

**Interactive comment on “Contributions of the direct supply of belowground seagrass detritus and trapping of suspended organic matter to the sedimentary organic carbon stock in seagrass meadows” by Toko Tanaya et al.**

**Anonymous Referee #1 Received and published: 5 January 2018**

### **General comments**

Comment #1: The paper was well executed and written and presented novel data on seagrass carbon dynamics. Particularly, this paper fills in a much needed gap on tropical blue carbon ecosystems and the contribution of belowground biomass (esp. sheathes) to carbon stocks, the latter often erroneously overlook or lumped in as the sediment carbon stock. It would be interesting to expand on this study by looking at similar variables at deeper depths so that (a) it is comparable to global studies that look at 30-100 cm depths, and (b) we can understand better the long-term contributions of seagrass and allochthonous OC were living biomass isn't present and detritus has been processed more by microbial remineralization. There are some concerns about the lumping of different vegetation types into a site average, but otherwise these are minor revisions.

Reply #1:

Thank you for your helpful comments. Please see our Reply #12 to your main concern.

### **Specific comments**

#### **Abstract**

Comment #2: Lines 3, 11, 13: What is meant by enrichment? Looking at the next sentences, 'accumulation' may be a more accurate term. Change throughout the manuscript.

Reply #2: Concur.

Change #2: We have changed the term as per your suggestion.

Comment #3: Line 5: 'bodies' is an uncommon term for seagrasses and should be 'plants' or 'biomass' here and throughout the manuscript.

Reply #3: Concur.

Change #3: We have deleted ‘bodies’ or changed it to ‘plants’ or ‘biomass’ as per your suggestion.

Comment #4: It will be helpful to describe what species of seagrass are being studied in the abstract.

Reply #4: Concur.

Change #4: We have added “*Thalassia hemprichii* dominated” before “back-reef” and “*Enhalus acoroides* dominated” before “estuarine sites” (page 2, line 7).

Comment #5: Line 16: no need to hyphenate blue carbon. Change throughout the manuscript as well.

Reply #5: Concur.

Change #5: We have removed the hyphen as per your suggestion.

## **Introduction**

Comment #6: Lines 17-30: Consider Trevathan-Tackett et al. 2017 as a specific review of seagrass recalcitrance and the potential for contributing to OC stocks (doi: 10.3389/fpls.2017.00925); it will also be useful in the first section of the discussion. Also consider new research on provenance of OC in seagrass meadows using eDNA: Reef et al 2017 doi: 10.1002/lno.10499

Reply #6: Concur with Trevathan-Tackett et al. 2017. However, we did not cite Reef et al. 2017 because our focus is not on the detailed provenance of OC but on factors controlling OC.

Change #6: We have added “; Trevathan-Tackett et al., 2017” to page 3 line 17.

## **Methods**

Comment #7: How are you considering leaf detritus in these sediment measurements/calculations? In sections 2.2 it says it’s a part of the dead plant structures but not in the calculations. Is it assumed that 100% of the surface leaf detritus is exported and not buried?

Reply #7: Leaf detritus is included in the OC mass calculation (page 7 line 23 and lines 27–30) but not in the calculation of  $\delta^{13}\text{C}_{\text{sed}}$  (page 8 line 3). We have added the reason for its exclusion from the latter after the explanation of the calculation of  $\delta^{13}\text{C}_{\text{sed}}$ .

Change #7: We have added the following sentences (page 8 line 4): “We did not include leaf detritus in the calculation of  $\delta^{13}\text{C}_{\text{sed}}$  because (1) the leaf fragments were so small that we could not remove epiphytes from them, and (2) their mass was much smaller than that of the sheath and rhizomes and roots, so we considered its contribution to  $\delta^{13}\text{C}_{\text{sed}}$  to be negligible.”

Comment #8: Why is the C<sub>sed</sub> calculation multiplied by 1/3 (eqs. 6 & 8)?

Reply #8: We have multiplied by 1/3 because  $\text{OC}_{\text{f sed}}$  is the averaged OC mass of the three layers (surface, medium, and bottom) of fine sediment.

Change #8: We have added the following sentence (page 7 line 30): “ $\text{OC}_{\text{f sed}}$  is the averaged OC mass of the three layers (surface, medium, and bottom) of fine sediment”.

Comment #9: How do equations 7 and 8 relation to traditional mixing model methods to look at OC providence? Were the end-members (seagrass, POM, algae/coral, terrestrial) taken into account? It seems a waste not to use this stable isotope to quantitatively obtain OC contribution values.

Reply #9: We intentionally did not use the stable isotope mixing model because, in the case examined in the present study, it failed to reliably isolate the contribution of seagrass from those of algae and corals; rather, the strong negative correlations among the inferred values imply that one source is simply being traded off against the other. (see Parnell et al., 2010). We showed that the direct supply of belowground seagrass detritus was a major mechanism of  $\text{OC}_{\text{sed}}$  accumulation at the back-reef site from the contribution of belowground detritus to  $\text{OC}_{\text{dead}}$  and  $\delta^{13}\text{C}_{\text{sed}}$ , and from the relationships among  $\delta^{13}\text{C}_{\text{sed}}$ , biomass,  $\text{OC}_{\text{sed}}$  and  $\text{OC}_{\text{dead}}$  (pages 11 lines 23–30).

#### Reference

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L.: Source partitioning using stable isotopes: coping with too much variation, PLOS ONE, 5, e9672, 2010, doi: 10.1371/journal.pone.0009672.

## Results

Comment #10: Since section 3.2 only has one sentence, I'd suggest adding it to the next OC section.

Reply #10: Concur.

Change #10: We have added the sentence in section 3.2 to the next section and renumbered all sections in the Results.

Comment #11: One suggestion is to make a supplementary table(s) for the statistics. This would make reading the text easier.

Reply #11: We do not concur. We have left the statistics in the main text for the convenience of readers who wish to use the statistics to help them understand the results.

Comment #12: Where are the data on the differences between vegetated, unvegetated and bare OC stocks and fractions? This will be very important in the interpretation of OC<sub>bio</sub> and OC<sub>dead</sub>. This will give better resolution into the differences within and between back-reef and estuary regions.

Reply #12: Concur.

Change #12: As per your suggestion, we have added a figure showing the differences in total OC stock and its components between vegetated and no-vegetation (unvegetated and bare area) points (Fig. AC1). At both sites, OC<sub>bio</sub>, OC<sub>dead</sub>, OC<sub>fsed</sub>, OC<sub>sed</sub>, and OC<sub>total</sub> were significantly higher at points with vegetation than at points without vegetation. At points with vegetation, OC<sub>bio</sub>, OC<sub>dead</sub>, OC<sub>csed</sub> and OC<sub>total</sub> were significantly higher at the estuarine site than at the back-reef site, whereas OC<sub>fsed</sub> and OC<sub>sed</sub> were not different between the sites. Therefore, this revision further supports our conclusion described in the original manuscript (page 12 line 24). Figure AC1 replaces Figure 4 in the revised manuscript (page 27) and the figure caption (page 22 lines 12–14) as well as the relevant results (page 9 lines 2–11), discussion (page 12 line 24) and abstract (page 2 line 8) have been modified accordingly.

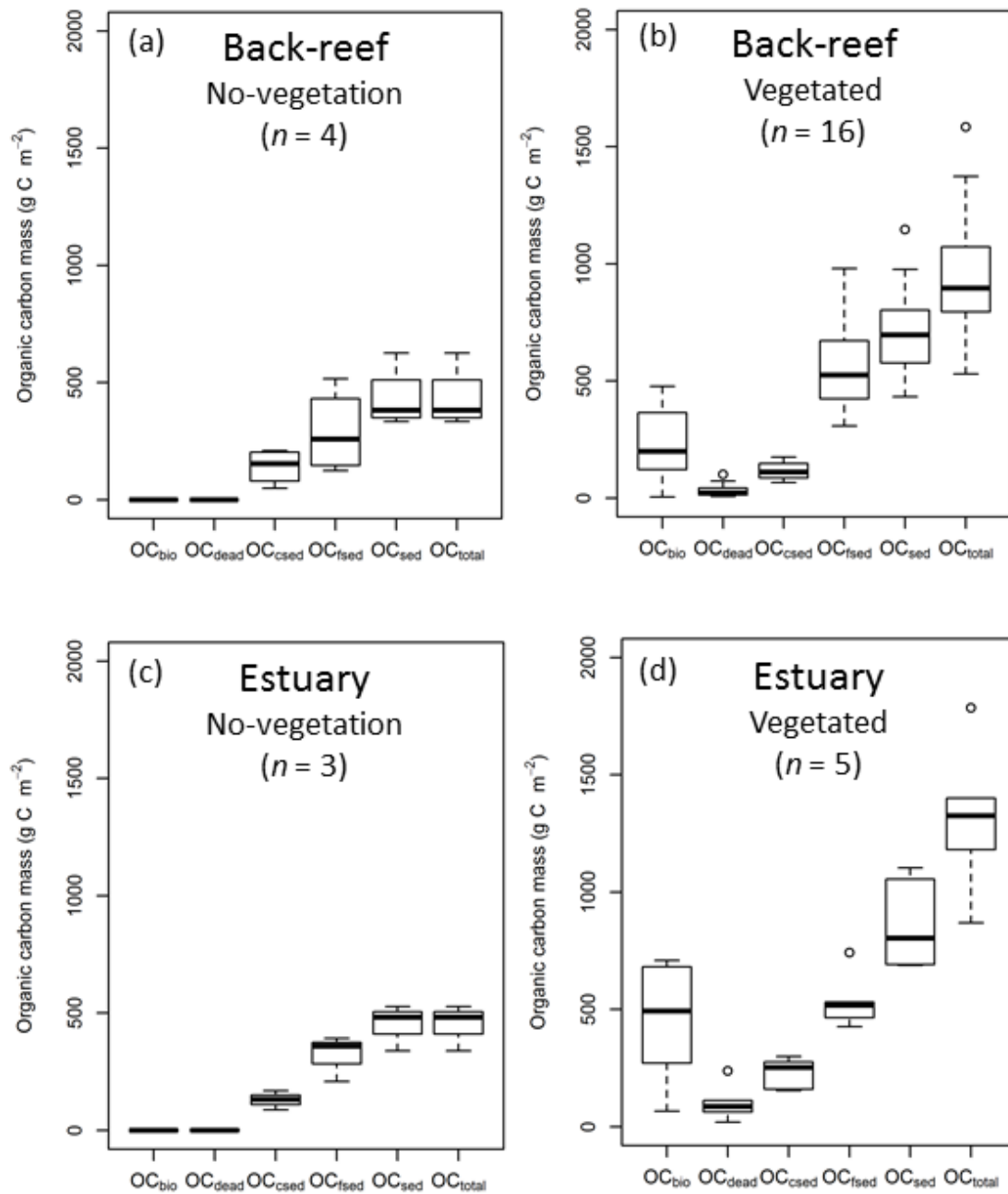


Figure AC1: OC mass (OC<sub>bio</sub>, OC<sub>dead</sub>, OC<sub>csed</sub>, OC<sub>fsed</sub>, OC<sub>sed</sub>, and OC<sub>total</sub>) at (a) no-vegetation (bare and unvegetated) points at the back-reef site, (b) vegetated points at the back-reef site, (c) no-vegetation points at the estuarine site, and (d) vegetated points at the estuarine site. Boxes show the 25% and 75% quantiles; horizontal bands inside the boxes are median values; whiskers show maximum and minimum values; and the open circles are outliers.

Comment #13: What about correlations between living AG:BG?

Reply #13: We concur that the relationship between living AG:BG should be added.

Change #13: We have added a description of the relationship in the manuscript (page 10 line 7): “We also found significant positive correlations between aboveground and belowground biomass ( $F_{1,18} = 94.10, P < 0.001, r^2 = 0.84$ )”. We added the following sentence after “(Fig. 7c).” (page 10 line 12): “We also found significant positive correlations between aboveground and belowground biomass ( $F_{1,6} = 78.40, P < 0.001, r^2 = 0.93$ )”.

### **Discussion**

Comment #14: Page 10, Lines 17-19: NO, we cannot assume constant to 1-m depth. There are important processes that affect OC down core, most notably the reduction on living biomass with depth, change in bulk density and microbial remineralization, so there is absolutely no meaning to the OC<sub>bio</sub> to OC<sub>total</sub> estimate. Please remove this sentence and calculation and find another more robust way to compare the OC<sub>bio</sub> data to previous literature.

