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Biogeosciences
Discussions

Interactive comment on “Radiocarbon isotopic evidence for assimilation of atmospheric CO$_2$ by the seagrass *Zostera marina*” *by* K. Watanabe and T. Kuwae

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We thank to referees’ critical review and constructive comments regarding our paper. The following list includes the alterations we have made to address your feedback. We believe that the manuscript has been substantially improved following adoption of your valuable suggestions. Please see also supplement file (revised manuscript). Yellow highlights show the revised points. The numbers of page and line are for the new version of manuscript.

Authors’ replies to the comments of Referee #1

1) P2L5: “DIC source” should be “C source for their photosynthesis”? Can Z. marina also use bicarbonate and/or carbonate as photosynthetic substrates?

1) Author’s reply: We have changed the sentence in response to the first part of your comment. (P2L5–6) Also, Z. marina can use bicarbonate as a photosynthetic substrate. We have therefore added the following sentences to the Introduction in response to the second part of your comment: “Under normal seawater pH conditions, the bicarbonate ion (HCO3−) is the most abundant inorganic carbon species, accounting for nearly 90% of the DIC pool (Plummer and Busenberg, 1982; Zeebe and Wolf-Gladrow, 2001). Some seagrass species indirectly use HCO3− under low-CO2(aq) conditions (Beer et al., 2002; Campbell and Fourqurean, 2013)” (P2L13–16); and “Seagrasses rely largely on aqueous CO2 [CO2(aq)] as a carbon source for photosynthesis in nature (Beer and Koch, 1996). Some seagrass species, however, can use bicarbonate ions (HCO3−) as a major carbon source (Beer et al., 2002; Beer and Rehnberg, 1997), although there is considerable interspecific variation in HCO3− utilization (Campbell and Fourqurean, 2013).” (P2L5–9) Moreover, we have added the following sentence to the Results and Discussion: “Z. marina also uses HCO3− as a carbon source under low-CO2(aq) conditions (Beer and Rehnberg, 1997)”. (P10L7–8)

2) P2L25: “ratios” should be inserted after “stable carbon isotope”

2) Author’s reply: We have inserted “ratios” in response to your comment. (P2L25)

3) P3L5: Needs more careful explanations. The difference of δ13C values among chemical species in DIC is important in this context. Chemical species in DIC (relative abundance of CO2 (aq), bicarbonate ion and carbonate ion) are controlled by in situ pH and water temperature. These species typically have distinctive delta 13C values under atmospheric equilibrium. The authors should check the following papers: “Plummer LN, Busenberg E. 1982. The solubilities of calcite, aragonite and vaterite in CO2-H2O solutions between 0 and 90°C, and an evaluation of the aqueous model for the system CaCO3-CO2-H2O. Geochimica et Cosmochimica Acta 46(6):1011–40.”; “Zhang J, Quay PD, Wilbur DO. 1995. Carbon isotope fractionation during gas-water

exchange and dissolution of CO2. Geochimica et Cosmochimica Acta 59(1):107–14."

3) Author's reply: We have added the following sentences in response to your comment: "The chemical species in the carbonate system (CO2(aq), HCO3$-$, and carbonate ion [CO32$-$]) have distinct δ13C values, and isotopic fractionations change depending on pH and temperature (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995). Because the δ13C of HCO3$-$ (0‰ is isotopically distinct from that of both CO2(aq) ($-$9‰ and Cair ($-$8‰ under normal seawater conditions (pH $\approx$ 8), high δ13C ($>-$10‰ in seagrasses shows that they use HCO3$-$ as a carbon source because isotopic discrimination during CO2 assimilation results in δ13C values that are always higher than those of the carbon sources. Although low δ13C ($<-$10‰ in seagrasses could be explained by the assimilation of both 13C-depleted CO2(aq) and Cair, quantification of the contribution of Cair is impossible because of the overlap between their δ13C values." (P3L5–14) In addition to the references suggested, we have also added: Zeebe, R. E. and Wolf-Gladrow, D.: CO2 in seawater: equilibrium, kinetics, and isotopes, in: Elsevier Oceanography Series 65, edited by: Halpern, D., Elsevier, Amsterdam, p.346, 2001.

