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Comment

## ***Interactive comment on “Dissolved organic carbon release by marine macrophytes” by C. Barrón et al.***

**C. Barrón et al.**

cristina.barron@uca.es

Received and published: 6 August 2012

Reviewer 3: \* General Comments: This manuscript seeks to combine scattered measurements of flux rates of DOC from seagrass beds (in the Phillipines, in Spain, and in Florida) with a literature review of DOC flux rates reported from seagrass beds and macroalgal beds. The ultimate synthetic goal of the authors is to combine this data to derive distributions of DOC flux rates and ultimately scale this data to provide estimates of flux of DOC from these systems worldwide. Generally speaking, this approach fails to derive accurate global estimates of DOC flux. The new data are quite sparse and are synthesized with published rates poorly. The final analysis oversells the conclusions which might be drawn from these data: it is inappropriate to calculate a global mean and variance of areal DOC flux from benthic chamber experiments conducted

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in many different habitats without a cohesive way to propagate error and incorporate biases from differences in experimental design.

Author comment: We agree with the reviewer in that (a) the limitations of the data set available to derive an global estimate need be acknowledged, and (b) error propagation techniques should be used to produce an error estimate in table 1 and 2. We agree that our estimate of global DOC production represents a first order approximation as it is derived from a limited number of estimates. However, we believe that this data set represents an improvement relative to that recently published by Maher and Eyre 2010, which was based on macrophytes from 2 sites in Spain, from Texas and their own data from the Southeastern coast of Australia, while our estimate is based in 66 individual flux estimates and 28 mean estimates from The Philippines, Norway, Five sites in Spain, Portugal, Florida, Greece and also the Texas and Australia. Our aim at calculating a first-order estimate of global flux is to assess if this process maybe globally relevant and, hoping that this will stimulate interest in resolving this process further. The discussion section will now include the text: “This range encompasses the global net DOC flux of 19 Tg Cyr<sup>-1</sup> estimated by Maher and Eyre (2010), based on a more limited data set on net DOC fluxes from eleven seagrass meadows including two sites in Spain, Texas and their own data from southeastern coast of Australia. The estimate provided here is based on 66 individual estimates from 28 mean seagrass meadow estimates from The Philippines, Norway, five sites in Spain, Portugal, Florida, Greece and also from Texas and Australia. Although an improvement relative to earlier estimates, the estimate of the global net DOC flux from seagrass meadows derived here should be considered a first-order estimate, which shows, however, that this process is a globally-relevant component of the carbon budget of the global coastal ocean and should, therefore, receive additional effort to further constrain the global flux.

Reviewer 3: \* Moreover, the authors have no consistent approach to how chambers are measured, doing such things as presenting multiple independent measures over successive days as equivalent independent measures as mean values derived from

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other studies conducted from multiple replicate chambers.

Author comment: We agree with the reviewer that it would have best to have the same experimental design, however in our case sometimes it was impossible to run night experiments due to logistics problems, and also procedures for DOC storage were improved along the years comprising the studies compiled in the manuscript. Differences in accuracy of estimates derived from different procedures for DOC storage are, however, an irrelevant source of variability when considering the range of values encompassed by the different communities summarised here. The synthesis on seagrass metabolic rates reported by Duarte et al. 2010 also comprises estimates derived from different experimental procedures. Restricting the analysis reported here to those derived using the same procedures would weaken, rather than strengthen, the present paper.

Reviewer 3: \* As I recommend below, I suggest that the authors remove from the title, abstract (final lines) and discussion/results (final paragraph) the work seeking to scale their analyses to global flux of DOC from seagrass and macroalgal beds.

