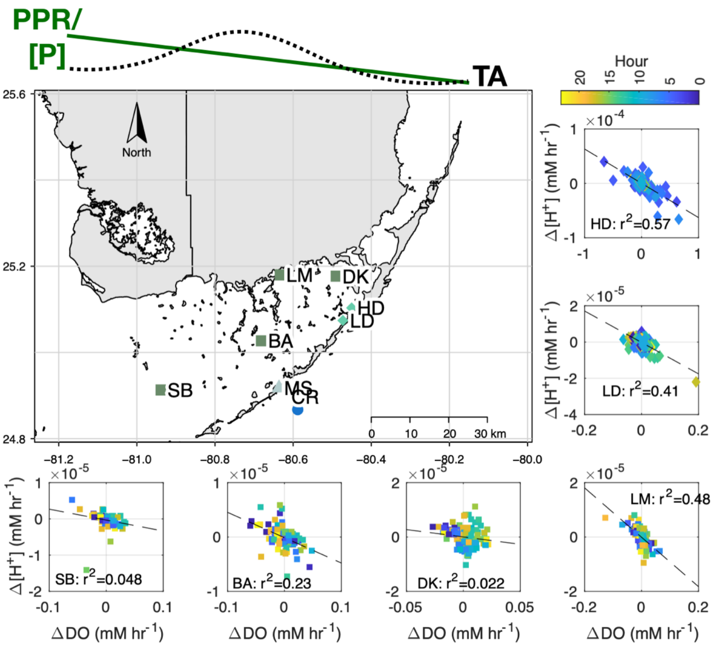

Figure 8. Map showing $\Delta[H^+]$ vs ΔDO relationship for sites associated with LTER (SB, BA, DK, LM) and the present study (high-density [HD] and low-density [LD]). At the top of the figure, we present the general east-to-west pattern in seagrass primary productivity (PPR), phosphorus content ([P]; Fourqurean et al., 1992), and TA (Millero et al., 2001) within Florida Bay. 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 1), 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. 9).

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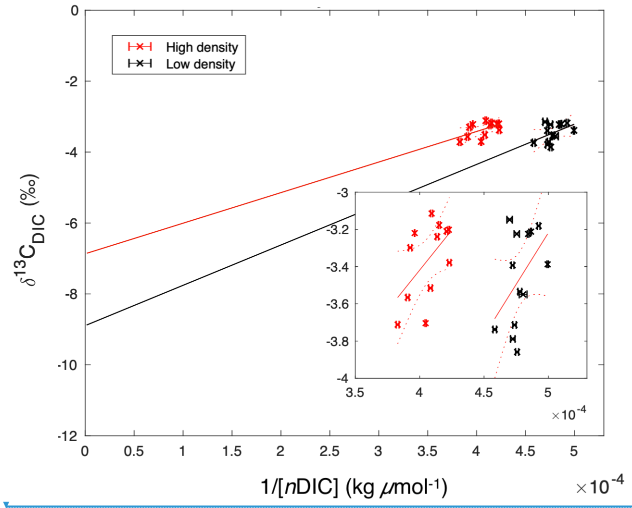

Figure 9. 'Keeling plot' of 1/[*n*DIC] versus $\delta^{13}C_{DIC}$, suggesting potential end-member isotopic values. These y-intercept $\delta^{13}C_{DIC}$ values were -6.9 ± 3.7 and -8.8 ± 6.8 ‰ (95% confidence interval) for the high- and low-density sites respectively. The inset figure is zoomed to 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.

4. Discussion

4.1 Drivers of NEP

Individual 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

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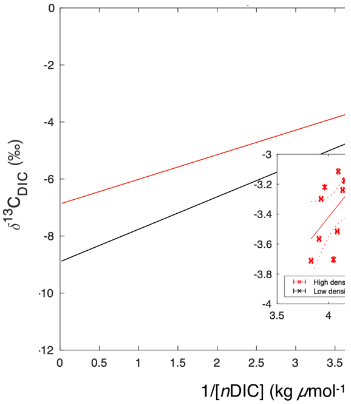

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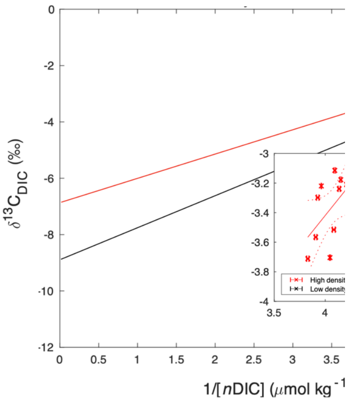