4) P3L21-22: "As any $\sim$ calculating Δ14C" should follow "because it is internally corrected by δ13C" and cite Stuiver and Polach (1977)

4) Author's reply: We have made the following changes to the sentence in question in response to your comment: "…by internal correction using δ13C values eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977)…" (P3L21–22)

5) P3L21: "Furthermore $\sim$ in ecosystems" Unnecessary sentence in this paper

5) Author's reply: We have removed this sentence in response to your comment.

6) P3L18-19: "The age of DIC" is confusing and not a good choice of words. "The 14C age of DIC" is more appropriate, but still unclear. I suggest the authors revise this sentence as "The Δ14C value of DIC generally differs from that of atmospheric CO2..."

6) Author's reply: We have changed this sentence as follows in response to your comment: "The Δ14C of DIC generally differs from that of atmospheric CO2". (P3L18–19)

7) P3L19-21: "long residence time in the ocean" should be replaced with "longer residence time of C in the aquatic environment than *** (reference)"

7) Author's reply: We have changed this sentence as follows in response to your comment: "…because of the longer residence time of carbon in aquatic ecosystems than in the atmosphere (Ishikawa et al., 2014; Stuiver and Braziunas, 1993)." (P3L19–21)

8) P3L24-25: "quantitative evidence of the assimilation of modern Cair by the seagrass, Zostera marina, by analyzing the Δ14C values" should be revised as "quantitative evidence that the seagrass Zostera marina assimilates modern Cair, based on the Δ14C values"

8) Author's reply: We have changed this sentence in response to your comment. (P3L24–25)

9) P4L16-17: "screw-cap glass culture bottles" Was the hermeticity of the bottles ensured?

9) Author's reply: We have changed the sentence as follows: "…500-mL hermetically-sealed glass bottles (Duran bottle; SCHOTT AG, Mainz, Germany)…" (P4L16–17)

10) P5L2-4: Was the surface of Z. marina leaves washed? Did the authors see biofilm covering Z. marina surface? If it is the case, terrestrial organic matter might be attached to the Z. marina surface and provided 14C-enriched C to bulk Z. marina samples. Then, negative relationship between Δ14C of (bulk) Z. marina and salinity can be also explained by the river transportation of terrestrial organic matter. That is, contribution of 14C-enriched terrestrial organic matter may be diluted along freshwater-seawater gradient

10) Author's reply: We washed away both the biofilm and any epiphytes covering the leaves to avoid contamination. We have added the following sentence to the Methods

and Materials section of the revised manuscript: “Both the biofilm and epiphytes covering the leaves were gently removed by hands with powder-free groves and washed off using ultrapure water (Milli-Q water; Millipore, Billerica, MA, USA).” (P5L2–4). In addition, we have added the following sentence to the Results and Discussion section in response to your comment regarding the negative relationship between Δ14C of Z. marina and salinity: “Furthermore, the negative relationship between salinity and Δ14Cseagrass cannot be explained by any residual contamination from terrestrial organic carbon on the leaves because the terrestrial POC was 14C-depleted (mean Δ14C of terrestrial POC, −74.7 ± 23.4‰.” (P8L15–18). We have also added sentences related to the POC sampling procedure to the Material and methods (P5L8–11).

11) P5L7: “plant” should be inserted between “the samples”

11) Author’s reply: We have changed this sentence in response to your comment. (P5L7)

12) P5L14: “ratios” instead of “ratio” and “concentrations” instead of “concentration”

12) Author’s reply: We have changed this sentence in response to your comment. (P5L14)

13) P7L12: I did not understand why and how the authors used GLM. Why was the objective variable the difference between the Δ14C values of the seagrass leaves and those of DIC? Why wasn’t single regression used for each of DIC and seagrass independently?

