

Author response to RC2, Pierre Polsenaere

GENERAL COMMENTS:

The submitted manuscript of Kindeberg et al. under review and discussion for the journal Biogeosciences presents summer benthic community metabolism and composition measurements over a “chrono-sequence” from bare sediments to different restored seagrass meadow development stages in a high temperate marine embayment in Sweden. Different flux techniques (aquatic Eddy Covariance, benthic chamber measurements) along with sediment and benthic fauna/flora characterization and associated computations (O_2 :DIC ratio, PQ and RQ, LUE, PI curves, etc.) were done to particularly resolve the links between carbon cycling and biodiversity in this restored seagrass meadow area.

The study presents very interesting in situ measurements, analysis and computations through a significant, detailed and well written manuscript. The latter is of particular importance as it identifies mechanisms and links between benthic carbon processes/fluxes and fauna/flora diversity over a restored seagrass meadow system and such coastal carbon research studies need to be done increasingly in the future; thus, I congratulate the authors for their work that is well suited for Biogeosciences journal.

I have two main general comments on the submitted manuscript (i) the first one concerns the lack of certain information especially on benthic flux measurements and computations described in the M&M section that could be given to help the readers to better follow in situ deployments done during the study. ii) my second and major concern is with regards to the chrono-sequence methodology and associated assumptions on which results and discussions relied. Indeed, as authors said, abiotic conditions during the four site measurements have to be not significantly different to truly endorse the four restored seagrass meadow development stage influence only on corresponding measured benthic carbon fluxes. Linear mixed effects models and associated statistical approaches have been used to address this fundamental purpose to validate the approach but associated results are not clear enough or even given in the manuscript as it stands. For instance, flow velocity (i.e. between bare/3years and 7 years/natural sites) as well as salinity and water temperatures during and between this summer week deployments experienced important and significant variations as rightly noticed by authors, that may have influenced benthic metabolism besides meadow habitat development itself. All these aspects need to be better ruled and discussed in the manuscript.

In this way, please see the specific comments below to help in the revision of the different sections and the overall manuscript.

We thank Dr. Polsenaere for his time and effort writing this thoughtful and constructive review. We are grateful for the encouraging words and appreciate the discussion on methodological constraints. While the chronosequence allows us to follow contrasting meadows occurring within very close proximity, our ability to measure metabolic fluxes in these different meadows under identical abiotic conditions is challenging and would probably require multiple eddy covariance systems running simultaneously. Unfortunately, this was not possible for this study.

Nevertheless, as rightly pointed out, the chronosequence is based on that general assumption that abiotic conditions that affect metabolism are similar and we agree that it is important to be upfront about this and the topic deserves more attention in the results and discussion.

We do see significant differences between sites with regards to flow velocity, temperature, DO and turbidity. However, these variables together only explain 20% of the variation in absolute oxygen fluxes and the majority of the variation is explained by some other feature(s) not included in the model. We focus the paper on trying to discern what those features may be, and we utilize our light-use-efficiency measurements – which accounts for day-to-day differences to some extent - as a step in our argumentation that it may be partly related to macrophyte structural complexity. While we have added paragraphs in the discussion pertaining to these caveats, we would like to expand on three separate lines of evidence of which we base our inference that any differences in flow velocity between deployments did not consistently explain the observed differences in metabolism between sites.

First, mean flow velocity was indeed different between deployments but the relationship with absolute oxygen fluxes was highly site-specific with generally low R^2 values (Bare=0.01; 3yr=0.10; 7yr<0.001; Nat= 0.20) and only in two of the sites (3yr and Nat) was the linear regression slope significantly different from 0. We have updated Fig. S3 (previously S2) to better illustrate this. When relationships are this weak the predictive capabilities are limited, and we would not be able to know what fluxes would have been under different conditions (e.g. lower flow velocity).

Second, our flow velocity measurements were obtained by the ADV mounted on the eddy covariance frame which was positioned within the site/meadow. Regrettably, we did not monitor flow outside of the sites (e.g. incoming flow to the bay). The flow velocities we measured will thus be modulated by the morphology of the bottom substrate (e.g. meadow density, canopy height etc.). Due to differences in drag (bed shear stress) of the different sites and differential modification of hydrodynamics, we cannot discriminate between the actual differences in environmental conditions (due to e.g. weather-induced currents) and what is due to the inherent effect of the bottom morphology on flow. Seagrass meadows are known to have a large impact on flow velocity, and the induced drag will modulate the turbulent flow above the canopy (e.g. Fonseca et al. 1982).