Reply #14: We deleted the sentence as per your suggestion. Instead, we compared OC<sub>bio</sub> and OC<sub>total</sub> in this study with the above + belowground seagrass biomass OC and sedimentary OC in the top 0.15-m-thick layer, respectively, reported in a previous study (Fourqurean *et al.*, 2012) (Table AC1).

Change #14: We have deleted the sentence (page 10 lines 17–21): “If we assume... (Fourqurean *et al.*, 2012)”. Instead, we compared data of OC<sub>bio</sub> and OC<sub>total</sub> in the present study with Fourqurean *et al.* (2012)’s data in the top 0.15-m-thick layer. We have added a new table (Table AC1) and the following sentence: “The averaged OC<sub>bio</sub> was significantly higher in this study than that in the previous study by Fourqurean *et al.* (2012) ( $W = 1691, P = 0.006$ ), whereas the averaged OC<sub>sed</sub> was significantly lower in this study than in the previous study at both vegetated and no-vegetation points (vegetation,  $W = 6952, P < 0.001$ ; no-vegetation,  $W = 225, P = 0.036$ ) (Table AC1). Hence, the contribution of OC<sub>bio</sub> to OC<sub>total</sub> at our sites was higher than the global average”. We also changed “the highest in globally compiled data” to “higher than in globally compiled data” in the abstract (page 2 line 8).

Table AC1. Values of seagrass biomass organic carbon and sedimentary organic carbon mass in globally compiled data (Fourqurean *et al.*, 2012) and this study (mean  $\pm$  SD, *n*).

	Vegetated		No-vegetation	
	Seagrass biomass OC (g C m <sup>-2</sup> ) mean $\pm$ SD ( <i>n</i> )	Sedimentary OC (g C L <sup>-1</sup> ) mean $\pm$ SD ( <i>n</i> )	Seagrass biomass OC (g C m <sup>-2</sup> ) mean $\pm$ SD ( <i>n</i> )	Sedimentary OC (g C L <sup>-1</sup> ) mean $\pm$ SD ( <i>n</i> )
Fourqurean <i>et al.</i> , 2012	251.4 $\pm$ 395.6 (251)	12.32 $\pm$ 8.04 (410)	-	8.08 $\pm$ 5.90 (43)
This study	283.0 $\pm$ 200.8 (21)	5.03 $\pm$ 1.32 (21)	-	2.93 $\pm$ 0.73 (7)

Comment #15: Second paragraph: Anoxic sediments that generally reduce decomposition rates also can lead to higher preservation of OC.

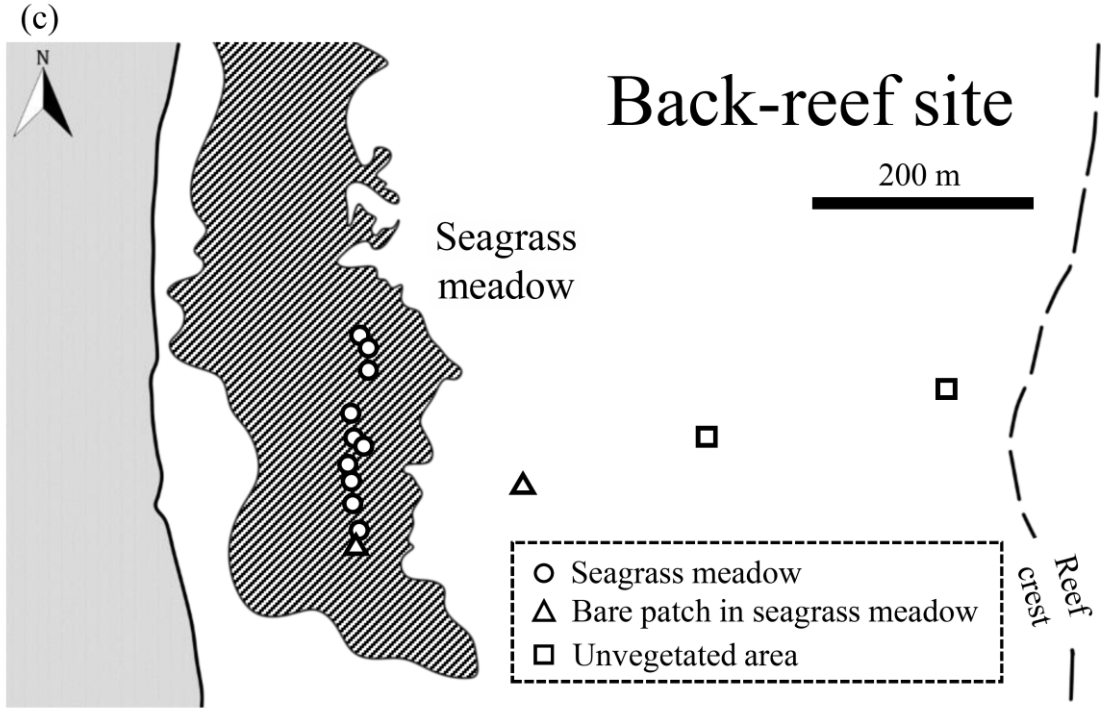
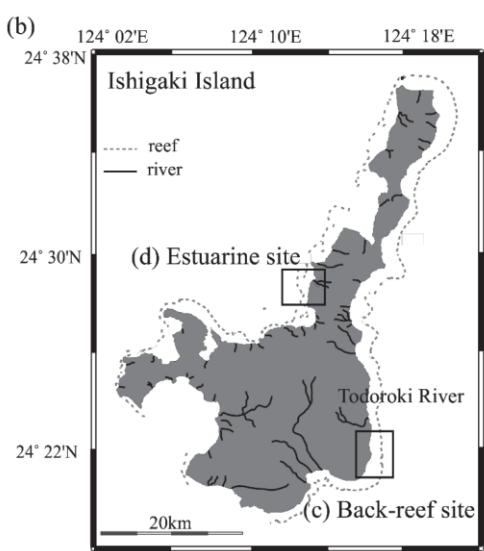
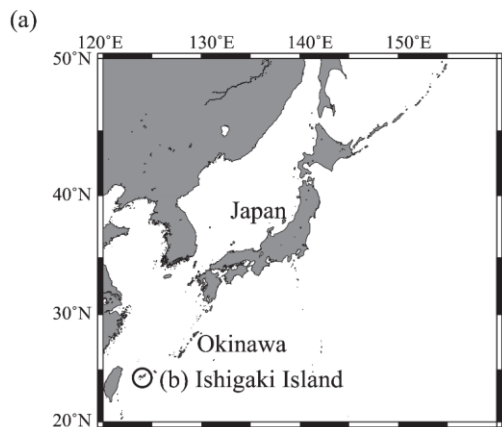
Reply #15: True, but we did not add a statement about this effect to the main text because we were addressing the differences in the characteristics of OC accumulation in sediment between aboveground and belowground seagrass detritus.

### Figures

Comment #16: Figure 1 is low quality and fuzzy and thus hard to read Figure 3: please define the abbreviations in the caption.

Reply #16: Concur.

Change #16: We have replaced Figure 1 with Figure AC2.





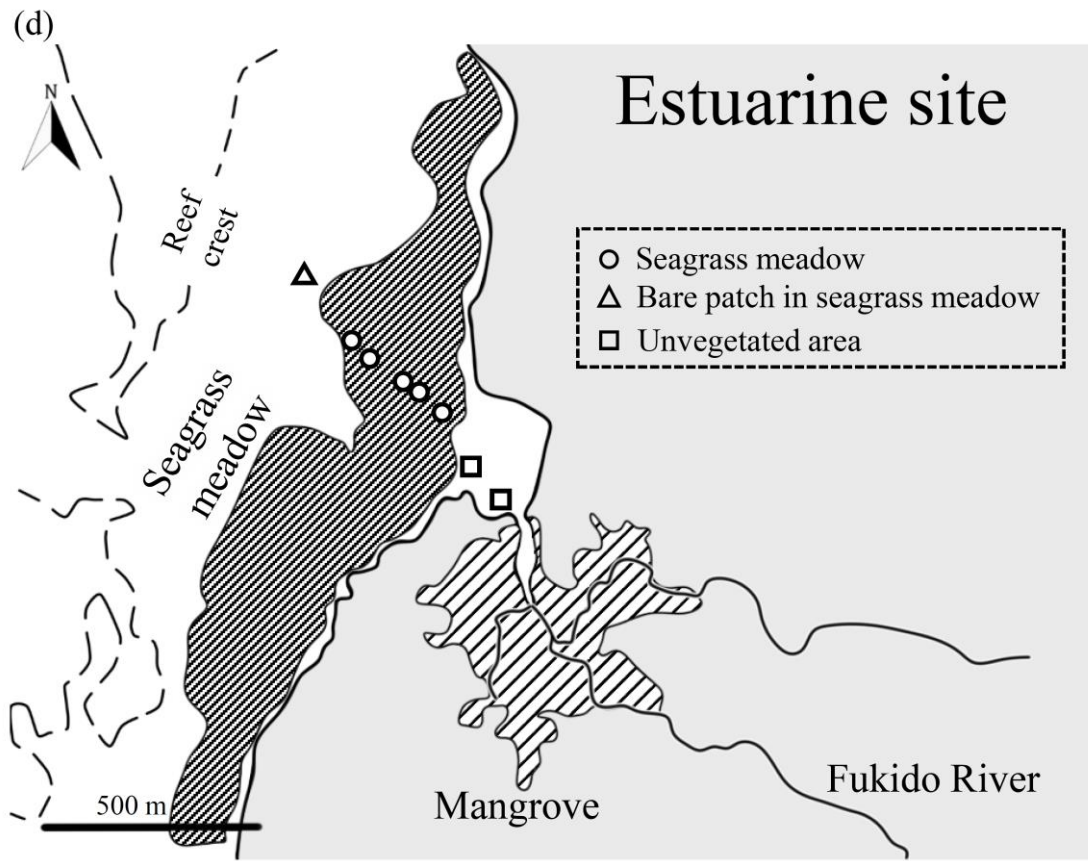


Figure AC2: Study sites. (a) (b) Study site location on Ishigaki Island, Japan. Sampling points at (c) the back-reef and (d) the estuarine site. At the back-reef site, the circle indicating the southernmost vegetated sampling point actually represents a cluster of six sampling points.

**Short comment by Rozaimi, M. and Hamdan N. H.**

School of Environmental and Natural Resource Sciences, Universiti Kebangsaan Malaysia,  
UKM Bangi, Selangor 43600, Malaysia

**General comments**

Comment #1: The study by Tanaya et al. reports findings in the context of blue-carbon science, specifically as a representation for the Indo-Pacific region. The authors demonstrated meticulous planning for the study and the manuscript is generally well-written. Our group is similarly involved in blue carbon studies and we draw some corollary between this study and our findings. In addition, we suggest some recommendations that may improve the authors' present and future outlook in this field. One of the highlights of this study is the argument on the contribution of biomass derived organic carbon (OC bio) to the organic carbon pool (OC total) as the highest globally (P2L8). The data is presented in percentages (i.e. 19% OC bio and 81% OC sed) rather than the actual organic carbon stocks. It may be apt to complement such comparisons with actual global stock values (in equivalent measures as grams C per meter squared or megagrams C per hectare).

Reply #1: Concur.

Change #1: We have revised the data presentation to include a comparison of OC mass in this study with that of a previous study (Fourqurean et al., 2012) as per your suggestion. We have removed the sentence (page 10 lines 17–21): “If we assume... (Fourqurean *et al.*, 2012)”. Instead, we have added a new table (Table AC1) and the following sentence: “The averaged OC<sub>bio</sub> was significantly higher in this study than that in the previous study by Fourqurean *et al.* (2012) ( $W = 1691$ ,  $P = 0.006$ ), whereas the averaged OC<sub>sed</sub> was significantly lower in this study than in the previous study at both vegetated and no-vegetation points (vegetation,  $W = 6952$ ,  $P < 0.001$ ; no-vegetation,  $W = 225$ ,  $P = 0.036$ ) (Table AC1). Hence, the contribution of OC<sub>bio</sub> to OC<sub>total</sub> at our sites was higher than the global average”. We have revised the phrase in the abstract (page 2 line 8) by replacing “the highest in globally compiled data” with “higher than globally compiled data”.