13) Author’s reply: We used a generalized linear model (GLM) rather than separate regressions for DIC and seagrass because we wanted to examine the difference between Δ14C values in the seagrass leaves and DIC. We have added the following sentences: “These differences provide evidence that the seagrasses assimilate Cair. A GLM was suitable for this study because both continuous (salinity) and categorical variables (seagrass leaves or DIC) were used as explanatory variables.” (P7L13–16)

In Table 1, the significance ($P < 0.001$) of “Type” (seagrass) indicates that the Δ14C value of the seagrass leaves was significantly higher than that of DIC, showing there is a contribution from Cair as a carbon source for the seagrass. For clarification, we have added model equations to Fig. 2a: Δ14CDIC = $-1.78 \times$ Salinity + 4.40, and Δ14Cseagrass = $-1.78 \times$ Salinity + (4.40 + 7.34).

14) P5L13: Were δ13C values of plants measured by EAIRMS whereas δ13C values of DIC measured by AMS? If so, provide a brief note that typical AMS is not optimized for δ13C measurements. A great care should be taken to compare δ13C values determined by EAIRMS and AMS. At least, the authors can check the difference between δ13C values of plants measured by EAIRMS and those by AMS. How much different were they?

14) Author’s reply: The analytical precision of AMS was within 0.7‰ for δ13C and the precision of IRMS was 0.2‰ for δ13C. Thus, IRMS is more suitable for δ13C measurements than AMS. However, as you point out, we must acknowledge that Δ14C was calculated from δ13C measured with IRMS, because different individual subsamples were used for each analysis in this study (e.g., the differences between δ13C by AMS and IRMS ranged from 0.1‰ to 3‰. In this version of the manuscript, we recalculated Δ14C from δ13C measured with AMS to avoid errors caused by using different individual subsamples. We have changed the relevant sentences in the Materials and Methods (P5L14–P6L9), the isotopic signatures (Δ14C and δ13C of seagrass), and the carbon-source mixing model results. Our recalculation shows that the contribution of Cair (mean, 17%) is lower than that in the previous version of the manuscript (22%) but the general conclusion (i.e., that Z. marina significantly assimilates Cair) is robust (P8L21–24).

15) P7L21: “at each of four stations” should be inserted before “as follows”?

15) Author’s reply: We have revised this sentence in response to your comment. (P7L21)

16) P8L3-4: The sentence “Δ14C DIC was calculated from the best GLM (Table 1).” should be revised as “As DIC and Z. marina were not collected from the same stations, the Δ14C DIC value as a C source of Z. marina in each station was estimated from the best GLM (Table 1).”

16) Author’s reply: We have added the following sentences: “Because DIC taken up by seagrasses is a mixture of DIC from two sources (terrestrial and oceanic) each having distinct Δ14C values, it is reasonable to use salinity as a proxy for the extent of mixing of these two sources as well as for the salinity gradient-based comparison between Δ14C of DIC and seagrass (Table 1). This comparison was therefore possible even though DIC and Z. marina samples were not necessarily collected from the same stations (Fig. 1).” (P7L7–12) We have also changed the following sentence in response to your comment: “The Δ14C values of DIC as the carbon source for Z. marina in the mixing model were estimated from the best GLM (Table 1).” (P8L3–4)

17) P8L19: Insert space between “theΔ14C”

17) Author’s reply: We have modified this sentence in response to your comment. (P8L19)

18) P8L24-P9L4: “As mean $\sim$ sampling sites” Unclear sentence

18) Author’s reply: We have changed this sentence as follows in response to your comment: “The contribution of Cair as a carbon source varied greatly even between samples from the same station (Fig. 2b). Because we did not determine the exposure time of each shoot in this study, we are unable to quantify any relationship between the contribution of Cair and air exposure time; however, the exposure time could mediate the assimilation of Cair (Clavier et al., 2011).” (P8L24–P9L4)

19) P9L10: “Nevertheless” should be replaced with “In any case”