Third, benthic chamber incubations largely preclude any effects of hydrodynamics on fluxes. Nevertheless, we observe a similar increasing trend in FO₂ (and decreasing FDIC) in light chambers going from bare to increasing meadow age and oxygen fluxes measured in chambers were not significantly different from eddy fluxes.

Accordingly, it is not possible to unequivocally disprove or validate the chronosequence assumptions based solely on the between-site differences in flow. We have tried to clarify these aspects throughout the results and discussion in the revised version of our manuscript.

Please see our detailed response to each of the comments below.

SPECIFIC COMMENTS:

Abstract

- 1.32: not clear what are these values? CR? NCP?

We have added that the values refer to NCP.

- 1.33: what about heterotrophic biomass?

This specific sentence regarding niche complementarity refers to macrophytes and we therefore choose to not bring up heterotrophic biomass in this context as to not confuse the readers. We have updated the sentence to clarify this: “While autotrophic biomass did not increase with meadow age, macrophyte diversity did, elucidating potential effects of niche complementarity among macrophytes on community metabolism.”

1. Introduction

This section is very good as it stands.

Thank you.

2. Material and methods

- 1.130: 1-4 m depth, is the studied zone subtidal? what about hydrodynamics, horizontal advection and influence of downstream and upstream systems? Please give general information on it.

We have clarified that the zone is subtidal and we have added information on tidal amplitude and salinity regimes. Regrettably, additional information on advective flows and hydrodynamics is scarce in the literature.

- 1.136: please give the exact distance between the four sites.

We have added the exact distances in section 2.2.1 of the Methods, as measured *a posteriori* using the distance measuring tool in the GIS software QGIS 3.6. Location and duration of each deployment is added to a new Table S1.

- 1.139-143: indeed, these aspects need to be addressed (see general comment above); also, please refer to Table S1 and Fig. S1 here.

Please see our response above and detailed responses below. We have added the suggested figure and table reference.

- 1.147-155: it is very important to refer here to Fig. S1, if not, we don't have any information on EC deployment beginnings and ends (days, dates, numbers, hours, durations at each site), these information have to be given in the text or at least in the Fig. S1 caption. A photo of the EC frame in situ deployed with habitats could be nice in the supplementary material as well.

We agree and have added the suggested information in a Table S1 and added BC deployments to Fig. S2 (previously Fig. S1). We also have a new Fig. S1 illustrating the EC and BC and their deployment in the field.

- 1.157-163: similarly, information according to benthic chamber incubations are lacking and must be given in this sub-section: the number of incubations at each site, the order of incubations between clear and dark chambers, the durations of each incubation, the dates of beginning and ending of each incubation, the correspondence with EC deployment (corresponding positions and times?), were they deployed simultaneously with EC measurements? A table with all these EC and BC information could be added in the MS (supplementary material besides the Table S1).

Please see the new Table S1, Fig. S1 and Fig S2 outlining this information.

- 1.193-194: why O₂ concentrations were not measured continuously inside the chamber during each incubation and only at the beginning and at the end of it? With regards to the laboratory experience testing the assumption concentrations change linearly with time, why could authors not test it in situ?

We wanted to measure O₂, TA and pH (DIC) concurrently from the same sample to get the most robust PQ and RQ. The reason we did not also use continuous loggers was due to logistical constraints and limits of our budget. Since we had six replicate chambers incubating simultaneously in the field we did not have sufficient equipment to continuously monitor DO concentrations in all of them. We therefore used discrete measurements and verified the linear response to time ex situ. We have since repeated incubations in another field study where we have deployed oxygen loggers (miniDOT optodes) within the chambers in situ and those data support the linear concentration changes we observed in the laboratory incubations (unpublished data).

- 1.207: the 2.3.4 Chlorophyll a subsection could be displaced right after the 2.3.1 Macrophytes one as we wonder here if microalgae (microphytobenthos) have been measured as well along with macroalgae at each station.

This is a good point and we have moved the chlorophyll a subsection up to subsection 2.3.1 and renamed this “2.3.1 Macrophytes and microphytobenthos”

- 1.227: how authors are sure the OM versus POC linear relationship they obtained for the top 0-2 cm sediment layer in the 12 samples is the same or is well suited for the other core slices? Is there no variability according to sediment depth for sure?

