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Interactive comment on "Macroalgal metabolism and lateral carbon flows create extended atmospheric CO_2 sinks" *by* Kenta Watanabe et al.

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Author response to RC2 by Dorte Krause-Jensen

We thank to your constructive comments. Below is reviewer's comments and our response to them.

GENERAL COMMENTS Comment #1: This study documents the exchange of dissolved carbon between a macroalgal habitat and adjacent waters. The study highlights that macroalgal metabolism and excretion of dissolved organic carbon (DOC) during the productive phase of the vegetation create low CO2 concentration and high DOC concentration that, via water exchange, propagates from the macroalgal habitat to waters beyond the habitat. The low CO2 concentrations created by macroalgae thereby

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contribute to increased air-sea CO2 uptake both at the habitat and beyond, and export of DOC beyond the habitat suggests a potential of this carbon to reach oceanic sinks. These findings add significantly to the limited field evidence of the effect of macroalgal metabolism on dissolved carbon concentrations and the size of macroalgal-associated carbon fluxes and potentials for C-sequestration. Such evidence is important to underpin the recent notion that macroalgae contribute significantly to global C-sequestration, with the majority of the sequestration being supported by dissolved organic carbon reaching oceanic C-sink. The combination of in-situ measurements and flux studies, degradation experiments and modeling strengthen the findings. The study can be improved by adding detail in the method description, presentation and discussion of results and reference to earlier findings.

Response: Thank you for your comments and suggestions. We have modified the manuscript considering your suggestions. Please see details below.

Change: We have modified the manuscript considering your suggestions. Please see details below.

SPECIFIC COMMENTS Methods Comment #2: Field surveys (l. 76-80): - Please specify that the field studies were conducted during a diurnal cycle in February and March, respectively, and please underline the timing of sampling as well as the timing of sunrise/sunset so that the reader knows how the diurnal cycle was represented. Please also mention that February /March is the local winter time.

Response: Field surveys were conducted only during the daytime. For estimating diurnal cycles including nighttime, we used a field-bag method (especially respiration rate) and mass balance modelling (L154). We collected water samples three times at 10:00, 13:00, and 16:00 during the daytime (approximately from 7:00 to 17:00).

Change: We have added this information in this paragraph. We have also showed that February and March are included in the local winter period in this paragraph.

Comment #3: Field bag experiments: - (l. 93) Was the ambient seawater for the macroalgal bags and control bags taken from the macroalgal site?

Response: Yes, the ambient seawater for every treatment was collected in the macroalgal bed.

Change: We have added this information in the paragraph.

Comment #4: Biomass, cover and species composition (l. 100-106): - How long were the transect lines? While cover was assessed every 10 m (in 1 x 1 m quadrates) it is not clear how biomass assessments relate to cover assessments. Were the five 0.5 x 0.5 m biomass samples taken within quadrates assessed for cover and where cover estimates documented dominance by sargassaceous algae? Or were the biomass samples placed e.g. randomly within the belt dominated by sargassaceous algae? Please add detail.

Response: Both transect lines were 120 m. Five quadrats (0.5 m $\times$ 0.5 m) for quantifying biomass were randomly located in the area dominated by Sargassum algae along each transect.

Change: We have added this information in this paragraph.

Comment #5: Degradation experiment - Why were the samples stored at room temperature (22âĎČ)? Did this correspond to the in situ temperature? Were the samples aerated or did they turn anoxic during the incubation? How were degradation rates calculated?

Response: In this study, we used room temperature (22âĎČ), which is higher than in situ temperature, for both study periods to compare the quality of organic matter. We made the headspace to keep sufficient oxygen for aerobic degradation.

Change: We have added the discussion about the effect of temperature on the microbial degradation of DOC in the discussion section. We have added the explanation about making headspace in this paragraph. We have added the equation for calculat-

ing degradation rates (k).

Comment #6: Mass balance modeling - It is not entirely clear how this modeling was conducted -please expand the explanation. As far as I understand, the modeling was conducted solely for the macroalgal site (and not for the off shore site), please state this clearly. –

Response: As you say, this modeling was conducted solely for the macroalgal site and the observed values of offshore site were used as the endmembers of inflowing carbon to the macroalgal bed.

Change: We added the sentences to clarify this point in this paragraph.

Comment #7: L. 154: Are the initial values for the macroalgal site estimated to be diurnal averages measured at the off shore site? (Please indicate in the text that the initial values are denotated "0" in the formula). (To clarify, I suggest moving the sentence (l. 163) "The values of DICO, TAlkO, and DOCO were the mean values at station H5." to follow l.154.)

Response: We agree with your suggestion.