19) Author’s reply: We have changed this sentence in response to your comment. (P9L10)

20) P10L2: “In particular isotopic fractionation” Cite Stuiver and Polach (1977) here

20) Author’s reply: We have added the appropriate citation in response to your comment. (P10L2)

21) P10L6-20: “As the $\sim$ Cair (-8 ‰” These values are determined by a certain combination of δ13C of atmospheric CO2, pH and water temperature with assumption that DIC equilibrates with atmospheric CO2. If pH and water temperature data are available, relative abundance and isotopic composition of each C species can be estimated. At least provide more detailed explanations with appropriate citations as I suggested in Introduction

21) Author’s reply: We have added Fig. 2d in response to your comment. We have modified the sentences in question as follows: “As the δ13C of HCO3$-$ was isotopically distinct from δ13C of both CO2(aq) and Cair (Fig. 2d) and as Z. marina also uses HCO3$-$ as a carbon source under low-CO2(aq) conditions (Beer and Rehnberg, 1997), the δ13C of the seagrass should change depending on the contribution of HCO3$-$ as a carbon source (Campbell and Fourqurean, 2009; Raven et al., 2002). However, it is not possible to distinguish the contribution of Cair from that of other carbon sources because the δ13C of Cair overlapped those of both HCO3$-$ and CO2(aq) (Fig. 2d). Furthermore, δ13C of both HCO3$-$ and CO2(aq) change through mixing between low-δ13C river water and high-δ13C seawater in brackish areas (Fig. 2d). In any case, there are large uncertainties when using δ13C to quantitatively estimate the contribution of Cair as a carbon source because the isotopic fractionation that occurs in the steps between the carbon source and organic plant compounds changes depending on the photosynthetic rate (Raven et al., 2002). The radiocarbon isotopic approach can avoid the uncertainties derived from both the contribution of HCO3$-$ as a carbon source and isotopic fractionation in carbon assimilation.” (P10L6–20) We have also added the related sentence: “The samples for measuring DIC concentration and TA were collected into 250-mL Duran bottles (SCHOTT AG), which were poisoned with saturated mercuric chloride solution (200 μL per bottle).” (P4L19–21) and a new sub-

section: “2.3 Carbonate system analysis” (P6L11–P7L4) to the Material and Methods.

22) P10L12-14: “seagrass with isotopic signatures” Unclear sentence. Revise

22) Author’s reply: We have changed this sentence as follows in response to your comment: “. . .the δ13C of the seagrass should change depending on the contribution of HCO3$-$ as a carbon source (Campbell and Fourqurean, 2009; Raven et al., 2002).” (P10L8–10)

23) Table 1: What is “Category (seagrass)”? Again, why was GLM used?

23) Author’s reply: “Category (seagrass)” indicates the difference between the intercept of the DIC model and that of the seagrass model. The significance ($P < 0.001$) of “Category (seagrass)” indicates that the Δ14C values of the seagrass leaves were significantly higher than that of DIC. A GLM was used because it can estimate the model equation and examine the significant differences simultaneously.

24) Fig. 2 (a): Provide regression formula for both DIC and seagrass

24) Author’s reply: We have added model equations to Fig. 2a. Δ14CDIC = $-1.78 \times$ Salinity + 4.40; Δ14Cseagrass = $-1.78 \times$ Salinity + (4.40 + 7.34).

25) Fig. 2 (c): Was the relationship between δ13C and salinity significant?

25) Author’s reply: Yes, this and the other relationships were significant. We have added the following sentence: “There were significant correlations between salinity and δ13C of DIC, HCO3$-$, CO2(aq) and the seagrass (Pearson’s correlation coefficient: $P < 0.001$; Fig. 2c, d).” (P10L5–6)

Authors’ replies to the comments of Referee #2

1) P2L6-9: Should acknowledge that HCO3- is also a viable carbon source, along with transport mechanisms associated with its use.