Indeed, we cannot know that this relationship is consistent across all core slices. We have added a sentence to clarify this: “This conversion is based on the assumption that the relationship persists with sediment depth and this introduces uncertainty in the POC values at depth.”

- 1.246: why authors used this flux formulation instead of the one taking into account surface and volume chambers and continuous O₂ concentration measurements? (see previous comment above)

Because we have in situ temperature, salinity and pressure for each sample, we can calculate seawater density (ρ) using the equation of state, which is included in the seacarb package. We can then obtain gravimetric oxygen concentrations such that:

$$O_2 (\mu\text{mol kg}^{-1}) = O_2 (\mu\text{mol L}^{-1}) / \rho (\text{kg L}^{-1})$$

With gravimetric concentrations, using seawater density and height ($\rho \cdot h$) in the flux calculation is equal to using volume divided by area (V/A) when using volumetric concentration units (e.g. mmol L^{-1}):

$$\frac{O_2 (\text{volumetric})}{dt} * \frac{Volume}{Area} = \frac{O_2 (\text{gravimetric})}{dt} * \rho * Height$$

Please see our response regarding continuous vs. discrete measurements of O₂ above.

- 1.249: authors computed salinity normalized TA and DIC fluxes, could they give here the range of salinity they measured at each site during the incubations please?

Salinity was constant at each site during incubations and the range between sites is given in section 3.1. We have added a reference to Table S2 next to the salinity-normalization equation.

- 1.260: that is why information previously asked in comments above are important to clearly understand what was in situ done in the study.

We agree and hope we have clarified this as per above.

- 1.315-326: Statistical (linear mixed-effects) models used to test the assumptions of similar or non-significant differences in environmental conditions during the 4 stations deployments, measurements are well described here, the presentation of the associated results in the manuscript is another story (see general comment above and other specific comments below). Authors could also better or in a clearer way present in the result section, their statistical tests and results to show if significant differences in environmental parameters (water temperature, turbidity, current, salinity) existed between each site.

We have added turbidity as a fixed factor in the formulation of the linear mixed effects model (Table S5). We have added turbidity data to Fig. S2 and updated results section 3.1 with a sentence referring to Turbidity results: “Turbidity was generally low but increased markedly at the Nat site, following a minor rain event prior to deployment (Fig. S2; Table S2). Yet, differences in turbidity did not have any detectable effects on seabed PAR (Fig. S2; Table S2)”.

As described above, salinity could not be included due to missing values. We hope that model results and interpretations are more clearly described now. We have made a new Table S5 with the model output including fixed and random effects.

- 1.331-332: what about microalgae, was it taken into account here in the carbon budget formulation (sigma algae)?

Microalgae were not considered here. We did not want to extrapolate from chlorophyll *a* content due to the uncertainties associated with this conversion.

3. Results

- 1.342-347: salinity variations from 24.7 to 28.9 are important and could have an (indirect) influence on benthic carbon metabolism at each sampled station. If rain events were minor as author said, could they give explanations on these salinity variations (hydrodynamics?) during this summer week please? Salinity remained constant during each individual deployment, at least between bare and 3 years sites and between 7 years and natural sites according to Fig. S1 (please complete the caption, insufficient information about colors, year, idem Fig. S2), however it is not clear, are there significant variation in salinity values among the four deployments? Please give the same results for all the other abiotic measured parameters (flow velocity, water temperature, turbidity, oxygen concentrations, aquatic PAR,

nutrients, TA, DIC etc.) summarized in a table to clearly rule these important considerations according to the author chrono-sequence assumption and possible interference with it in the associated results and discussion. In which sense have flow velocities varied (0,9-21 cm s⁻¹)? Yet, with these important flow velocity variations, I doubt flow velocity didn't influence at all measured benthic fluxes and did not partly explain associated flux differences observed between sampled stations besides (meadow development) habitats?