Table AC1. Values of seagrass biomass organic carbon and sedimentary organic carbon mass in globally compiled data (Fourqurean *et al.*, 2012) and this study (mean  $\pm$  SD, *n*).

	Vegetated		No-vegetation	
	Seagrass biomass OC (gC m <sup>-2</sup> ) mean $\pm$ SD ( <i>n</i> )	Sedimentary OC (gC L <sup>-1</sup> ) mean $\pm$ SD ( <i>n</i> )	Seagrass biomass OC (gC m <sup>-2</sup> ) mean $\pm$ SD ( <i>n</i> )	Sedimentary OC (gC L <sup>-1</sup> ) mean $\pm$ SD ( <i>n</i> )
Fourqurean <i>et al.</i> , 2012	251.4 $\pm$ 395.6 (251)	12.32 $\pm$ 8.04 (410)	-	8.08 $\pm$ 5.90 (43)
This study	283.0 $\pm$ 200.8 (21)	5.03 $\pm$ 1.32 (21)	-	2.93 $\pm$ 0.73 (7)

Comment #2: They then rounded off their study by stating below-ground biomass is a driver for sediment OC storage (P2L14-15). It may hold true for this specific study, which is represented by findings from two sites. The authors rightly indicated past studies (e.g. Kennedy *et al.*, 2004; 2010 and Howard *et al.* 2017 – P11L14) showed no relationships between seagrass biomass and sediment OC stocks. This is consistent with our recent study as well (Rozaimi *et al.* 2017). However, our other studies suggest otherwise whereupon biomass is indeed important in driving sediment OC stocks (Serrano *et al.* 2016; Rozaimi *et al.* 2013). Tanaya *et al.* provided possible explanations on why they had different outcomes (P11) but alternatively, it may be plausible that their study sites may simply have exceptional sediment OC storage characteristics compared to other Indo-pacific seagrass meadows.

Reply #2: Concur.

Change #2: We have added the following sentence (page 11 line 18): “although we could not exclude the possibility that our sites may have specific sedimentary OC storage characteristics different from those of other Indo-Pacific seagrass meadows”. We added the relevant literature (Rozaimi *et al.* 2017) after “Howard *et al.*, 2017” (page 11 line 14).

Comment #3: Further to the above, it has to be clearly noted this study reports findings from surficial sediments (up to 16 cm depth: P5L5). This depth is within the range of vertical rhizomal growths for Indo-pacific seagrass rhizomes (especially *T. hemprichii*). So clearly autochthonous inputs play an important role in retaining seagrass-derived OC within this depth layer. However, the context of the authors’ findings within 15 cm sediment depths up-scaled to

1 m, on the assumption that sediment OC density is constant (P10L18) may be too broad an assumption. In our published results (Rozaimi et al. 2017), we found variability in surficial downcore OC content (up to 30 cm sediment depth, albeit as %OC) as well as changing  $\delta^{13}\text{C}$  sediment signatures with increasing sediment depth. In other studies (Rozaimi et al. in preparation), we did not find consistency in downcore OC content or OC density in cores up to 1 m. Conventionally, the scaling-up approach is employed (and admittedly we have used scaling-up approaches to model sediment OC stocks up to 1 m) to contextualise findings relative to regional and global estimates as that in Fourqurean et al. (2012). The authors' assumption in this regard may be corroborated if other evidence can be presented to support the notion of past seagrass occurrences in their study site (re: Serrano et al. 2016; Belshe et al. 2017). Or simply, such investigations may be room for improvements in the authors' future work.

Reply #3: Concur.

Change #3: We have deleted the sentence as per your suggestion. Instead, we have added a new table (Table AC1), see Change #1.

Comment #4: On a final note, it is particularly interesting the authors have data (though not apparently analysed as yet) that can be used in stable isotope mixing models. Mixing models have been increasingly used to account for the contributions of seagrass derived-OC to bulk sediment organic pool and could thus offer alternative insights to the authors' findings. We do wonder how the authors' approach in this study hold up compared to approaches such as stable isotope analysis in R (SIAR; e.g Watanabe and Kuwae 2015; Rozaimi et al. 2017) or eDNA approaches (Reef et al 2017). The lack of reference to SIAR, at least, is somewhat peculiar since there are co-authors in this current study, who are familiar with SIAR (i.e. Watanabe and Kuwae 2015). Overall, we view this study as interesting and may well be citable in future blue carbon endeavours.

Reply #4: We intentionally did not use the stable isotope mixing model because, in the case examined in the present study, it failed to reliably isolate the contribution of seagrass from those of algae and corals; rather, the strong negative correlations among the inferred values imply that one source is simply being traded off against the other. (see Parnell et al., 2010). We showed that the direct supply of belowground seagrass detritus was a major mechanism of  $\text{OC}_{\text{sed}}$  accumulation

at the back-reef site from the contribution of belowground detritus to  $OC_{dead}$  and  $\delta^{13}C_{sed}$ , and from the relationships among  $\delta^{13}C_{sed}$ , biomass,  $OC_{sed}$  and  $OC_{dead}$  (pages 11 lines 23–30).

#### Reference

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L.: Source partitioning using stable isotopes: coping with too much variation, PLOS ONE, 5, e9672, 2010, doi: 10.1371/journal.pone.0009672.

#### **General technical comments:**

Comment #5: Seagrass “bodies” is a peculiar term to use

Reply #5: Concur.

Change #5: We have deleted ‘bodies’ or replaced it with ‘plants’ or ‘biomass’.

Comment #6: On the use of “enrichment”: conventionally, communications in this regards may construe the presence of higher quantity of 13-C atoms (i.e. enriched samples) relative to non-enriched samples. In the text, readers may find some confusion on whether the authors refer to 13-C enrichment, or simply linguistic reference to higher amounts of a particular entity.

Reply #6: Concur.

Change #6: We have changed the term as per your suggestion.

Comment #7: P4L14-22: Content more suited in the Introduction section

Reply #7: We do not agree. We did not move these sentences because they are too long and detailed to be included in the introduction.

Comment #8: P11L14: A word missing after OC (perhaps OC stocks?)

Reply #8: Concur.

Change #8: We have modified “OC” to “%OC or OCmass” (page 11 line 14) as per your suggestion.

Comment #9: P21 Table 2: On data entries as 0.00 \_ 0.00: do these data refer to nil values, or data values less than 0.001?

Reply #9: The data entries of 0.00 are values less than 0.01 g cm<sup>-3</sup>.

Change #9: We have changed the units of dry density from “g cm<sup>-3</sup>” to “mg cm<sup>-3</sup>” to avoid having entries of 0.00 (Table AC2).

Table AC2: Organic carbon content,  $\delta^{13}\text{C}$ , and dry density of each sediment and dead plant component at the back-reef and estuarine sites.

	Back reef			Estuary		
	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )
	%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )		%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )	
Fine sediment	0.37 $\pm$ 0.13 (60)	-12.8 $\pm$ 0.8 (60)	893 $\pm$ 303 (60)	0.42 $\pm$ 0.20 (24)	-17.4 $\pm$ 3.6 (24)	760 $\pm$ 294 (24)
Coarse sediment	0.32 $\pm$ 0.13 (20)	-12.8 $\pm$ 1.1 (20)	292 $\pm$ 152 (20)	0.26 $\pm$ 0.08 (8)	-15.9 $\pm$ 1.5 (8)	475 $\pm$ 142 (8)
Dead leaf	24.80 $\pm$ 3.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.05 $\pm$ 0.04 (20)	23.31 $\pm$ 3.86 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	0.03 $\pm$ 0.04 (8)
Dead sheath and rhizome	21.29 $\pm$ 4.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.55 $\pm$ 0.63 (20)	27.52 $\pm$ 1.75 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	1.44 $\pm$ 1.86 (8)
Dead root	19.25 $\pm$ 1.67 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.26 $\pm$ 0.25 (20)	19.94 $\pm$ 5.89 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	0.31 $\pm$ 0.35 (8)

<sup>a</sup>Total of sheath and rhizomes, and root.

<sup>b</sup>At one sampling point (FS1) where the dominant species was different, the values were dead leaf, 25.77%; dead sheath and rhizome, 19.05%; and dead root, 19.21%.

Comment #10: P28 Figure 5: Axis labels are too small

Reply #10: Concur.

Change #10: We have enlarged axis labels of Figure 5 (Figure AC3).

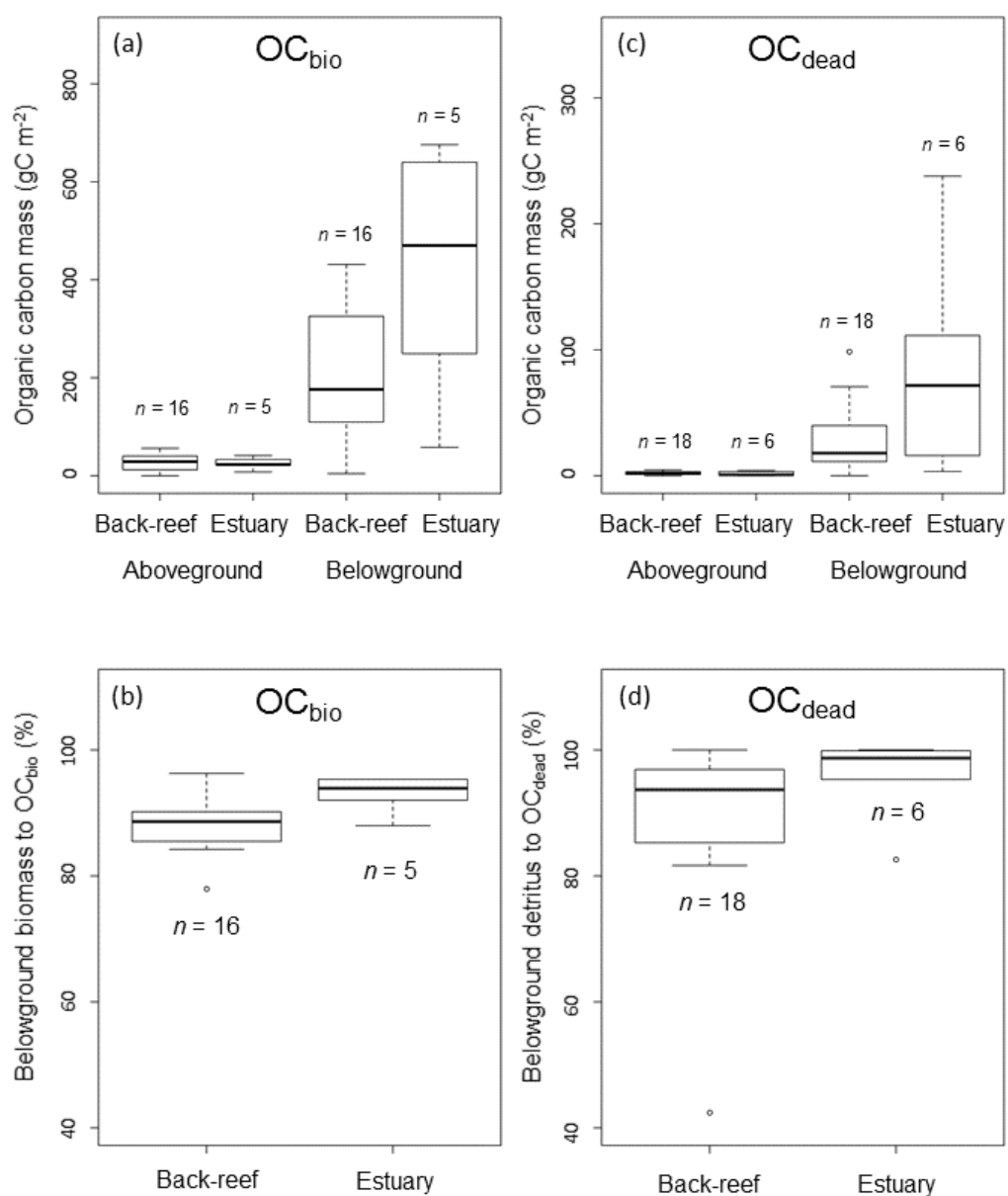


Figure AC3: (a) OC<sub>bio</sub> (sum of aboveground and belowground biomass) (g C m<sup>-2</sup>); (b) contribution of belowground biomass to OC<sub>bio</sub> (%); (c) OC<sub>dead</sub> (sum of above- and belowground detritus) (g C m<sup>-2</sup>); and (d) contribution of belowground detritus to OC<sub>dead</sub> (%). Boxes show the 25% and 75% quantiles; horizontal bands inside the boxes are median values; whiskers show maximum and minimum values; and open circles show outliers.