Author response: We agree, and acknowledge in the manuscript, that our estimate carries considerable uncertainty and represents a first-order-approximation, and we agree that it should be de-emphasized in the paper. Hence, we have now removed it from the title and abstract. However, removing this estimate altogether from the manuscript would be a disservice, as one estimate of a global net DOC release, that of Maher and Eyre (2010), is already available, and our estimate represents a clear improvement relative to that one. In the same way as the estimate of Maher and Eyre (2010) provided an impetus for our study, we believe that our estimate, improved relative to that of Maher and Eyre (2010), provides additional reassurance that this is a globally-relevant process and may, therefore, provide additional motivation to further constrain this estimate. Moreover, we have improved our first-order estimate of a global flux by using error propagation to better reflect the uncertainty associated with our estimate. By removing the global estimate from the title and abstract we de-emphasize this component

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of the paper already.

Reviewer 3: \* However, the additional meta-analyses done in this paper, including analyzing relationships between temperature and DOC flux, analyses of the distribution of flux rates among studies, and the relationships between DOC flux and GPP/R/NCP are very useful and I believe sufficiently well-analyzed to allow the reader to draw meaningful conclusions. In addition, the comparative analysis of seagrass beds with macroalgal beds is useful because, despite the inaccuracies in the absolute magnitude of DOC, it allows a rough conclusion that macroalgal beds release significantly more net DOC than do seagrass beds, either due to decreased heterotrophic activity (likely in sediments) or to increased productivity and shunting of carbon to exudates. The new data on community DOC flux from various habitats are appropriately collected and a valuable contribution. However, the methods are very sloppy, and unacceptable as currently presented. I have made specific comments below to clarify the time frames of DO change measurement, the raw DOC concentrations, and to reconsider how volume differences in the chambers are accounted for and might have affected changes in DOC. In addition there is limited metadata presented on the locations or status of the seagrass beds analyzed, which is inexcusable and should be corrected (as other reviewers have noted, temperature is not even included in Table 1). Aside from minor technical issues (discussed below) I think that the data are worth publishing, though clearly not groundbreaking.

Author comment: We agree that the analysis from the individual rates are more robust than the global estimate discussed above. See specific reply to each item below.

Reviewer 3: \* Specific Comments and technical corrections: - Please change the title; the current title implies that this paper does a thorough analysis of DOC release by marine macrophytes when it does not.

Author comment: We agree. The title has been changed to “Patterns in dissolved organic carbon release by marine macrophytes” to highlight the component of the results

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presented that the reviewer considered strongest.

Reviewer 3: \* - The final sentence in the abstract should be removed - the authors have provided no reason for readers to believe that these estimates are comprehensive, accurate, or justifiable in any way, and without that numbers like this are dangerous in an abstract because they can be so easily misused.

Author comment: We agree, and have removed the last sentence as requested.

Reviewer 3: \* - No raw DOC concentrations are provided. In this age it would be appropriate to provide tables of raw values so that readers could assess contamination issues and also see the magnitude of DOC releases which were being measured before normalization for volume (problematic in its own right) and surface area of the substrate.

Author comment: We will add a table, as supplementary materials, with the average initial and final DOC concentrations of the benthic chambers we run.

Reviewer 3: I recommend that Unpubl be replaced with "this study" in these tables.

Author comment: We agree. We will use "this study" in table 1 and 2.

Reviewer 3: \* Fig 1 - The histogram bins should be made smaller, perhaps by a factor of 4. This will give a far more informative idea of the variance for the reader.

Author comment: We will change the histogram as requested, and we will make bins every 5 mmol C m<sup>-2</sup> d<sup>-1</sup>.

Reviewer 3: \* Fig 2 - How was this figure produced when so little temperature data is presented in Tables 1 and 2? I recommend eliminating this figure, or at the very least noting clearly here and in the results that only a handful of the studies in Table 1 were used in the analysis (refs 3,5,6,7 only). More importantly, the authors' own additional measurements do not appear to include temperature ancillary data, which eliminates them from the analyses.

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Author comment: We agree, and have removed this figure and provide additional temperature data in table 1, as requested (see also reply to similar comment by reviewer 1).

Reviewer 3: \* Fig 3,4 - lines are both solid? Fig 4 - report exact p-values please in the methods

Author comment: We will change lines in fig 3. As suggested by another reviewer we will remove the regression lines that are not significant in figure 4. We will also report the exact p-values in the method section.