1) Author’s reply: We have added the following sentences in response to your comment: “Under normal seawater pH conditions, the bicarbonate ion (HCO3$-$) is the most abundant inorganic carbon species, accounting for nearly 90% of the DIC pool (Plummer and Busenberg, 1982; Zeebe and Wolf-Gladrow, 2001). Some seagrass species indirectly use HCO3$-$ under low-CO2(aq) conditions (Beer et al., 2002; Campbell and Fourqurean, 2013), using one or both of the following suggested mechanisms: (1) extracellular dehydration of HCO3$-$ into CO2(aq) via membrane-bound enzymes (Beer and Rehnberg 1997); or (2) electrogenic proton (H+) extrusion into an boundary layer on the leaf surface, facilitating HCO3$-$/H+ cotransport (Hellblom et al. 2001).” (P2L13–20)

2) P4L22: Why the difference in sites between the DIC sampling stations and the Z. marina stations?

2) Author’s reply: We have added the following sentence as explanation: “Because DIC taken up by seagrasses is a mixture of DIC from two sources (terrestrial and oceanic) each having distinct Δ14C values, it is reasonable to use salinity as a proxy for the extent of mixing of these two sources as well as for the salinity gradient-based comparison between Δ14C of DIC and seagrass (Table 1). This comparison was therefore possible even though DIC and Z. marina samples were not necessarily collected from the same stations (Fig. 1).” (P7L7–12).

3) P5L1: How was this determined?

3) Author’s reply: We have added the following sentence to explain how biomass was determined: “The aboveground wet-weight biomass of the seagrass, estimated from randomly thrown quadrats (0.0625 m2), ranged from 400 to 4300 g m$-$2.” (P4L23–P5L1)

4) P4L13: State number of independent samples per station both for DIC samples and seagrass biomass.

4) Author’s reply: We have added the following sentences: “At each station, one water

sample was collected. . ." (P4L13) and "Three or four independent samples of seagrass leaves were collected at each station." (P5L1–2)

5) P5L2-4: Any epiphyte loading on the seagrass leaf surface?

5) Author's reply: We washed the leaves of both biofilm and epiphytes to avoid contamination. We have added the following sentence: "Both the biofilm and epiphytes covering the leaves were gently removed by hands with powder-free groves and washed off using ultrapure water (Milli-Q water; Millipore, Billerica, MA, USA)." (P5L2–4).

6) P8L22: Please clarify where 46% Cair contribution comes from? This value seems rather high. While carbon fixation clearly occurs during emersion, prior work has suggested reduced maximal photosynthetic rates during air exposure (Clavier 2011), particularly in cases of desiccation (Leuschner et al 1998). Furthermore, Fig 2b. displays rather high within station variation on the relative contribution of Cair, to what might you attribute such variation?

6) Author's reply: The value of 46% was the maximum from all samples. For clarification, we have shown the range of the Cair contribution (P1L19, P8L22). The estimated values have been modified in this revision of the manuscript as the result of a recalculation (please see our response to comment 14 from Referee #1). We have added the following sentences in response to your comment: "Our high estimate of the Cair contribution (mean, 17%) was unexpected because prior works suggest that photosynthetic rates of seagrasses in intertidal zones decrease during air exposure (Clavier, 2011), particularly in cases of desiccation (Leuschner et al., 1998). However, the leaves of subtidal seagrass are never desiccated because of the presence of the thin film of water, which reduces the negative effects of air exposure (i.e., desiccation)." (P10L24–P11L4) As you point out, there is large variation in the Cair contribution, even between samples from the same station. We believe that this variation results from the variation in exposure time. We have added the following sentences: "The contribution of Cair as a carbon source varied greatly even between samples from the same station (Fig. 2b).

Because we did not determine the exposure time of each shoot in this study, we are unable to quantify any relationship between the contribution of Cair and air exposure time; however, the exposure time could mediate the assimilation of Cair (Clavier et al., 2011)." (P8L24–P9L4)

7) P10L6-10: Citations statements are primarily derived from interspecific distinctions. Given that you're comparing the same species, how might salinity gradients influence resultant δ13C values? What about terrestrially derived sources of isotopically light δ13C from the decomposition of organic matter.