**Interactive comment on “Contributions of the direct supply of belowground seagrass detritus and trapping of suspended organic matter to the sedimentary organic carbon stock in seagrass meadows” by Toko Tanaya et al.**

**Anonymous Referee #2**

**Received and published: 18 March 2018**

### **General comments**

Comment #1: This study aims to assess the mechanisms constraining organic carbon storage at two sites in Japan colonised by seagrass meadows quantifying the different pools of organic carbon that contribute to sediment organic carbon stock in seagrass sediments (and unvegetated sediments). The study demonstrates that seagrass structure and detritus constrain sediment organic carbon stores at the study sites. The manuscript is well written. However, I have some comments that I list in detail below.

### **Specific comments**

Introduction. Page 3, line 33/Page 4, line 1. It is not clear in this sentence if the authors mean organic carbon or carbonate of calcareous organisms.

Reply #1: We have already clearly explained the meaning in the original manuscript: “OC derived from calcareous organisms” (page 3 line 33 and page 4 line 1).

Comment #2: Introduction. I suggest to re-write the last paragraph of the introduction to highlight the novel aspects of the study.

Reply #2: Concur.

Change #2: We have revised the phrase “the relationship between seagrass and the sedimentary OC stock” to “the pathways of sedimentary OC accumulation in seagrass meadows, especially the direct supply of belowground seagrass detritus” (page 4 line 7), and we added “along a seagrass biomass gradient” at the end of the paragraph (page 4 line 9).

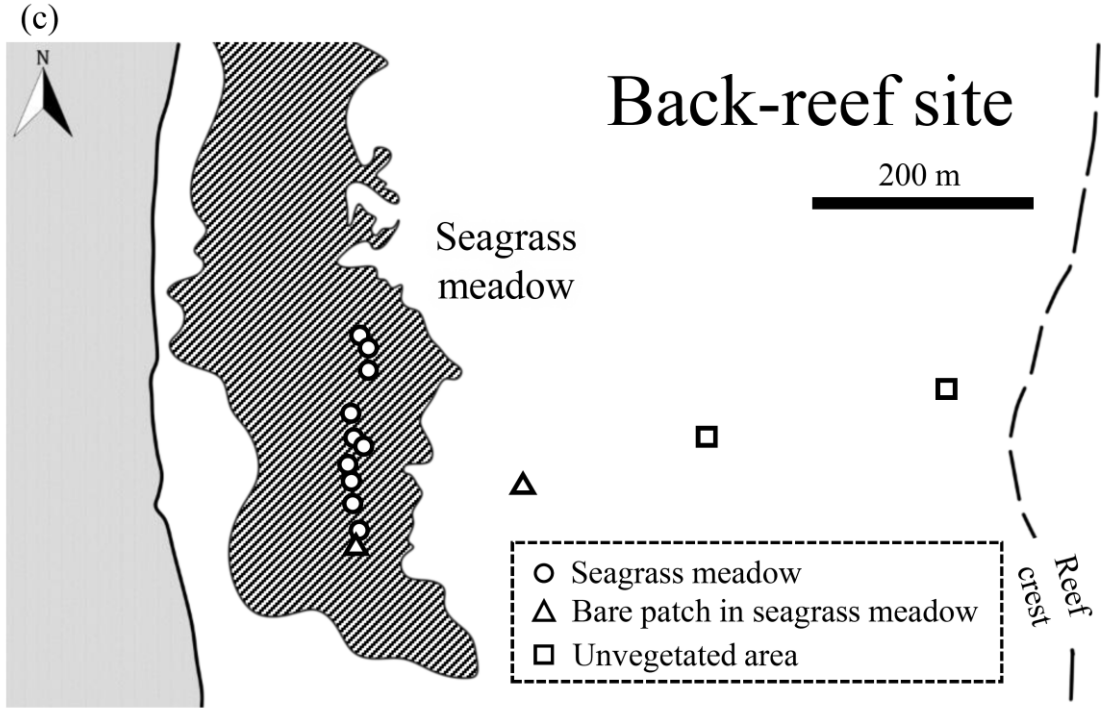
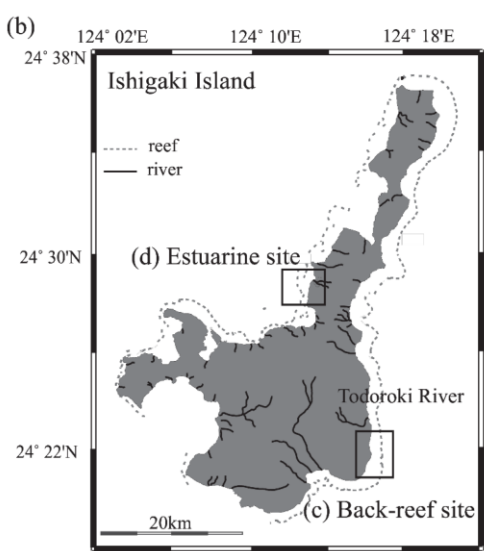
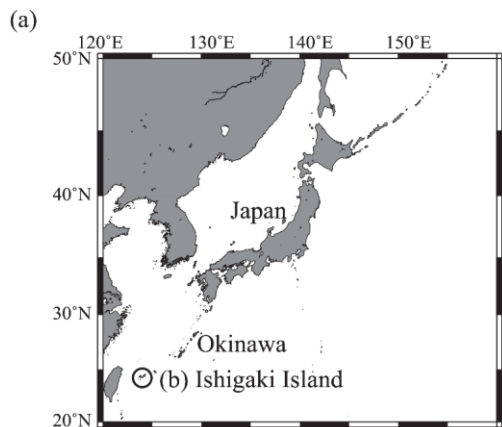
Comment #3: Methods. Study site. The first paragraph could be moved to the introduction.

Reply #3: We do not agree. We did not move the paragraph because the description is too long and detailed to be included in the introduction.

Comment #4: Methods, page 4 last paragraph and Fig. 1. The location of the river mouth of Todoroki River relative to the sampling site is not clearly shown in the figure. This prevents to understand why the terrestrial input in this site is low. Similarly, the location of the small river discharging into the estuary is not clear in the image.

Reply #4: Concur.

Change #4: We have replaced Figure 1 with Figure AC2.



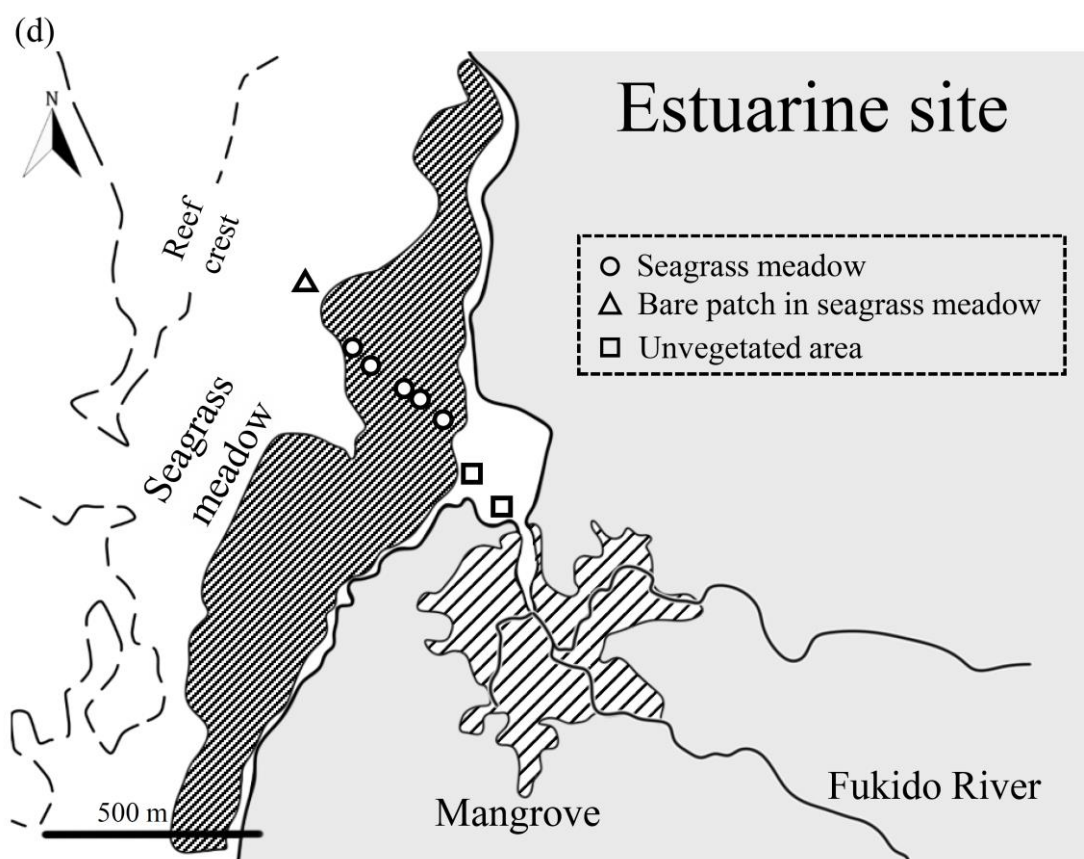


Figure AC2: Study sites. (a) (b) Study site location on Ishigaki Island, Japan. Sampling points at (c) the back-reef and (d) the estuarine site. At the back-reef site, the circle indicating the southernmost vegetated sampling point actually represents a cluster of six sampling points.

Comment #5: Methods. Page 5. It is not clear the type of organic material included in the fraction  $OC_{csed}$ . If it contained the carbonate from skeletons of corals, foraminifera, and other calcareous organisms it should not be considered in the organic carbon pool.

Reply #5: We have already explained that the carbonate was not included in  $OC_{csed}$  in the original manuscript (page 6 line 24).

Change #5: We have added “OC in the” before “coarse (> 1 mm diameter) sediments” (page 5 line 18) for clarity.

Comment #6: Methods. Page 5. Line 24. “We merged dead plant structures attached to live seagrass bodies into  $OC_{bio}$ ”. How much did dead plant structures attached to living biomass

weight? How much was it in comparison to mass of the seagrass dead compartment? Could this affect the OC results across compartments?

Reply #6: We have already explained in the original manuscript that their mass was usually very small (page 5 line 25).

Comment #7: Methods. Page 5, last paragraph. At each site, samples were collected in vegetated, unvegetated patches within the meadows and bare sediment. However the results in the box plots (Figs. 4 and 5) are presented per site, without indicating if they correspond to vegetated, unvegetated patches or bare sediment. I think it would be relevant to present these results indicating if the sediments were vegetated or not.

Reply #7: Concur.

Change #7:

As per your suggestion, we have added a figure showing the differences in total OC stock and its components between vegetated and no-vegetation (unvegetated and bare area) points (Fig. AC1). At both sites,  $OC_{bio}$ ,  $OC_{dead}$ ,  $OC_{fsed}$ ,  $OC_{sed}$ , and  $OC_{total}$  were significantly higher at points with vegetation than at points without vegetation. At points with vegetation,  $OC_{bio}$ ,  $OC_{dead}$ ,  $OC_{csed}$  and  $OC_{total}$  were significantly higher at the estuarine site than at the back-reef site, whereas  $OC_{fsed}$  and  $OC_{sed}$  were not different between the sites. Therefore, this revision further supports our conclusion described in the original manuscript (page 12 line 24). Figure AC1 replaces Figure 4 in the revised manuscript (page 27) and the figure caption (page 22 lines 12–14) as well as the relevant results (page 9 lines 2–11), discussion (page 12 line 24) and abstract (page 2 line 8) have been modified accordingly. We have added an explanation of vegetation in the caption of Figure 5: Figure 5 (a) and (b) show the data of vegetated sampling points and Figure 5 (c) and (d) show the data of vegetated and bare sampling points.