Reviewer 3: \* Fig 5 - This figure should be removed and the results should simply state that there is no significant relationship.

Author comment: As suggested, we will remove Fig 5 as net DOC fluxes were independent of level of shading in *Thalassia testudinum* communities in Homossassa (Florida).

Reviewer 3: \* Fig 6 - Remove minor tick marks from axis - misrepresents the axis as continuous when it is categorical.

Author comment: We will remove the minor ticks from the x-axis.

Reviewer 3: \* 1532 P1 - present the literature review at the end of the methods in a separate section (in other words, separate experimental and review methods)

Author comment: We will separate the literature review from the new data we present in the methods section.

Reviewer 3:\* 1532/7 - How long after cementing were the chambers allowed to acclimate?

Author comment: This sentence will appear now in the method section, "Plastic bags were fitted to the PVC ring 6 hours after the underwater cement was placed."

Reviewer 3:\* 1533/4 - exact times of the incubations are necessary

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Author comment: We will report exactly the duration of the incubations run simulating dark conditions when night incubations were not possible to set up. The sentence will read “Most of the DOC fluxes during dark were collected from incubations spanning the entire night period. However, night incubations could not be set up in some locations and dark DOC fluxes were collected from short-term (less than 6 h) incubations of benthic chambers covered by 5 dark plastic bags to prevent light penetration, run in parallel to clear ones. Incubations under these conditions lasted around 2 to 3 hours in The Philippines, around 4 hours in Ria Formosa, and between 3 to 4 hours in Florida. The only exception was the benthic chamber covered by the dark plastic bags conducted with *Cymodocea nodosa*, which was incubated close to 24 h (Barrón et al., 2004).”

Reviewer 3: \* 1533/10 - what was the thickness (mil) of the plastic on bags?

Auhor comment: We will add this sentence in the method section, “The plastic bags were made from a 115  $\mu\text{m}$  thick transparent laminated plastic”.

Reviewer 3: \* 1533/28 - concentrations depend on volume of chamber (ranged from 5-20L) and amount of seagrass. How would you normalize for this in rates of change, as rates of concentration change will depend on starting concentrations (concentration dependency)? It would be better to calculate flux rates by correcting for volume disparities. How did other studies accomplish this? Can you compare your rates with other studies on this basis?

Author comment: At the beginning of this paragraph we describe: “The volume of each chamber was estimated by injecting 5 ml a phosphate solution (0.25 mol l<sup>-1</sup>), and analysing a water sample for phosphate concentration after mixing at the end of the incubation.” And at the end of this paragraph after describing the injecton of the phosphate solution we will add this sentence: “The volume of the benthic chambers ranged from 5 to 20 l. The volume of each benthic chamber was used to calculate the net DOC fluxes, which were compared with net DOC fluxes reported in the literature. “

Reviewer 3: \* 1534/2-4 - What does this statement "DOC samples retrieved subse-

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quently..." refer to? The samples from the chambers were handled one way as stated in previous sentence, so what does this mean?

Author comment: First DOC samples were stored frozen and after May 2001 we kept them acidified until analyses. Now the sentences reads: "DOC samples collected from March 2001 to May 2001 were kept frozen in acid washed material (glass vials encapsulated with silicone-teflon caps) until analysed. DOC samples collected subsequently were kept acidified with 2 mol L<sup>-1</sup> HCl at room temperature in acid-washed sealed ampoules, a procedure which improved accuracy in DOC determinations".

Reviewer 3: \* 1534/13 - Precision estimates for the instrument? Stability? HTCO TOC systems are notoriously imprecise and require careful and constant calibration and stability assessment.

Author comment: In line 6 we will add this sentence: "DOC samples in the Shimadzu TOC-5000A (Benner and Strom, 1993) were run manually to avoid contamination and everyday a calibration with potassium hydrogen phthalate was done. For each DOC sample 3 to 5 replicate injections were made, with an associated variation coefficient of <2% across all injections.". Also, as stated in the methods section, the accuracy of the estimates was verified using standards. And we have this sentence: "DOC standards provided by Dennis A. Hansell and Wenhao Chen (University of Miami) of 44–45 and 2  $\mu$ M DOC were used to assess the accuracy of the analyses"

Reviewer 3: \* 1534/15 - it is not clear how "hourly" rates were estimated. Over what time frame? Were these instantaneous rates (measured over just a few hours in day and night) or were these diel changes (ie differences from one dawn to the next)? Much more detail must be provided.