Regarding the effect of differing salinity, we agree that e.g. precipitation, submarine groundwater discharge can indeed influence carbon metabolism, and especially total alkalinity (TA). However, comparing our salinity normalized TA flux with non-normalized TA flux equals a mean offset of only 0.7±1.9 mmol m⁻² hr⁻¹. Furthermore, we do not observe any relationship between salinity and O₂ or DIC fluxes across our benthic chamber incubations. To clarify how abiotic conditions varied, we have made a new Table S2 which includes the mean±sd of PAR, temperature, flow velocity, DO, salinity and turbidity measured during EC deployments. We have also updated the captions to Figure S1 and S2 (now Fig. S2 and S3) and added the incubations to Fig. S2. Regarding nutrients, TA and DIC we only have data on these from the incubations, and the ambient concentrations at the onset of each incubation is listed in Table S3.

Flow velocity did indeed correlate positively with oxygen flux across all sites. However, flow velocity explained 20% or less of the variance, and there was no linear relationship in Bare (R²=0.01; p=0.22) and 7 yr (R²<0.001; p=0.98). Because of the contrasting relationships with oxygen flux we cannot constrain the effect it may have had on between-site differences in metabolism.

- 1.348-351: here again, authors have to be clearer, did they measure significant differences in salinity, TA, DIC and DIN between the four sites and between which sites, or not? Please better do the link between these parameter variations and the hydrodynamic of the site. Values given 1.351 do not correspond to Table S1 for bare sediment?

DO values on 1.351 correspond to mean ambient DO as measured during EC deployments whereas Table S3 (previously Table S1) are starting conditions of the incubations only (which lasted 3 hours out of the 48 hours of EC deployment). We have clarified this in the sentence and refer to the new Table S2, which includes mean±sd of abiotic conditions including post hoc tests of differences between sites from one-way ANOVAs.

- 1.362-369: authors clearly computed significant relationships between benthic fluxes and flow velocities, moreover their linear mixed effect models indicated PAR and flow velocity explain a large portion of the variation in O₂ fluxes, along with other parameters not included in the model. All these considerations and results should be addressed in a clearer way and discussed after in the discussion section. Is it possible to include in the model parameters such as salinity, temperature for instance?

We agree with the suggestions and realize that the manuscript would benefit from increased clarity on these aspects.

Indeed, there is a significant positive relationship between flow and abs(O₂_flux) across the entire study, but site-specific relationships range from non-existent to significantly positive (see above). We anticipate PAR to explain a large part of daytime O₂ fluxes in autotrophic environments as a primary driver of photosynthesis and this we discuss in relation to our P-I

curves. Importantly, there were no between-site differences in neither daily integrated PAR nor the mean PAR during deployments (Fig. 5, S2). Regarding the phrasing that flow and PAR explain a large proportion of the variation in O₂ fluxes this is in relation to the other abiotic variables in the model (i.e. Temp and now also turbidity). It still does not explain a lot of the total variation in O₂ fluxes, only 20% as mentioned above. We updated the text to clarify this:

“Consequently, site R² values were low ranging from <0.001 – 0.20 (Fig. S3). Further analysis through linear mixed effects modelling indicated that while temperature, PAR, turbidity and flow velocity explained 20% of the variation in hourly |F_{O₂}| across all sites, the random effect Site was highly significant (LRT = 20.9, p < 0.001) suggesting that some other feature, not included in the model, contributed to the observed differences in oxygen fluxes between sites (Table S5).”

Regarding the last part, we have updated the model to also include turbidity and created a new table S5 with the model formulation and its results. As for salinity, we unfortunately do not have continuous salinity measurements at all sites during EC deployments due to a logger malfunction and we can therefore not include it in the model of hourly O₂ fluxes. Nevertheless, salinity was highest in Nat and 3 yr and lowest in 7yr and Bare which is not consistent with any trend in daily metabolism for those sites.

- 1.443 and Fig. 4: only fauna in the sediment (infauna) was encountered at the bare site?

Epifauna (using the mesh net frames) was only sampled in the seagrass. We have clarified this in the methods section (2.3.2) and added “n.d.” to the bare site in Fig. 4 b & d.

- 1.453 and 1.459: how authors explain that large within site POC variability?

We could only speculate but such large small-scale spatial variability is not uncommon for POC profiles in eelgrass meadows in the area (e.g. Röhr et al. 2016; Dahl et al. 2020).

- 1.484 and Table 3: interesting modelling tested by authors, have you tried to test these models with other parameters than the meadow age?

Yes, and similar relationships emerged with e.g. macrophyte diversity (as illustrated in Fig. 6). Here we focus on meadow age as we believe it corresponds best to our main hypothesis and aim of the study.