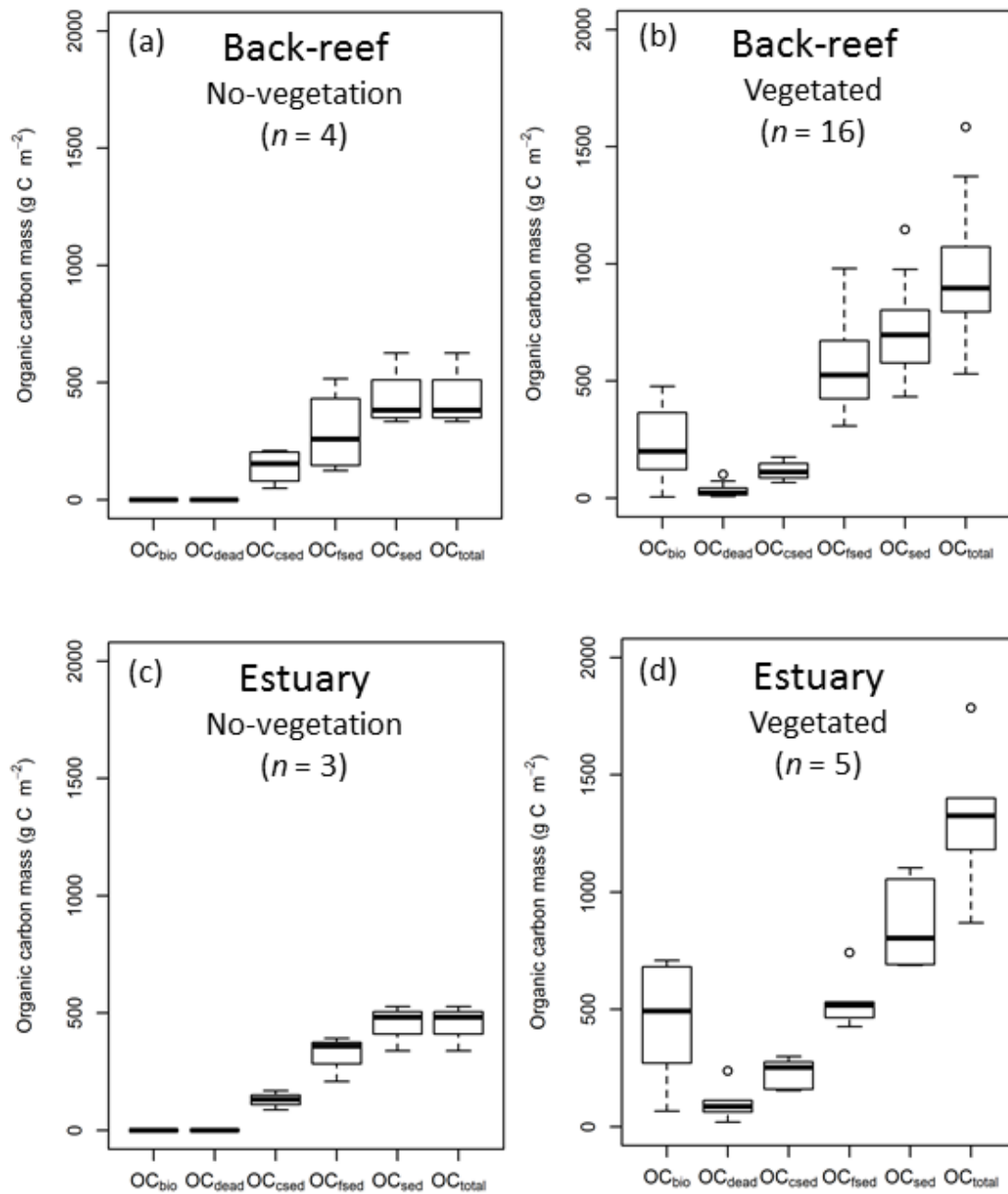


Figure AC1: OC mass ( $\text{OC}_{\text{bio}}$ ,  $\text{OC}_{\text{dead}}$ ,  $\text{OC}_{\text{csed}}$ ,  $\text{OC}_{\text{fsed}}$ ,  $\text{OC}_{\text{sed}}$ , and  $\text{OC}_{\text{total}}$ ) at (a) no-vegetation (bare and unvegetated) points at the back-reef site, (b) vegetated points at the back-reef site, (c) no-vegetation points at the estuarine site, and (d) vegetated points at the estuarine site. Boxes show the 25% and 75% quantiles; horizontal bands inside the boxes are median values; whiskers show maximum and minimum values; and the open circles are outliers.

Comment #8: Table 2. In this table the density of dead plant material is 0.00 \_ 0.00 g cm<sup>-3</sup>. I believe that these components did have some dry density but lower than 0.00 g cm<sup>-3</sup>. In order to be able to provide their dry density, the units could be expressed in mg cm<sup>-3</sup>.

Reply #8: Concur.

Change #8: We have changed the units of dry density from “g cm<sup>-3</sup>” to “mg cm<sup>-3</sup>” to avoid entries of 0.00 (Table AC2).

Table AC2: Organic carbon content,  $\delta^{13}\text{C}$ , and dry density of each of sediment and dead plant component at the back-reef and estuarine sites.

	Back reef			Estuary		
	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )
	%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )		%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )	
Fine sediment	0.37 $\pm$ 0.13 (60)	-12.8 $\pm$ 0.8 (60)	893 $\pm$ 303 (60)	0.42 $\pm$ 0.20 (24)	-17.4 $\pm$ 3.6 (24)	760 $\pm$ 294 (24)
Coarse sediment	0.32 $\pm$ 0.13 (20)	-12.8 $\pm$ 1.1 (20)	292 $\pm$ 152 (20)	0.26 $\pm$ 0.08 (8)	-15.9 $\pm$ 1.5 (8)	475 $\pm$ 142 (8)
Dead leaf	24.80 $\pm$ 3.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.05 $\pm$ 0.04 (20)	23.31 $\pm$ 3.86 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	0.03 $\pm$ 0.04 (8)
Dead sheath and rhizome	21.29 $\pm$ 4.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.55 $\pm$ 0.63 (20)	27.52 $\pm$ 1.75 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	1.44 $\pm$ 1.86 (8)
Dead root	19.25 $\pm$ 1.67 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	0.26 $\pm$ 0.25 (20)	19.94 $\pm$ 5.89 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	0.31 $\pm$ 0.35 (8)

<sup>a</sup>Total of sheath and rhizomes, and root.

<sup>b</sup>At one sampling point (FS1) where the dominant species was different, the values were dead leaf, 25.77%; dead sheath and rhizome, 19.05%; and dead root, 19.21%.



Comment #9: Discussion. How much was the OC sediment stock at the studied seagrass meadows and at the bare sites? How do the OC stocks in the seagrass sediments found in this study compare with global seagrass OC sed stocks?

Reply #9: Concur. We have added these results and a corresponding explanation.

Change #9: We have removed the sentence (page 10 lines 17–21): “If we assume... (Fourqurean *et al.*, 2012)”. Instead, we compared data of OC<sub>bio</sub> and OC<sub>total</sub> in the present study with Fourqurean *et al.* (2012)’s data in the top 0.15-m-thick layer. We have added a new table (Table AC1) and the following sentence: “The averaged OC<sub>bio</sub> was significantly higher in this study than that in the previous study by Fourqurean *et al.* (2012) ( $W = 1691$ ,  $P = 0.006$ ), whereas the averaged OC<sub>sed</sub> was significantly lower in this study than in the previous study at both vegetated and no-vegetation points (vegetation,  $W = 6952$ ,  $P < 0.001$ ; no-vegetation,  $W = 225$ ,  $P = 0.036$ ) (Table AC1). Hence, the contribution of OC<sub>bio</sub> to OC<sub>total</sub> at our sites was higher than the global average”. We also changed “the highest in globally compiled data” to “higher than in globally compiled data” in the abstract (page 2 line 8).

Table AC1. Values of seagrass biomass organic carbon and sedimentary organic carbon mass in globally compiled data (Fourqurean *et al.*, 2012) and this study (mean  $\pm$  SD,  $n$ ).

	Vegetated		No-vegetation	
	Seagrass biomass OC (g C m <sup>-2</sup> )	Sedimentary OC (g C L <sup>-1</sup> )	Seagrass biomass OC (g C m <sup>-2</sup> )	Sedimentary OC (g C L <sup>-1</sup> )
	mean $\pm$ SD ( $n$ )	mean $\pm$ SD ( $n$ )	mean $\pm$ SD ( $n$ )	mean $\pm$ SD ( $n$ )
Fourqurean <i>et al.</i> , 2012	251.4 $\pm$ 395.6 (251)	12.32 $\pm$ 8.04 (410)	-	8.08 $\pm$ 5.90 (43)
This study	283.0 $\pm$ 200.8 (21)	5.03 $\pm$ 1.32 (21)	-	2.93 $\pm$ 0.73 (7)

Comment #10: Discussion. What is the contribution of the different potential OC sources (seagrass, algae, corals, suspended POM and terrestrial POM) to OC in the sediment at both sites (and discriminating between vegetated and bare sediment)? The fraction of the different sources to the compartments of coarse and fine sediment could be estimated using mixing models. These estimates could be incorporated in a revised Fig. 8.

Reply #10:

We intentionally did not use the stable isotope mixing model because, in the case examined in the present study, it failed to reliably isolate the contribution of seagrass from those of algae and corals; rather, the strong negative correlations among the inferred values imply that one source is simply being traded off against the other. (see Parnell *et al.*, 2010). We showed that the direct supply of belowground seagrass detritus was a major mechanism of  $OC_{sed}$  accumulation at the back-reef site from the contribution of belowground detritus to  $OC_{dead}$  and  $\delta^{13}C_{sed}$ , and from the relationships among  $\delta^{13}C_{sed}$ , biomass,  $OC_{sed}$  and  $OC_{dead}$  (pages 11 lines 23–30).

Reference

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L.: Source partitioning using stable isotopes: coping with too much variation, PLOS ONE, 5, e9672, 2010, doi: 10.1371/journal.pone.0009672.

Comment #11: Conclusions. Kennedy et al 2010 and several other papers demonstrate that the contribution of particle trapping and seagrass material to sediment organic carbon widely varies across seagrass meadows, from meadows where allochthonous carbon is the main source to others where the sediment organic carbon pool is dominated by seagrass material. Therefore,

there is evidence in the literature that seagrass carbon can be an important source to sediment organic carbon.

Reply #11: Although previous studies showed the provenance of sedimentary OC, they did not show the pathway of sedimentary OC (page 3 lines 28–30). We empirically showed that not only suspended-particle trapping but also the direct supply of belowground seagrass detritus can be a dominant organic carbon accumulation pathway in seagrass sediments.

### **Minor comments**

Comment #12: Minor comments Abstract- line 7. It should say that the stable carbon isotope ratio was measured in OC sources as well as in OC<sub>sed</sub>.

Reply #12: Concur.

Change #12: We have added “and its potential OC sources” after “( $\delta^{13}\text{C}$ ) of OC<sub>sed</sub>” (page2 line 7)

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# Contributions of the direct supply of belowground seagrass detritus and trapping of suspended organic matter to the sedimentary organic carbon stock in seagrass meadows

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**Abstract.** Carbon captured by marine living organisms is called “blue carbon”, and seagrass meadows are a dominant blue carbon sink. However, our knowledge of how seagrass increases sedimentary organic carbon (OC) stocks is limited. We investigated two pathways of OC ~~enrichment~~accumulation: trapping of organic matter in the water column and the direct supply of belowground seagrass detritus. We developed a new type of box corer to facilitate the retrieval of intact cores that preserve the structures of both sediments (including coarse sediments and dead plant structures) and live seagrass ~~es~~bodies. We measured seagrass density, total OC mass (OC<sub>total</sub>) [= live seagrass OC biomass (OC<sub>bio</sub>) + sedimentary OC mass (OC<sub>sed</sub>)], and the stable carbon isotope ratio ( $\delta^{13}\text{C}$ ) of OC<sub>sed</sub> and its potential OC sources at Thalassia hemprichii dominated back-reef and Enhalus acoroides dominated estuarine sites in the tropical Indo-Pacific region. At points with vegetation, OC<sub>bio</sub> accounted for 2519% and OC<sub>sed</sub> for 7581% of OC<sub>total</sub>; this contribution of OC<sub>bio</sub> to OC<sub>total</sub> is higher than the highest in globally compiled data. Belowground detritus accounted for ~90% of the OC mass of dead plant structures (>2 mm in size) (OC<sub>dead</sub>). At the back-reef site, belowground seagrass biomass, OC<sub>dead</sub>, and  $\delta^{13}\text{C}$  of OC<sub>sed</sub> ( $\delta^{13}\text{C}_{\text{sed}}$ ) were positively correlated with OC<sub>sed</sub>, indicating that the direct supply of belowground seagrass detritus is a major mechanism of OC<sub>sed</sub> ~~enrichment~~accumulation. At the estuarine site, aboveground seagrass biomass was positively correlated with OC<sub>sed</sub> but  $\delta^{13}\text{C}_{\text{sed}}$  did not correlate with OC<sub>sed</sub>, indicating that trapping of suspended OC by seagrass leaves is a major mechanism of OC<sub>sed</sub> ~~enrichment~~accumulation there. We inferred that the relative importance of these two pathways may depend on the supply (productivity) of belowground biomass. Our results indicate that belowground biomass productivity of seagrass meadows, in addition to their aboveground morphological complexity, is an important factor controlling their OC stock. Consideration of this factor will improve global blue -carbon estimates.