Author comment: We will clarify in the method section that for the hourly rates we incubated during the whole night and day. The sentence will read: "Hourly rates of respiration (R) and NCP were estimated from the difference in oxygen concentration change in the chambers during the whole night and day, respectively". Also, we will

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reference at the end of the paragraph the paper Barrón and Duarte (2009) where we provide additional details on the benthic chambers used and the calculations done.

Reviewer 3: \* 1537/11-13 Is this a correlation or a regression? Be clear.

Author comment: In this paragraph we show results of the linear regression between community respiration and net DOC fluxes in seagrass and macroalgal communities shown in Fig 4. We will change the sentence, which will read: “Community respiration and net DOC flux were significantly correlated in seagrass communities. ...”

Reviewer 3: \* 1537 P2. This paragraph is poorly constructed. First off, neither system showed a relationship between shading intensity and flux rates. Say this first. Then move into a presentation of the nuances of the Phillipines experiments: 1) net DOC source under all irradiances after 2 days (save one), 2) net DOC consumption under all irradiances (save 2?) after 6 days, 3) Wilcoxon test to show fluxes differed between day 2 and 6.

Author comment: As suggested by the reviewer we will reorganize the paragraph. First we will comment that there is no relation between light levels and net DOC release in *Thalassia testudinum* from Homossassa (as suggested by this reviewer this Fig. 5 will be removed). Then we will show the relation between the mixed seagrass meadow in the Philippines and after we will show the details of the effect of light on this mixed seagrass after 2 and 6 days of shading.

Reviewer 3: \* 1538/P1: See the work by Haas et al (2011, PLoS ONE) for accurate estimates of DOC release as a proportion of primary production without using <sup>14</sup>C (ie separate measurements) and incorporate into this discussion. Various of these authors (Esp. Haas, but also Naumann and Wild) have published rates of macroalgal DOM release. Although these are not community release rates they are still informative to contrast. I believe that Haas et al. 2010 (MEPS) also has seagrass release rates.

Author comment: We agree that these are relevant papers. They report experiments

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to estimate dissolved organic carbon release by isolated marine macrophytes without using the  $^{14}\text{C}$  technique. We will now refer to Hass et al. 2010, 2011 in page 1531 line 9. Also we will add in page 1538 at the end of the first paragraph the sentence: “Recently Hass et al. (2010, 2011) reported a net DOC release of  $1.3 \pm 0.5 \text{ mmol C m}^{-2} \text{ h}^{-1}$  and  $0.2 \pm 0.2 \text{ mmol C m}^{-2} \text{ h}^{-1}$  using fragments of seagrass and algal specimens, respectively, incubated in beakers under natural daylight conditions that were heal for 48 hours prior to the incubation period. The corresponding references will be added in the Reference section.

Reviewer 3: \* 1541/24-end - As noted above I patently object to the scaling up to global DOC release rates from macrophyte/macroalgal communities. These are small scale studies and should not be scaled as such beyond a comparative approach (such as that done in the preceeding sentences to compare seagrass beds with macroalgal communities). Certainly it is inappropriate to propose that this DOC is advected as export from coastal habitats or to imply that it is not immediately recycled locally without better evidence. I would remove this statement also from the abstract.

Author comment: We concur that the global estimate should de-emphasized an its uncertainties acknowledge, and have removed this from the title and abstract. However, we still hold it on the results/discussion, as it represents an improvement relative to that reported, on the basis of a more limited data set by Maher & Eyre, 2010, which otherwise would continue to be the sole estimate available in the literature. See additional details in the reply above.

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Interactive comment on Biogeosciences Discuss., 9, 1529, 2012.

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