Change: We have moved this sentence and rephrased as follows: "The values of DICO, TAlkO, and DOCO were the mean values at the offshore station (H5). The initial values in the simulation were defined as the average values at the offshore site (station H5). Namely, DIC(0), Talk(0), and DOC(0) were DICO, TAlkO, and DOCO, respectively."

Comment #8: L. 160-162: Please explain in more detail how the central parameters GCP, R and CC were determined (based on start/end and light/dark and macroalgal/control measurements) and which day length was applied. Regarding the calculation of calcification – please also see e.g. Wahl et al. 2018.

Response: We agree with your suggestion. We used the day length shown in Table 2.

Change: We have added the equations and explanations for metabolic parameter es-

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timation.

Comment #9: - Please make it clear in the methods section how the two different model scenarios (i.e. with and without considering water exchange, blue line and black line of Fig 5) were calculated.

Response: We agree with your suggestion.

Change: We have added the explanation of two different model scenarios in this paragraph.

Results Comment #10: (3.1) Carbonate system and DOC - Net community calcification: Did your study allow calculating potential differences in calcification between light and dark settings? - Fig. 2: Are the two lines significantly different?

Response: Because we used both transparent and dark bags for measuring net community calcification (NCC), we can calculate NCC rates in both light and dark settings (Table S3 in the Supplement). As we have discussed in the previous manuscript, it is difficult to discuss the differences in NCC values between light and dark settings because the uncertainty of NCC derived from the measurement precision were comparable to the observed values. We did not conduct statistical analysis here because we did not intend to discuss the differences between March and February.

Change: We have not changed manuscript about this comment.

Comment #11: (3.2) Biomass/cover - Fig. 3: Relationships between cover and biomass (relates to the question on how biomass samples were taken): How come that the highest biomass in panel a corresponds to the lowest coverage in panel b? And that the lowest biomass of sargassaceous algae (and highest relative biomass of “others” in panel c corresponds to a high (absolute and relative) cover of sargassaceous algae in panel d? Are there any significant biomass-cover relationships?

Response: As we replied to the question on how biomass samples were taken, quadrats for biomass and coverage were randomly located along each transect. We,

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thus, think that the heterogenous colonization of macroalgae (e.g., patches) caused the inconsistence. Because we used only the averaged biomass for the mass balance modelling, this inconsistence between biomass and coverage did not change the conclusion.

Change: We have added the explanation about how biomass samples were taken in Materials and methods section.

Comment #12: (3.3) Degradation of DOC - l. 211-212 “Degradation rates (k) estimated by exponential fitting were 0.0044 d$-$1 and 0.0018 d$-$1 in February and March, respectively.” Please clarify how degradation rates were calculated by e.g. chnagingt he sentence to “Degra-dation rates (k) estimated by exponential fitting of XXX vs XXX were 0.0044 d$-$1 and 0.0018 d$-$1 in February and March, respectively.” and specify XXX.

Response: Thank you for your comment. We have added the explanation.

Change: We have added the explanation and equation for calculating Degradation rates (k) in Materials and methods section.

Comment #13: Fig. 4: - It is notable that DOC concentrations of the control bags were similar between Feb and Mar while DOC concentrations of macroalgal bags differed considerably, with final concentrations being about 140 μM in Feb and <100 μM in Mar. Please discuss this in the discussion section..

Response: The difference in the initial DOCM concentrations of macroalgae bags between February and March would be caused by the differences in the biomass and water volume in the experimental bags. We have added the discussion about this point.

Change: We have added the discussion about this point as follows: “The difference in the initial DOCM concentrations of macroalgae bags between February and March would be caused by the differences in the biomass and water volume in the experimen-

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tal bags (Fig. 4a, b). The variation of DOC concentration may affect the degradation rates via resource limitations for microbial activity (e.g., Arrieta et al., 2015). The understanding of the fate of macroalgal DOC will be supported by assessing physical and biochemical factors regulating the microbial degradation of DOC."

Comment #14: - The 4th control sampling for March has high variability – might one sample be contaminated and should be omitted? - Panel c: Should the line-fit not be exponential?

Response: Thank you very much for your comment. We conducted triplicate analyses per one sample for DOC measurement using TOC analyzer to reduce the analytical uncertainty and used the average of this triplicate. One of the samples for 30-day control contained an erroneous value within the triplicate, which caused this unintentional high variability. We have omitted this erroneous value and modified Fig. 4b, c and related sentences. We rechecked every data and the others were not erroneous. After this correction, plots became suitable for exponential fitting (R2 was improved).

Change: We have omitted the erroneous value and modified Fig. 4b, c and related sentences.