7) Author's reply: As you point out, the decomposition of terrestrially-derived organic carbon affects the positive relationship between salinity and δ13C of seagrass. However, DIC released from the decomposed terrestrial organic matter should be reflected in the δ13C values of bulk DIC. We have added the following sentence regarding the influence of salinity: "Furthermore, δ13C of both HCO3$-$ and CO2(aq) change through mixing between low-δ13C river water and high-δ13C seawater in brackish areas (Fig. 2d)." (P10L12–14)

8) P10L6-10: Without detailed data from laboratory incubation, I find these conclusions difficult to make given the reasons that you have already described in regards to δ13C isotope analysis. There appears to be many relevant citations missing from the references. I suggest the authors incorporate additional studies, and provide a more comprehensive discussion of this topic.

8) Author's reply: We have added Fig. 2d in response to your comment. We have modified the sentences in question as follows and moved them to the Introduction: "Because the δ13C of HCO3$-$ (0‰ is isotopically distinct from that of both CO2(aq) ($-$9‰ and Cair ($-$8‰ under normal seawater conditions (pH $\approx$ 8), high δ13C ($>-$10‰ in seagrasses shows that they use HCO3$-$ as a carbon source because isotopic discrimination during CO2 assimilation results in δ13C values that are always higher than those of the carbon sources. Although low δ13C ($<-$10‰ in seagrasses could be explained by

the assimilation of both 13C-depleted CO2(aq) and Cair, quantification of the contribution of Cair is impossible because of the overlap between their δ13C values." (P3L8–14) We have added the following sentences to the Results and Discussion: "As the δ13C of HCO3$-$ was isotopically distinct from δ13C of both CO2(aq) and Cair (Fig. 2d) and as Z. marina also uses HCO3$-$ as a carbon source under low-CO2(aq) conditions (Beer and Rehnberg, 1997), the δ13C of the seagrass should change depending on the contribution of HCO3$-$ as a carbon source (Campbell and Fourqurean, 2009; Raven et al., 2002). However, it is not possible to distinguish the contribution of Cair from that of other carbon sources because the δ13C of Cair overlapped those of both HCO3$-$ and CO2(aq) (Fig. 2d). Furthermore, δ13C of both HCO3$-$ and CO2(aq) change through mixing between low-δ13C river water and high-δ13C seawater in brackish areas (Fig. 2d)." (P10L6–14)

Authors' replies to the comments of Referee #3

1) P9L10-11: The comments about surface water Δ14C-DIC seem like they could be a fairly significant over-simplification: what about the seasonal role of currents with markedly different Δ14C, e.g. to the south the dynamics of the Oyashio and Tsugaru Warm Current (Kuroshio) can lead to variation in Δ14C-DIC in surface waters that covers the range of values observed in this study. The potential role of seasonal variability in Δ14C-DIC needs to be better explored – for instance could oceanic intrusion perhaps explain the Δ14C variations in seagrass leaves independent of the hypothesized utilization of atmospheric CO2?

1) Author's reply: As you point out, the seasonal change of oceanic context (e.g., currents) could affect the application of our approach. We have added the following sentences to address this issue: "However, the seasonal dynamics of Δ14CDIC could affect the application of this approach because it is only applicable when the Δ14C values for endmembers (seawater DIC, freshwater DIC, and Cair) are distinct (not overlapping) as they were in May and July 2014 during this study. We could not use the Δ14C approach to quantify the Cair contribution in September or November 2014 in Furen

Lagoon because the Δ14CDIC of seawater increased to near Δ14Cair and there was overlap between the two (Fig. 3). The overlapping in the range of values, induced by variations in the Δ14CDIC of seawater, likely caused by the dynamics of the Oyashio (mean Δ14CDIC, -41‰ $Aramakietal.,2001)andtheSoyawarmcurrent($Δ14CDIC $>$ 50‰ $Aramakietal.,2007)(Fig.1).Thevariationin$Δ14CDIC of seawater could also be affected by seasonal stratification via regulation of the upwelling of low-Δ14C bottom water. The applicability of the Δ14C technique to other areas will depend on the Δ14C dynamics of endmembers." (P9L11–22)