## 1 Introduction

The carbon captured by marine living organisms has been termed “blue carbon” (Nelleman et al., 2009). Among marine ecosystems, the organic carbon (OC) accumulation rate of vegetated coastal systems such as seagrass meadows, mangrove forests, and salt marshes is estimated to be higher than that of terrestrial forests (Mcleod et al., 2011). The global total OC stock contained in the top 1 m of sediment and in the plant biomass in these vegetated ecosystems is estimated to be 0.63–8.54 Pg C (Pendleton et al., 2012). Thus, vegetated ecosystems are expected to contribute greatly to the mitigation of global warming. In this regard, seagrass meadows have attracted particular attention because they are one of the most dominant blue-carbon sinks (Kennedy et al., 2010; Fourqurean et al., 2012). However, the OC stock of a seagrass meadow is highly variable, depending on geographical region (Miyajima et al., 2015), seagrass species (Lavery et al., 2013), microlocation within a seagrass patch (Ricart et al., 2015), and the patch scale (Miyajima et al., 2017). Hence, to develop a precise methodology of OC estimation and reduce the uncertainty of the global estimate, it is necessary to understand the factors controlling OC stocks in seagrass meadows (Duarte et al., 2013).

Seagrass meadows enhance the accumulation of sedimentary OC by directly supplying of abundant OC from their high production (Duarte et al., 2010), by reducing sediment resuspension, and by promoting sedimentation of autochthonous and allochthonous OC in the water column (Agawin and Duarte, 2002; Gacia and Duarte, 2001; Gacia et al., 2003; Hendriks et al., 2008). However, our knowledge of the factors that mediate the sequestration of sedimentary OC by seagrass meadows is limited. For example, the chemical recalcitrance of the supplied organic matter ([Trevathan-Tackett et al., 2017](#); Watanabe and Kuwae, 2015) and the specific surface area of the sediment (Miyajima et al., 2017) are factors that control the sedimentary OC stock in seagrass meadows. Recent studies have also shown that, in addition to chemical and physical factors, biological factors such as primary productivity, seagrass shoot density, and the amount of leaf material (as indicated by the leaf area index) also affect the sedimentary OC stock (Samper-Villarreal et al., 2016; Serrano et al., 2014; Serrano et al., 2016b). In addition, an increase in the amount of leaf material may enhance the trapping of suspended OC and, thus, the accumulation of sedimentary OC (Dahl et al., 2016; Gacia et al., 1999). An increase in seagrass density may also cause an increase in seagrass production per unit area and thus enhance the direct supply of seagrass-derived OC. However, few previous studies have analyzed the controlling factors and provenance of sedimentary OC along a seagrass biomass gradient (Kennedy et al., 2004; Kennedy et al., 2010; Samper-Villarreal et al., 2016; Howard et al., 2017). Kennedy et al. (2004, 2010) and Howard et al. (2017) found no significant relationship between seagrass biomass and sedimentary OC, whereas Samper-Villarreal et al. (2016) concluded that autochthonous sedimentary OC increased as the leaf area index increased. However, they did not show the mechanism (pathway) by which seagrass-derived OC became sedimentary OC; that is, they did not show whether the seagrass trapped seagrass-derived OC suspended in the water column or directly supplied seagrass-derived carbon to the sediments.

To assess the effect of seagrass on the sedimentary OC stock, it is important to examine all stock components, including live and dead above- and belowground biomass in the sediment column, and their origins. For this reason, it is

necessary to retrieve intact cores, because both macroscopic plant materials (Miyajima et al., 1998) and OC derived from calcareous organisms such as corals, foraminifera, molluscs, and coralline algae (Ingalls et al., 2003; Versteegh et al., 2011) occur in the coarse sediment fraction (sand and gravels), especially in tropical seagrass meadows around coral reefs (Suzuki, 2005). However, to our knowledge, all previous studies have only examined some of the stock components: for example, the fine sediment fraction (<1–2 mm diameter) (Hemminga et al., 1994; Miyajima et al., 2015; Kennedy et al., 2004; Ricart et al., 2015), dead plant structures (Cebrian et al., 2000), surface sediment (Barron et al., 2004), and small subsamples from a core (Dahl et al., 2016).

In this study, to investigate [the pathways of sedimentary OC accumulation in seagrass meadows, especially the direct supply of belowground seagrass detritus](#)~~the relationship between seagrass and the sedimentary OC stock~~, we used intact cores that included all ~~seagrass~~-live and dead ~~bodies~~-seagrasses and sediments and then performed the OC mass and stable carbon isotope analyses of all components of the cores to examine the origin of the OC [along a seagrass biomass gradient](#).

## 2 Materials and Methods

### 2.1 Study sites

To assess the relationship between seagrass and the sedimentary OC stock, we chose tropical Indo-Pacific seagrass meadow sites. Globally, the tropical Indo-Pacific region is the world's largest bioregion and contains the highest diversity of seagrasses, which are distributed predominantly on coral reef flats (Short et al., 2007). Globally, the total documented seagrass area is 164,000 km<sup>2</sup> (Green and Short, 2003), and the total seagrass area in the Indo-Pacific region, excluding Australia, where both tropical and temperate seagrasses are distributed, is around 32,400 km<sup>2</sup>, or about 20% of the total area. Furthermore, given that about half of the documented seagrass habitat in Australia is composed of tropical seagrasses (Kirkman, 1997), the total area of tropical Indo-Pacific seagrass habitat reaches approximately 116,000 km<sup>2</sup>, accounting for 70% of the global seagrass area. Thus, accurate estimation of the blue-carbon stock of seagrasses in the tropical Indo-Pacific region is important for the estimation of the global seagrass carbon stock. However, in spite of the geographical importance of this region, reports on seagrass OC stocks there are limited (Lavery et al., 2013; Miyajima et al., 2015).

We obtained cores from two Indo-Pacific tropical seagrass meadow sites from 13 to 23 August 2014. The two sites, a back-reef site (Shiraho reef) and an estuarine site (Fukido estuary), both located around Ishigaki Island, Okinawa, southwestern Japan, (Fig. 1), have different allochthonous carbon input amounts. The back-reef site is situated on a well-developed reef flat about 1 km wide, where seagrass meadows, dominated by *Thalassia hemprichii*, are distributed between 100 and 300 m from the shoreline. The site is about 2 km south of the mouth of the Todoroki River, and most sediments transported by the river accumulate on its north side (Mitsumoto et al., 2000) because the prevailing current, which is controlled by large channels in the reef, is northward (Tamura et al., 2007). Therefore, terrestrial sediment input to the back-reef site is

low. The mud (silt + clay) content of the surface sediment of the seagrass meadows at the site ranges from 1.2% to 3.9% (mean 2.3%) (Tanaka and Kayanne, 2007). The estuarine site is located near the mouth of a small river, which is bordered by small mangrove forests. The freshwater inflow is low, so water exchange between the river and estuary is controlled mainly by tidal motion (Terada et al., 2007). The dominant seagrass species at the site is *Enhalus acoroides*. The mud content of the surface sediment in the seagrass meadows at the estuarine site ranges from 0.9% to 6.4% (mean 3.6%) (Tanaka and Kayanne, 2007).

## 2.2 Core sampling

We developed a new box corer to facilitate the retrieval of intact cores that preserve sedimentary structures as well as above- and belowground live and dead seagrass es-bodies (Fig. 2). The box corer is 15 cm wide, 15 cm deep, and 17 cm high and is made of stainless steel so that it can cut through roots and rhizomes. A shutter 1 cm above the bottom of the corer is designed to cut through the relatively hard belowground seagrass es-bodies, making it possible to obtain intact cores. The corer also has a lid to prevent the loss of surficial sediments from the core during underwater sampling. The corer is large enough to retrieve all components of the OC stock whole: shoots, live and dead above- and belowground seagrass es-bodies, and old skeletal OC in sand and gravel derived from calcareous organisms such as corals, foraminifera, molluscs, and coralline algae. Most cores obtained with the corer were about 15 cm long, but we were not able to insert the core to its full length at three sampling points because of the presence of large gravels in the sediment. We were able to collect all of the seagrass biomass at these points, however.

To measure the total OC mass ( $OC_{total}$ ), we quantified three components of the box corer samples (Fig. 3): (1) live seagrass es-bodies ( $OC_{bio}$ ); (2) dead plant structures (>2 mm in size: dead seagrass leaves, sheaths, rhizomes, and roots detached from live structures) ( $OC_{dead}$ ); and (3) OC in the coarse (>1 mm diameter) sediments (excluding dead plant structures >2 mm in size) ( $OC_{csed}$ ). We also collected samples with cylinder cores so that we could obtain depth profiles of OC in the fine (<1 mm diameter) sediments (including dead plant structures <1 mm in size) ( $OC_{fsed}$ ). It was technically impossible to obtain these profiles with the box corer because of its large surface sampling area and the high density of the belowground structures (Fig. 2c). The samples retrieved by the box corer were immediately sieved through a 1mm mesh sieve *in situ* to obtain the >1 mm fractions of the  $OC_{bio}$ ,  $OC_{dead}$  and  $OC_{csed}$ . Live sSeagrass es-bodies have air-filled lacunae so that they float; thus, we considered buoyant seagrass es-bodies captured by the sieve to be  $OC_{bio}$  (Borum et al., 2006). We merged any dead plant structures attached to live seagrass es-bodies into  $OC_{bio}$  because their mass was usually very small. We collected a cylinder core 10–16 cm long with an acrylic pipe (internal diameter 6.6 cm) from a point immediately adjacent to each box core. We subdivided each cylinder core into 1-cm-long subsamples from the surface to the bottom of the core.

We obtained 20 paired samples (one box and one cylinder core) from the back-reef site and eight paired samples from the estuarine site. At the back-reef site, we collected 16 paired samples from vegetated points in the seagrass meadows, two from bare patches in the seagrass meadows, and two from unvegetated areas (Fig. 1 cb). Similarly, at the estuarine site, we



collected five paired samples from vegetated points, one pair from a bare point, and two paired samples from unvegetated areas near the river mouth (Fig. 1de).

Potential sources of sedimentary OC ( $OC_{sed}$ ) were also collected at both sites and analyzed for  $\delta^{13}C$ . Samples of seagrass leaves were collected from all dominant seagrass species at each site: *T. hemprichii*, *Cymodocea rotundata*, *C. serrulata*, and *Halodule uninervis* at the back-reef site, and *E. acoroides*, *T. hemprichii*, and *C. serrulata* at the estuarine site. Samples for determining the  $\delta^{13}C$  of algae and corals were taken from epiphytes, benthic microalgae, and the dominant coral species (mainly *Acropora* spp. and *Porites* spp.) at the sites. Epiphytes were collected from the seagrass leaves by using a stainless steel scraper, and benthic microalgae were extracted from the surface sediment (up to approximately 1-mm depth) by the method of Kuwae et al. (2008). All obtained samples were stored in polyethylene bags at  $-20^{\circ}C$  until analysis.

We used the published  $\delta^{13}C$  data of suspended OC (collected about 1 km off the outer reef edge of Ishigaki Island) and of terrestrial particulate organic matter (POM; collected from the Fukido River, Ishigaki Island) from Miyajima et al. (2015). We assumed that the published  $\delta^{13}C$  data were normally distributed.