Comment #15: - How were degradation rates calculated. Based on fits of data in panel c? Why not based on fitting an exp decline curve to the macroalgal data of panel a and b?

Response: We have added the explanation and equation for calculating Degradation rates (k) in Materials and methods section. The focus on this degradation experiment was the quantification of refractory DOC derived from macroalgae. We decided not to fit exponential decay curve to the data of macroalgal bags in Fig. 4a, b because DOC of macroalgal bags contained ambient DOC.

Change: We have added the explanation and equation for calculating Degradation rates (k) in Materials and methods section.

Comment #16: (3.4) Carbon budgets - L. 214. “The mass balance models simulated the temporal changes in carbonate chemistry and DOC concentration (Fig. 5).” It is not clear how the mass balance models did this - please expand in the methods section and also elaborate a bit more here. Please explain that the model simulations represent both the situation when water exchange is taken into account (blue lines in Fig. 5) and the situation when it is not (black lines in Fig. 5). - I also suggest adding more detail to the legend of Fig. 5 to specify the significance of the blue line (in contrast to the black line), which is not mentioned in the current version of the legend.

Response: We have added the explanation of two different model scenarios in Materials and methods and result section. We have added the explanation for the model improvement (the change in the RMSEs of every parameters) by considering water exchange in this paragraph and the legend of Fig. 5. In the previous version of our manuscript, model fitting was performed by minimizing RMSEs solely for DIC model but it may cause the uncertainty in other parameters (i.e., TAlk, DOC, and fCO2). We have modified this model fitting method as follows: “EXr was determined by fitting the models so as to minimize the root mean squared error (RMSE) compared with the observed values. RMSEs were calculated for the z-scores of DIC, TAlk, DOC, and fCO2 values, which were standardized anomalies from the mean observed values divided by the standard deviations. The EXr value that minimize the averaged RMSEs for these four parameters was determined for each survey.” This modification has changed the results of water exchange rate and carbon budgets but the conclusion has not been changed.

Change: We have added the explanation of two different model scenarios in Materials and methods and result section. We have added the explanation for the model improvement (the change in the RMSEs of every parameters) by considering water exchange in this paragraph and the legend of Fig. 5. We have modified the model fitting method and the related results (Fig. 5 and Table 3).

Comment #17: l. 216: How were the spans in hourly exchange rates calculated (35-

48% and 50-76%) and why is the range not reported in Table 3 (35% and 50% is reported without any range).

Response: EXr values were constants estimated for each survey but EXtide values were variables changing depending on water height. For clarification, we have rephrased this sentence as follows: “Hourly water exchange rates (the sum of EXtide and EXr) were...”. We have showed the temporal change in water exchange rate (the sum of EXtide and EXr) in Fig. 5.

Change: We have rephrased this sentence as follows: “Hourly water exchange rates (the sum of EXtide and EXr) were...”. We have showed the temporal change in water exchange rate (the sum of EXtide and EXr) in Fig. 5.

Comment #18: - It follows from the modeling approach (l. 223-224) that “DIC budgets driven by water exchange indicated a net input of DIC from offshore to the macroalgal bed (Fig. 6 and Table 3.)”, - because otherwise the DIC levels at the macroalgal site would have been considerably lower that what was measured (as shown in Fig 5). However, the abstract says “The exported water lowered CO2 concentrations in the offsite surface water and enhanced atmospheric CO2 uptake.”, and I think this statement needs be better underpinned by results and discussion.

Response: Thank you for your suggestion. We agree that our study did not directly demonstrate the enhancement of CO2 uptake in offshore site by the macroalgal bed.

Change: We rephrased the sentence in Abstract as follows: “These results indicate that the exported water would potentially lower CO2 concentrations in the offshore surface water and enhance atmospheric CO2 uptake.”

Discussion Comment #19: - Net calcification (NCC): Please discuss /mention what may constitute the NCC: calcareous algae in the algal bed, mussels. . . ? Did you identify any variation in NCC between light and dark incubations? – please mention/discuss. For these discussions I suggest referring/comparing to e.g. Wahl et al 2018 & Duarte

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& Krause-Jensen 2018.

Response: As described above, we can calculate NCC rates in both light and dark settings (Table S3 in the Supplement). However, it is difficult to discuss quantitatively the differences in NCC values between light and dark settings because the uncertainty of NCC derived from the measurement precision were comparable to the observed values. We also think that the quantitative comparison between our data and a previous work is difficult. However, we have cited this previous work about the calcification in macroalgal beds.

Change: We have cited this previous work about the calcification in macroalgal beds.