4. Discussion

This section is good and well-written. However, with regards to my general and specific comments, it is important to add a sub-section or clear elements on the limitation of the chrono-sequence approach used here due to environmental condition difference observed during this summer week measurement between the four sampled sites.

We agree with the lack of clarity on these topics and have added a paragraph discussing the limitations of the chronosequence approach with respect to abiotic conditions:

“The chronosequence approach employed in this study utilizes the unique opportunity of assessing contrasting restored seagrass habitats of different ages that exist within a close

distance from each other (Fig. 1). This enables comparisons between near-identical geomorphology, bathymetry, hydrodynamics and seawater characteristics. However, due to logistical limitations we were unable to measure all four sites simultaneously leading to a temporal mismatch of these comparisons. Consequently, this introduces the risk of potential environmental changes between deployments. Importantly, if the change in environmental conditions is conducive to altered benthic metabolism it can influence the comparison along the chronosequence (i.e between sites). The combined effect of abiotic variables, including PAR, flow velocity, seawater temperature and turbidity accounted for 20 % of the variation in O₂ fluxes, as measured by the eddy covariance. Noticeably, PAR reaching the seabed did not differ between sites, despite varying levels of turbidity (Fig. 2; Fig. S2). Salinity was higher in the 3 yr and Nat site compared to 7 yr and Bare (Table S2; Fig. S2). However, due to missing data, we could not evaluate its impacts on oxygen fluxes within the model. However, we found no discernable effects on either oxygen or carbon fluxes during our incubations, suggesting that variability in salinity was not a driving factor of metabolism. Flow velocity peaked in Nat and 7 yr sites but while there was a positive relationship between |F_{O₂}| and flow in Nat and 3 yr site, no such relationship was evident in the 7yr or Bare site (Fig. S3). Nonetheless, we cannot decisively rule out the potential role of varying flow velocities in the observed differences in benthic metabolism between sites.”

- 1.514-515: please specify here, as the link between both contributions from macrophytes diversity and benthic fauna communities to benthic carbon metabolism is hard to dissociate.

This paragraph is merely an opening paragraph to the following discussion and the roles of macrophyte and fauna diversity in carbon metabolism are specifically addressed in section 4.3 and 4.4, respectively.

- 1.516: it could also be interesting to discuss and compare expected results that could arise at other seasons than summer?

Yes indeed. We have added a sentence as the end of section 4.1 to this effect: “Further research should address whether these relationships are consistent across seasons and what role differing macrophyte phenologies play.”

- 1.521-522: values could be given here in the discussion as a reminder.

We agree and have added the average GPP, CR and NCP of the three seagrass sites.

- 1.548-549: the sentence is not clear, please specify; if both GPP and CR increased, it does not always imply an autotrophic diversity increase?

Yes, since we are discussing the absolute value (|CR|) the correlation is indeed positive for both. We have updated the text to clarify this: “Irrespective of traditional seagrass metrics such as seagrass shoot density and biomass, GPP and |CR| consistently increased in magnitude with meadow age which in turn corresponded to higher autotrophic diversity and macroalgal biomass.”

- 1.578-580: please specify these other biogeochemical processes or delete this sentence since as authors said it is speculative at this stage.

We have removed this sentence.

- 1.585-588: please specify.

We have specified the average (\pm sd) incubation time of 3.0 ± 0.1 hours but unfortunately, we do not have exact data on acclimation times for each chamber. We started incubations 30 minutes after deployment and the order of chambers was random to avoid making acclimation times a potential systematic error.

TECHNICAL CORRECTIONS:

- 1.44: delete “crisis” and “the” and replace “crisis” by “crise”

- 1.126-128 and Fig. 1: where are a) and b) - 1.371: the weakest instead of “lowest”

- Fig. 3 caption: delete “of” before oxygen - 1.375: $-21 \text{ mmol m}^{-2} \text{ d}^{-1}$

- 1.452: in “the” natural meadow core instead of “a”

We thank the reviewer for catching these typographical errors and we have corrected them accordingly, except the last comment (1.452) in which the value refers to one core out of three. Here we have changed to “one”.

References:

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M. S. Fonseca, J. S. Fisher, J. C. Zieman and G. W. Thayer, 1982. Influence of the seagrass, *Zoster marina* L, on current flow. *Estuarine Coastal and Shelf Science* 1982 Vol. 15 Issue 4 Pages 351

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