### 2.3 OC and stable isotope analysis

We identified live seagrass ~~bodies~~ to the species level and separated aboveground biomass (leaf blades) from belowground biomass (leaf sheathes, rhizomes, and roots). Then we dried all parts at  $60^{\circ}C$  and weighed them. Box corer sediments were dried at  $60^{\circ}C$  and sieved through a 2-mm-mesh sieve, and the included dead plant structures ( $>2$  mm in size) were picked out and weighed. To ensure homogeneity of subsamples, the coarse sediments (excluding the dead plant structures) were first crushed to approximately 1-mm grains with a jaw crusher (Jaw Crusher PULVERISETTE 1 Model I classic line, FRITSCH, Ltd., Idar-Oberstein, Germany) and then divided into 16 or 64 subsamples with a splitter (Simple microsampler, Iwamoto Mineral, Ltd., Tokyo, Japan). The cylinder core samples were subdivided into surface (0–1 cm depth), intermediate (5–8 cm depth), and bottom (9–16 cm depth) layers and dried at  $60^{\circ}C$ . For the  $OC_{f_{sed}}$  analysis, each layer was sieved through a 1mm mesh sieve and then subdivided into two or four subsamples with the splitter. All subsamples used for chemical analyses were weighed and then powdered and homogenized in an agate mill.

For OC analysis, the homogenized samples were placed in silver containers (to prevent the loss of acid-soluble OC in carbonate sediments) and pretreated with hydrochloric acid to remove carbonates (Yamamuro and Kayanne, 1995). First, each sample was weighed in a silver container and its weight was adjusted to about 20 mg. Then, 1 N HCl was carefully and gradually added until bubbles were no longer seen, and the sample was dried at  $60^{\circ}C$  overnight and at  $105^{\circ}C$  for 1 h. The dried sample was then wrapped in tin foil. We measured the total OC concentration and the stable carbon isotopic ratio of each sample with an elemental analyzer-connected isotope ratio mass spectrometer (FLASH EA 1112 / DELTA<sup>plus</sup> Advantage, Thermo Electron, Inc., Massachusetts, USA). The stable carbon isotope ratio ( $\delta^{13}C$ ) is reported as the relative per mil deviation

from VPDB (Vienna Pee Dee Belemnite). The analytical precision of the isotope ratio mass spectrometer, based on the standard deviation of  $\delta^{13}\text{C}$  values of internal reference replicates, was  $<0.2\%$ .

## 2.4 Determination of the mass and $\delta^{13}\text{C}$ of OC

5 We calculated  $\text{OC}_{\text{total}}$  per unit area ( $\text{g C m}^{-2}$ ) at each sampling point by summing the  $\text{OC}_{\text{bio}}$  and  $\text{OC}_{\text{sed}}$  components in the top 0.15 m (Fig. 3) as follows:

$$\text{OC}_{\text{total}} = \text{OC}_{\text{bio}} + \text{OC}_{\text{sed}}. \quad (1)$$

10  $\text{OC}_{\text{bio}}$  was calculated as,

$$\text{OC}_{\text{bio}} = \sum_i (a_i x_i + b_i y_i), \quad (2)$$

where  $a_i$  and  $b_i$  are the averaged OC concentrations ( $\text{g C g}^{-1}$  DW) of the aboveground and belowground biomass, respectively, of the  $i$ th seagrass species collected at three different sampling points (except *C. serrulata*, which was collected at only one sampling point at the estuarine site), and  $x_i$  and  $y_i$  are the aboveground and belowground biomass ( $\text{g m}^{-2}$ ), respectively, of the  $i$ th seagrass species. The biomasses of *Syringodium isoetifolium*, *Halophila ovalis*, and an unidentified species at the back-reef site accounted for  $<0.1\%$  of the total biomass, so they were excluded from this calculation. The averaged OC concentrations and aboveground and belowground biomass dry weights are summarized in Table 1.

20  $\text{OC}_{\text{sed}}$  was calculated as follows:

$$\text{OC}_{\text{sed}} = \text{OC}_{\text{dead}} + \text{OC}_{\text{csed}} + \text{OC}_{\text{fsed}}. \quad (3)$$

The terms of Eq. (3) were calculated by the following equations:

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$$\text{OC}_{\text{dead}} = \frac{1}{100} (\% \text{OC}_{\text{leaf}} \times \rho_{\text{leaf}} + \% \text{OC}_{\text{shr}} \times \rho_{\text{shr}} + \% \text{OC}_{\text{root}} \times \rho_{\text{root}}) \times h, \quad (4)$$

$$\text{OC}_{\text{csed}} = \frac{1}{100} (\% \text{OC}_{\text{csed}} \times \rho_{\text{csed}}) \times h, \quad (5)$$

$$\text{OC}_{\text{fsed}} = \frac{1}{3} \times \frac{1}{100} (\% \text{OC}_{\text{fseds}} \times \rho_{\text{fseds}} + \% \text{OC}_{\text{fsedm}} \times \rho_{\text{fsedm}} + \% \text{OC}_{\text{fsedb}} \times \rho_{\text{fsedb}}) \times h, \quad (6)$$

30 where %OC is the concentration of OC (%DW) ( $n = 3$ );  $\rho$  is the dry density ( $\text{g DW m}^{-3}$ ) of each component (indicated by subscripts: leaf, dead leaf; shrh, dead sheath and rhizome; root, dead root; csed, coarse sediment; fseds, fine sediment of the

surface layer; fse dm, fine sediment of the intermediate layer; fse db, fine sediment of the bottom layer), and  $h$  is the sample thickness (0.15 m). OC<sub>fse d</sub> is the averaged OC mass of the three layers (surface, medium, and bottom) of fine sediment.

$\delta^{13}\text{C}$  of OC<sub>sed</sub> ( $\delta^{13}\text{C}_{\text{sed}}$ ) at each sampling point was calculated as follows:

$$5 \quad \delta^{13}\text{C}_{\text{sed}} = \frac{1}{\text{OC}_{\text{sed}}} (\text{OC}_{\text{dead}} \times \delta^{13}\text{C}_{\text{dead}} + \text{OC}_{\text{cse d}} \times \delta^{13}\text{C}_{\text{cse d}} + \delta^{13}\text{C}_{\text{fse d}}), \quad (7)$$

where  $\delta^{13}\text{C}_{\text{dead}}$  is the averaged  $\delta^{13}\text{C}$  value of dead plant structures (sheath and rhizomes, and roots) at the back-reef and estuarine sites. We did not include leaf detritus in the calculation of  $\delta^{13}\text{C}_{\text{sed}}$  because (1) the leaf fragments were so small that we could not remove epiphytes from them, and (2) their mass was much smaller than that of the sheath and rhizomes and roots, so we

10 considered its contribution to  $\delta^{13}\text{C}_{\text{sed}}$  to be negligible. We used the  $\delta^{13}\text{C}_{\text{dead}}$  value at each site for the calculation of  $\delta^{13}\text{C}_{\text{sed}}$ . The standard deviation (SD) of  $\delta^{13}\text{C}_{\text{sed}}$  derived from the SD of  $\delta^{13}\text{C}_{\text{dead}}$  was smaller than 0.1%.  $\delta^{13}\text{C}_{\text{cse d}}$  is the  $\delta^{13}\text{C}$  value of OC<sub>cse d</sub>.  $\delta^{13}\text{C}_{\text{fse d}}$  is the averaged  $\delta^{13}\text{C}$  value of OC<sub>fse d</sub> multiplied by the OC mass of each layer and was calculated as follows:

$$15 \quad \delta^{13}\text{C}_{\text{fse d}} = \frac{1}{3} \times \frac{1}{100} (\% \text{OC}_{\text{fse ds}} \times \rho_{\text{fse ds}} \times \delta^{13}\text{C}_{\text{fse ds}} + \% \text{OC}_{\text{fse dm}} \times \rho_{\text{fse dm}} \times \delta^{13}\text{C}_{\text{fse dm}} + \% \text{OC}_{\text{fse db}} \times \rho_{\text{fse db}} \times \delta^{13}\text{C}_{\text{fse db}}) \times h. \quad (8)$$

The averaged values of the organic carbon concentration,  $\delta^{13}\text{C}$ , and dry density of sediment and dead plant structures are summarized in Table 2.

### 3 Results

#### 20 3.1 Seagrass biomass and species composition at each site

At the back-reef site, the average ( $\pm$ SD) aboveground and belowground biomass values were  $74 \pm 45$  g DW m<sup>-2</sup> ( $n = 16$ ) and  $675 \pm 450$  g DW m<sup>-2</sup> ( $n = 16$ ), respectively (Table 1). The dominant species was *T. hemprichii*, accounting for 76.7% of the total biomass; *C. rotundata* (18.0%), *C. serrulata* (3.3%), *H. uninervis* (1.7%), *H. ovalis* (<0.1%), *S. isoetifolium* (<0.1%), and an unidentified species (<0.1%) were minor components at the back-reef site. At the estuarine site, the average aboveground and belowground biomass were  $70 \pm 34$  g DW m<sup>-2</sup> ( $n = 5$ ) and  $1354 \pm 847$  g DW m<sup>-2</sup> ( $n = 5$ ), respectively (Table 1). The dominant species was *E. acoroides*, accounting for 92.3% of the total biomass; *T. hemprichii* (7.0%), *C. serrulata* (0.6%), and *H. uninervis* (<0.1%) were minor components.

### 3.2 OC density in the fine sediments

The average OC density ( $\text{g C cm}^{-3}$ ) did not differ significantly among the fine sediment layers at either the back reef (paired  $t$ -test, Bonferroni adjusted  $P > 0.05$ ) or the estuarine site (Wilcoxon signed rank test, Bonferroni adjusted  $P > 0.05$ ).

#### 5 3.2.3 OC mass

The average OC density ( $\text{g C cm}^{-3}$ ) did not differ significantly among the fine sediment layers at either the back-reef (paired  $t$ -test, Bonferroni adjusted  $P > 0.05$ ) or the estuarine site (Wilcoxon signed rank test, Bonferroni adjusted  $P > 0.05$ ). The averaged  $\text{OC}_{\text{bio}}$ ,  $\text{OC}_{\text{dead}}$ ,  $\text{OC}_{\text{fscd}}$ ,  $\text{OC}_{\text{scd}}$ , and  $\text{OC}_{\text{total}}$  values were significantly higher at points with vegetation than at points without vegetation at both the back-reef site ( $\text{OC}_{\text{bio}}$ ,  $t = -6.23$ , d.f. = 15,  $P < 0.001$ ;  $\text{OC}_{\text{dead}}$ ,  $W = 0$ ,  $P < 0.001$ ;  $\text{OC}_{\text{fscd}}$ ,  $t = -2.61$ , d.f. = 18,  $P = 0.018$ ;  $\text{OC}_{\text{scd}}$ ,  $t = -2.85$ , d.f. = 18,  $P = 0.011$ ;  $\text{OC}_{\text{total}}$ ,  $t = -3.44$ , d.f. = 18,  $P = 0.003$ ) (Fig. 4a and Fig. 4b) and the estuarine site ( $\text{OC}_{\text{bio}}$ ,  $t = -3.61$ , d.f. = 4,  $P = 0.022$ ;  $\text{OC}_{\text{dead}}$ ,  $W = 0$ ,  $P = 0.036$ ;  $\text{OC}_{\text{fscd}}$ ,  $t = -2.59$ , d.f. = 6,  $P = 0.041$ ;  $\text{OC}_{\text{scd}}$ ,  $t = -3.33$ , d.f. = 6,  $P = 0.016$ ;  $\text{OC}_{\text{total}}$ ,  $t = -4.24$ , d.f. = 6,  $P = 0.005$ ) (Fig. 4c and Fig. 4d). At points with vegetation, the averaged  $\text{OC}_{\text{bio}}$ ,  $\text{OC}_{\text{dead}}$ ,  $\text{OC}_{\text{fscd}}$  and  $\text{OC}_{\text{scd}}$  and  $\text{OC}_{\text{total}}$  values did not significantly differ between the sites ( $\text{OC}_{\text{bio}}$ ,  $W = 73$ ,  $P > 0.05$ ;  $\text{OC}_{\text{dead}}$ ,  $W = 65$ ,  $P > 0.05$ ;  $\text{OC}_{\text{fscd}}$ ,  $t = 0.670.33$ , d.f. = 1926,  $P > 0.05$ ;  $\text{OC}_{\text{scd}}$ ,  $t = -0.521.53$ , d.f. = 1926,  $P > 0.05$ ;  $\text{OC}_{\text{total}}$ ,  $t = 0.86$ , d.f. = 26,  $P > 0.05$ ), whereas the averaged  $\text{OC}_{\text{bio}}$ ,  $\text{OC}_{\text{dead}}$ ,  $\text{OC}_{\text{cscd}}$  and  $\text{OC}_{\text{total}}$  values were significantly higher at the estuarine site ( $191 \pm 75 \text{ g C m}^{-2}$ ) than at the back-reef site ( $123 \pm 45 \text{ g C m}^{-2}$ ) ( $\text{OC}_{\text{bio}}$ ,  $t = -2.25$ , d.f. = 19,  $P = 0.036$ ;  $\text{OC}_{\text{dead}}$ ,  $W = 11$ ,  $P = 0.015$ ;  $\text{OC}_{\text{cscd}}$ ,  $t = -2.984.86$ , d.f. = 2619,  $P < 0.006001$ ;  $\text{OC}_{\text{total}}$ ,  $t = -2.34$ , d.f. = 19,  $P = 0.030$ ) (Fig. 4b and Fig. 4d). This higher  $\text{OC}_{\text{cscd}}$  at the estuarine site was resulting from the higher density of coarse sediments there than at the back reef site ( $t = -2.92$ , d.f. = 26,  $P = 0.007$ ), because the %OC of  $\text{OC}_{\text{cscd}}$  was not different between the sites ( $W = 103$ ,  $P > 0.05$ ) (Table 2). At points with vegetation,  $\text{OC}_{\text{total}}$  ranged from 531.334 to 1785  $\text{g C m}^{-2}$  across both sites.  $\text{OC}_{\text{scd}}$ , which ranged from 433.334 to 1147  $\text{g C m}^{-2}$  and was the main component of  $\text{OC}_{\text{total}}$ , accounted for  $75.181.3 \pm 135.17\%$  DW of  $\text{OC}_{\text{total}}$ . Hence, the contribution of the live seagrass body itself ( $\text{OC}_{\text{bio}}$ ) was minor ( $24.948.7 \pm 13.145.7\%$  DW).  $\text{OC}_{\text{fscd}}$  was the major component of  $\text{OC}_{\text{scd}}$ , accounting for  $55.68.3 \pm 124.58\%$  DW of  $\text{OC}_{\text{total}}$ ;  $\text{OC}_{\text{cscd}}$  and  $\text{OC}_{\text{dead}}$  were minor components, accounting for  $15.09.6 \pm 7.243.7\%$  DW and  $4.53.4 \pm 4.10\%$  DW of  $\text{OC}_{\text{total}}$ , respectively.