Comment #20: - L. 276-8: Please elaborate a bit on this in relation to the differences observed between the Feb and Mar measurements. Regarding “growth phase”, please mention that the study took place during the productive period.

Response: In the present study, both surveys were conducted during the productive period but the averaged biomass per individual S. horneri used for field bag experiment was different (February, 353 g WW; March 260 g WW), which may indicate the difference in growth phase.

Change: We have added this explanation in this paragraph.

Comment #21: - L. 305-307: Regarding the comparation between DOC turnover times: Were the experimental conditions similar?

Response: Wada et al. (2008) calculated degradation rates for 30 days incubation, which was shorter than our study. Thus, we recalculated degradation rates for 30 days incubation and compared with Wada et al. (2008). This recalculation results also showed the same trend.

Change: We have modified this sentence according to this recalculation. We have also added the sentence regarding the temporal change in degradation rate during the DOC degradation referring a previous study.

Comment #22: - Please discuss reasons for the big difference in the residual concentrations of DOC in the degradation of material from Feb and March.

Response: As described above, the difference in the residual DOCM concentrations of macroalgae bags between February and March would be caused by the differences in the initial DOCM concentrations.

Change: We have added the discussion about this point as follows: “The difference in the initial DOCM concentrations of macroalgae bags between February and March would be caused by the differences in the biomass and water volume in the experimental bags (Fig. 4a, b). The variation of DOC concentration may affect the degradation rates via resource limitations for microbial activity (e.g., Arrieta et al., 2015). The understanding of the fate of macroalgal DOC will be supported by assessing physical and biochemical factors regulating the microbial degradation of DOC.”

Comment #23: - C-sequestration. L. 322-323: I suggest mentioning that a first-order assessment suggested that almost 70% of global macroalgal C-sequestration is attributable to DOC export to the deep sea (Krause-Jensen & Duarte 2016).

Response: We agree with your suggestion.

Change: We have added this mentioning in this paragraph as per your suggestion.

Comment #24: Figs/Tables (in addition to what is mentioned above) Fig 5 legend: Please mention that details regarding rates are available in Table 1. I suggest moving Table 1 to supplementary material as the data are already presented in Fig 5.

Response: We showed Table 1 separately from Fig. 5 for clearly representing the difference in parameters between the macroalgal bed and the offshore by using statistical analysis. We believe that Table 1 should be also represented in main manuscript.

Change: We have not changed our manuscript.

Comment #25: Fig 6 & Legend: Please change “Carbon flows..” to “Dissolved carbon

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flows..” Please add explanation of RDOCm. Unclear what is the difference between NDR and DOC. The legend says that (107) and (88) represents DOC flows; then why are the numbers at the DOC arrows different? Please mention how the data were generated (model, degradation exp, bag exp – maybe using different colors). Mention that the same calcification rates are reported both as calcification rates and as inorganic biomass growth. Mention if the NCP is the sum of macroalgal and planktonic NCP.. Mention that details regarding rates of C-metabolism are available in Table 2 and details on water exchange rates are available in Table 3.

Response: Because we also showed air–water CO2 gas exchange flux in this figure, we think that “Carbon flows...” is better explanation. We agree with the other comments.

Change: We have modified the legend as follows: “Carbon flows and community metabolism (NCP, net community production; NCC, net community calcification; NDR, net DOC release) in the macroalgal bed. NCP, NCC, and NDR were calculated using the results of field bag experiments (details are available in Table 2). Biomass growth in terms of organic carbon (OC) was calculated by subtracting NDR from NCP. Biomass growth in terms of inorganic carbon (IC) was same as NCC. DIC and DOC flows via water exchange were estimated by mass balance modelling (details are available in Table 3). Community metabolism, biomass growth, and DOC outflow indicates the sum of macroalgal and planktonic carbon flows. The parentheses show the carbon flows solely due to macroalgae. Carbon fluxes were calculated in units of mmoles per square meter of the surface area of the macroalgal bed per day. RDOCM indicates refractory DOC released by macroalgae.”

References Comment #26: - It would be appropriate to mention the pioneer study by Smith 1981 suggesting that lowering of CO2 concentrations by macroalgae leads to increased CO2 uptake and to highlight that the current study not only confirms that this is the case but also takes the finding further by documenting that the effect extends beyond the macroalgal habitat.

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Response: We agree with your comment.

Change: We have added the explanation about the previous study by Smith (1981).