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m^{-2} h^{-1}). Over the entire study period, however, cumulative NEP_{DIC} was negative at both sites (Fig. 5b), indicating that heterotrophic conditions dominated in both seagrass meadows during these two sampling campaigns. CO_2 fluxes were positive at both sites, indicating a net release of CO_2 from the water to the atmosphere (Table 1). Seagrass aboveground primary productivity rates were between 1.5-2 µmol C m^{-2} h^{-1} at both sites (Table 1), 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 by sediment processes (including seagrass belowground productivity, which was not measured during this study). 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 7). 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 (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 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 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

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 1, we did observe a variety of bivalves and tube-building polychaetes that may have contributed to the high NEC at 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)

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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]), 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. 9). Furthermore, this isotopic analysis implicitly assumes a closed system, which clearly is not the case in Florida Bay.

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From these lines of evidence, we infer that OC remineralization in sediments, combined with carbonate dissolution 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 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. When expressed as aerial fluxes, sediment-water DIC exchange was 79 and 109% of average NEP_{DIC} 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 (Hines and Lyons 2007; Holmer et 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. The extent to which these anaerobic TA-generating processes also affect our NEC estimates is largely dependent on the fraction of reduced species that are re-oxidized in oxygenated micro-zones within surface sediments. 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 (Manzello et al., 2012; Unsworth et al., 2012; Hendriks et al., 2014; Cyronak et al., 2018; Koweek et al., 2018; Pacella et al., 2018).

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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 meadows, 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., 1967). Early work suggests that calcareous sediments in Florida Bay can be separated into distinct zones of calcareous

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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 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 not homogeneous throughout Florida Bay or throughout the year.

4.3 Regional Implications and Future Outlook

Variations in TA and DIC exports affect the carbonate system buffering of adjacent systems, further complicating the relationship between NEP_{DO} and NEP_{DIC}. In Fig. 8, 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 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 study; 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 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

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of new in-situ approaches that rely on variations in pH and DO alone to infer rates of biogeochemical processes (e.g. Long et al., 2015b).

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 valuable, studies based solely on rates of C_{org} burial integrate processes over long time scales, and may miss the impact of seagrass NEP and NEC on air-water CO_2 exchange and lateral $CO_{2(water)}$ and TA export. 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 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.

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. We found negative NEP_{DIC} and NEC at both sites, indicating that despite typical values of seagrass biomass and productivity (Table 1), both sites were net heterotrophic and net dissolving over the study period. Multiple lines of evidence point to sediment respiration and carbonate dissolution (Fig. 9) 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. Because of this, we express caution in interpreting our NEC results as strictly net production of $CaCO_3$; it appears that TA generated by anaerobic processes in the sediment likely influenced our estimates of NEC. Further studies should refine our estimates of benthic DIC and TA fluxes from seagrass sediments (with benthic chambers [present study], underwater eddy covariance [Long et al., 2015b; Yamamoto et al., 2015], or pore-water modeling), and compare these values to other component fluxes of NEP and NEC (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,

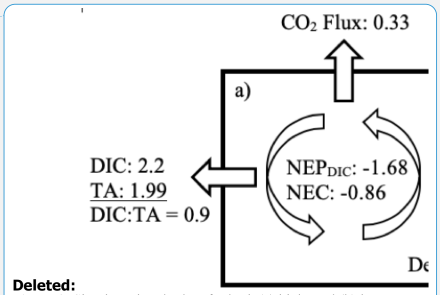

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Figure 9. Simple carbon budget for both (a) high- and (b) low-density sites, including inferred lateral DIC and TA export (mmol m^{-2} h^{-1}). Values of NEP and CO_2 flux were averaged for RC01 and Ho06 k_{600} parameterizations, from which DIC lateral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

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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 (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}. Unfortunately, given the very limited temporal scope of this study, just 8 days, it is impossible to extend the results of this study to longer time scales. At present, we cannot determine whether the seagrass ecosystem at this site is net dissolving and heterotrophic throughout the year, or even across seasons. 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.

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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.

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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

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. We acknowledge the thoughtful comments and suggestions of three anonymous reviewers. Funding for this study was acquired by JF, and additionally through the DAAD (#57429828) from funds of the German Federal Ministry of Education and Research (BMBF).

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Competing Interests

The authors declare no conflicts of interest

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