We have also added the following two references to the reference list:

Aramaki, T., Watanabe, S., Kuji, T., and Wakatsuchi, M.: The Okhotsk-Pacific seawater exchange in the viewpoint of vertical profiles of radiocarbon around the Bussol' Strait, Geophys. Res. Lett., 28, 3971–3974, 2001.

Aramaki, T., Senjyu, T., Togawa, O., Otosaka, S., Suzuki, T., Kitamura, T., Amano, H., and Volkov, Y. N.: Circulation in the northern Japan Sea studied chiefly with radiocarbon, Radiocarbon, 49, 915–924, 2007.

2) Another major issue in the context of the potential seasonal variation in Δ14C of the DIC in an oceanographic context (as above) is the leaf turnover time: what is the turnover time of the leaf carbon, i.e. what season does the tissue sampling reflect, and does this change spatially into the lagoon?

2) Author's reply: We have added the following sentence in response to your comment: "The Δ14Cseagrass could reflect Δ14CDIC from May to July because Z. marina leaves start to grow in early May at the study site, with the turnover time of leaves being 30–90 days (mean, 60 days; Hosokawa et al., 2009)." (P8L13–15)

3) A location map is needed, showing the sampling sites and the location of the bay in relation to the open ocean etc. All of this is very important for the readers' interpretation, especially given the possible seasonal influence of ocean current dynamics on

Δ14C of the DIC as above.

3) Author's reply: We have added a location map (Fig. 1) and the appropriate text regarding this map and sampling locations (P4L13, P4L22, P5L9).

4) Abstract; P1L19: What does the 46 % refer to if the mean is 22 %?

4) Author's reply: The value of 46% was the maximum for all samples. For clarification, we have included the range of the Cair contribution (P1L19, P8L21–22). The estimated values have been modified as a result of recalculation (please see our response to comment 14 from Referee #1).

5) P2L1: Second "their" seems superfluous.

5) Author's reply: We have removed this word in response to your comment.

6) P2L21: If the diffusion rate of CO2 is lower in water, how does a water layer promote CO2 uptake? A layer of water would seem to reduce uptake by limiting diffusion.

6) Author's reply: We argue that CO2 uptake is promoted under conditions with a thin layer or film of water on the leaf surface during low tide, in contrast to a thick water layer when leaves are submerged during high tide. We have modified the sentence in question as follows for clarification: "During low tide, air-exposed aquatic macrophytes have a thin film of water between the air and their leaves, which promotes the uptake of Cair, in contrast to high tide, when there is a thick water layer inhibiting the uptake of Cair (Ji and Tanaka, 2002)." (P2L21–24)

7) P4L16: The use of "dispensed" here is strange.

7) Author's reply: We have replaced "dispensed" with "collected". (P4L16)

8) P9L10-11: The application of the technique here, and certainly other areas of the Pacific, depends on much more thorough understanding of Δ14C dynamics in response to oceanic forcing.

8) Author's reply: As you point out, the utility of the approach depends on a much more thorough understanding of Δ14C dynamics. We have added the following sentence: "The applicability of the Δ14C technique to other areas will depend on the Δ14C dynamics of endmembers." (P9L21–22)

9) P11L10: It could also be argued that a more thorough oceanographic context is required to adequately interpret tracers like Δ14C in this context.

9) Author's reply: We have added the following sentences in response to your comment: "Other applications may include determining the origin of the DIC source (e.g., terrestrial or oceanic) in deeper seagrass systems. However, adequate determinations will require separation and stability in the endmember values (e.g., in oceanographic contexts and in the dynamics of Δ14C in coastal waters)." (P11L12–15)

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C4535/2015/bgd-12-C4535-2015-supplement.pdf

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