Comment #27: - Line 60-61: “However, the effects of macroalgal metabolism on the carbonate system in both macroalgal beds and adjacent water bodies have not been quantified”: Please consider rephrasing to say e.g. that there is limited field evidence on this.. Earlier studies have documented effects of macroalgal metabolism on the carbonate system (e.g. Wahl et al. 2018, Middelboe et al. 2007, Krause-Jensen et al. 2015 & 2016, Duarte & Krause-Jensen 2018), and some of these provide evidence of how diurnal/temporal variations in macroalgal metabolism affect calcification. You may also want to mention that there are recent studies on particulate organic carbon (POC) fluxes from macroalgae (e.g. Filbee-Dexter et al. 2018, Pedersen et al. 2019, Queirós et al. 2019...) but less information on DOC fluxes despite the expected major importance of macroalgal DOC fluxes for carbon sequestration.

Response: We agree with your comment.

Change: We have rephrased this sentence as follows: “Indeed, some previous studies have shown that macroalgal beds act as sinks for atmospheric CO2 (Delille et al., 2009; Ikawa and Oechel, 2015; Koweek et al., 2017) and contribute to global carbon fluxes (Smith, 1981; Krause-Jensen and Duarte, 2016). Macroalgal metabolism regulates diurnal and temporal variations in carbonate chemistry and affects calcification by calcifiers inhabiting in macroalgal beds (Middelboe et al., 2007; Krause-Jensen et al., 2015, 2016; Duarte and Krause-Jensen, 2018; Wahl et al., 2018). However, there is limited field evidence for how the effects of macroalgal metabolism on the carbonate system extend to adjacent water bodies.” We also added references about POC export in the Discussion section.

TECHNICAL CORRECTIONS Comment #28: l. 17-18: I suggest adding field measurements of carbon species to the set of applied methods.

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Response: We agree with your comment.

Change: We have added “field measurements of carbon species” to the set of applied methods in Abstract as per your suggestion.

Comment #29: l. 22: Please change “offsite” to “offshore”

Response: We agree with your comment.

Change: We have changed “offsite” to “offshore” in Abstract.

Comment #30: l. 36: “is more labile” – I suggest rephrasing to underline the variable lability of macroalgal carbon.

Response: We agree with your comment.

Change: We have modified this sentence as follows: “Organic matter produced by macroalgae shows variable lability but are generally more labile than that produced by vascular plants”

Comment #31: l. 39: “is comparable to..” – or larger than?

Response: We agree with your comment.

Change: We have changed “is comparable to” to “larger than” according to the data shown in these references.

Comment #32: l. 40-41: “Macroalgal beds therefore have the potential to sequester substantial amounts of carbon in marine systems”: This does not follow logically from the previous sentences – please rephrase.

Response: We agree with your comment.

Change: We have rephrased this sentence as follows: “Macroalgal beds therefore have the potential to regulate carbon dynamics in coastal ecosystems.”

Comment #33: l. 49-50: please mention also the estimated contribution of DOC

to macroalgal C-sequestration in this previous study to highlight the hypothesis that macroalgal DOC is of major importance.

Response: We agree with your comment.

Change: We have added the estimated contribution of DOC to macroalgal C-sequestration (33%) in this sentence.

Comment #34: l. 67: "..this macroalgae is the dominant group in temperate regions": should it be e.g.". . .the Sargassaceae family of macroalgae is a dominant group in temperate regions"?

Response: We agree with your comment.

Change: We have rephrased this sentence as follows: "we focused on Sargassum beds because they are one of the dominant macroalgal habitats in temperate and tropical regions"

Comment #35: l. 74: "at a water depth.." –> "at water depths.."

Response: We agree with your comment.

Change: We have modified this wording as per your suggestion.

Comment #36: l. 188 and 193: Please add "(Table 1)" at the end of the sentence.

Response: We agree with your comment.

Change: We have added "(Table 1)" at the end of the sentences as per your suggestion.

Comment #37: l. 236: Please add after "(Table 2)" e.g.: ".., the rest being attributable to planktonic NCP."

Response: We agree with your comment.

Change: We have added modified this sentence as per your suggestion.

Comment #38: l. 241: please substitute "known" by "shown".

Response: We agree with your comment.

Change: We have changed “known” to “shown” in this sentence as per your suggestion.

Comment #39: l. 275/6: I suggest changing “The inhibition of macroalgal R by low water temperatures during the winter can explain the relatively high NCP values during the productive period at our study site (Table 1 and 2).” to “The inhibition of macroalgal R by low water temperatures during the productive winter can explain the relatively high NCP values observed at our study site (Table 1 and 2).”

Response: We agree with your comment.

Change: We have rephrased this sentence as per your suggestion.

Comment #40: - should “irradiation” be “irradiance”?

Response: We agree with your comment.

Change: We have changes “irradiation” to “irradiance” through the manuscript.

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