The average aboveground and belowground biomass in  $\text{OC}_{\text{bio}}$  did not differ significantly between the sites (Fig. 5a) (aboveground biomass,  $t = 0.30$ , d.f. = 19,  $P > 0.05$ ; belowground biomass,  $t = -1.75$ , d.f. = 4.67,  $P > 0.05$ ). Belowground biomass accounted for  $89.1 \pm 4.4\%$  DW of  $\text{OC}_{\text{bio}}$  (Fig. 5b). The averaged biomasses of aboveground (i.e., leaf) and belowground (i.e. sheath and rhizome, and root) detritus in  $\text{OC}_{\text{dead}}$  did not differ significantly between the sites (aboveground detritus,  $t = 0.60$ , d.f. = 7.82,  $P > 0.05$ ; belowground detritus,  $W = 28$ ,  $P > 0.05$ ) (Fig. 5c). The biomass of belowground detritus accounted for  $90.8 \pm 12.0\%$  DW of  $\text{OC}_{\text{dead}}$  (Fig. 5d). The biomasses of sheath and rhizome, and root accounted for  $65.5 \pm 19.2\%$  DW and  $25.3 \pm 16.0\%$  DW of  $\text{OC}_{\text{dead}}$ , respectively.

### 3.34 $\delta^{13}\text{C}$ of OC

The average  $\delta^{13}\text{C}_{\text{sed}}$  at the back-reef site ( $-12.6 \pm 0.7\text{‰}$ ) was significantly higher than that of the estuarine site ( $-16.6 \pm 3.1\text{‰}$ ) ( $t = 3.61$ , d.f. = 7,  $P = 0.008$ ), and it was also significantly higher than the  $\delta^{13}\text{C}$  values of algae and corals ( $-15.2 \pm 1.9\text{‰}$ ) ( $W = 2753$ ,  $P < 0.001$ ), suspended POM ( $-21.9 \pm 1.6\text{‰}$ ) ( $t = 15.45$ , d.f. = 8,  $P < 0.001$ ), and terrestrial POM ( $-28.7 \pm 1.5\text{‰}$ ) ( $t = 29.25$ , d.f. = 8,  $P < 0.001$ ). However, average  $\delta^{13}\text{C}_{\text{sed}}$  at the back-reef site was significantly lower than  $\delta^{13}\text{C}$  of seagrass ( $-9.2 \pm 1.3\text{‰}$ ) ( $t = -12.64$ , d.f. = 57,  $P < 0.001$ ) (Fig. 6). Average  $\delta^{13}\text{C}_{\text{sed}}$  at the estuarine site did not differ significantly from  $\delta^{13}\text{C}$  of algae and corals ( $W = 457$ ,  $P > 0.05$ ), but it was significantly higher than  $\delta^{13}\text{C}$  of both suspended POM ( $t = 4.36$ , d.f. = 14,  $P < 0.001$ ) and terrestrial POM ( $t = 10.05$ , d.f. = 14,  $P < 0.001$ ), and significantly lower than  $\delta^{13}\text{C}$  of seagrass ( $t = -6.66$ , d.f. = 8,  $P < 0.001$ ). The average  $\delta^{13}\text{C}$  among fine sediment layers did not differ significantly at either the back-reef site (Wilcoxon signed rank test, Bonferroni adjusted  $P > 0.05$ ) or the estuarine site (paired  $t$ -test, Bonferroni adjusted  $P > 0.05$ ).

### 3.45 Relationships among biomass, OC mass, and $\delta^{13}\text{C}$

At the back-reef site, we found significant correlations between  $\text{OC}_{\text{sed}}$  and DW-based (not carbon-based) biomass ( $F_{1,18} = 11.63$ ,  $P = 0.003$ ,  $r^2 = 0.39$ ) (Fig. 7a),  $\text{OC}_{\text{sed}}$  and aboveground biomass ( $F_{1,18} = 16.38$ ,  $P < 0.001$ ,  $r^2 = 0.48$ ) (Fig. 7b),  $\text{OC}_{\text{sed}}$  and belowground biomass ( $F_{1,18} = 10.95$ ,  $P = 0.004$ ,  $r^2 = 0.38$ ) (Fig. 7c),  $\text{OC}_{\text{sed}}$  and  $\text{OC}_{\text{dead}}$  ( $F_{1,18} = 4.55$ ,  $P = 0.047$ ,  $r^2 = 0.20$ ) (Fig. 7d), and  $\text{OC}_{\text{sed}}$  and  $\delta^{13}\text{C}_{\text{sed}}$  ( $F_{1,18} = 11.51$ ,  $P = 0.003$ ,  $r^2 = 0.39$ ) (Fig. 7e). We also found significant correlations between  $\delta^{13}\text{C}_{\text{sed}}$  and belowground biomass ( $F_{1,18} = 4.68$ ,  $P = 0.044$ ,  $r^2 = 0.21$ ) (Fig. 7f), and between  $\delta^{13}\text{C}_{\text{sed}}$  and  $\text{OC}_{\text{dead}}$  ( $F_{1,18} = 13.18$ ,  $P = 0.002$ ,  $r^2 = 0.42$ ) (Fig. 7g). We also found significant positive correlations between aboveground and belowground biomass ( $F_{1,18} = 94.10$ ,  $P < 0.001$ ,  $r^2 = 0.84$ ). At the estuarine site, we found significant correlations between  $\text{OC}_{\text{sed}}$  and aboveground biomass ( $F_{1,6} = 8.18$ ,  $P = 0.029$ ,  $r^2 = 0.58$ ) (Fig. 7b) and between  $\text{OC}_{\text{sed}}$  and  $\text{OC}_{\text{dead}}$  ( $F_{1,6} = 6.94$ ,  $P = 0.039$ ,  $r^2 = 0.54$ ) (Fig. 7d) but not between  $\text{OC}_{\text{sed}}$  and biomass ( $F_{1,6} = 3.08$ ,  $P > 0.05$ ,  $r^2 = 0.34$ ) (Fig. 7a),  $\text{OC}_{\text{sed}}$  and belowground biomass (Fig. 7c) ( $F_{1,6} = 2.94$ ,  $P > 0.05$ ,  $r^2 = 0.33$ ), or  $\text{OC}_{\text{sed}}$  and  $\delta^{13}\text{C}_{\text{sed}}$  ( $F_{1,6} = 0.040$ ,  $P > 0.05$ ,  $r^2 < 0.01$ ) (Fig. 7e). The slope of the regression line of  $\text{OC}_{\text{sed}}$  against aboveground biomass did not differ significantly between the sites (ANCOVA,  $F = 1.09$ , d.f. = 1,  $P > 0.05$ ) (Fig. 7b), and that of  $\text{OC}_{\text{sed}}$  against  $\text{OC}_{\text{dead}}$  also did not differ significantly between the sites ( $F = 0.36$ , d.f. = 1,  $P > 0.05$ ) (Fig. 7d). We also found significant positive correlations between aboveground and belowground biomass ( $F_{1,6} = 78.40$ ,  $P < 0.001$ ,  $r^2 = 0.93$ ).

## 4 Discussion

### 4.1 Components of OC stock in seagrass meadows

Our results showed that the sedimentary OC mass ( $OC_{sed}$ ) was the main component of the total organic carbon mass ( $OC_{total}$ ; i.e., all stock components: live and dead above- and below-ground biomass and sediments) at our study sites. ~~If we assume that the density of sedimentary OC is constant to 1 m depth, then we can estimate the relative contribution of  $OC_{bio}$  to  $OC_{total}$  to be  $5.6 \pm 3.9\%$  (excluding unvegetated sampling points). This contribution of  $OC_{bio}$  to  $OC_{total}$ , which is the highest among globally compiled data (range,  $0.6 \pm 0.1\%$  to  $2.5 \pm 1.4\%$ ; Fourqurean et al., 2012), is attributable to the relatively high  $OC_{bio}$  and low  $OC_{sed}$  at our sites (Fourqurean et al., 2012). The averaged  $OC_{bio}$  was significantly higher in this study than that in the previous study by Fourqurean et al. (2012) ( $W = 1691$ ,  $P = 0.006$ ), whereas the averaged  $OC_{sed}$  was significantly lower in this study than in the previous study at both vegetated and no-vegetation points (vegetation,  $W = 6952$ ,  $P < 0.001$ ; no-vegetation,  $W = 225$ ,  $P = 0.0396$ ) (Table 3). Hence, the contribution of  $OC_{bio}$  to  $OC_{total}$  at our sites was higher than the global average. The high  $OC_{bio}$  was due to the well-developed belowground biomass, which accounted for  $90.8 \pm 3.9\%$  of DW-based biomass  $OC_{bio}$  at our sites. This value is also among the highest among globally compiled data (Duarte and Chiscano, 1999). Possible reasons for the exceptional development of belowground biomass include (1) morphological plasticity for resistance to high wave energy (Fonseca and Bell, 1998), which is supported by the low mud content at our sites compared to that reported by previous studies (Koch, 2001; Serrano et al., 2016a), and (2) nutrient limitation, which can lead to more allocation of biomass to belowground parts to enable the plant to acquire nutrients in deeper sediment layers (Lee et al., 2007). The low  $OC_{sed}$  may be attributable to (1) high wave energy in association with increased OC lability due to the low specific surface area of sediments (Miyajima et al., 2017) and (2) the low gross primary production/respiration (P/R) ratio in this geographical region (Duarte et al., 2010).~~

Belowground detritus (i.e., sheath and rhizome, and root) was the major component of  $OC_{dead}$ , accounting for  $90.8 \pm 12.0\%$  of  $OC_{dead}$  at our sites. This result is consistent with a previous report on *Cymodocea nodosa* (Cebrian et al., 2000) and suggests that belowground detritus is more easily stored in the sediment than aboveground detritus. A mechanism supporting this hypothesis might be either (1) a higher belowground biomass and an associated higher supply of seagrass detritus or (2) higher recalcitrance of belowground detritus. Here, a higher supply is more likely because at our sites the belowground biomass is among the highest reported values for each species (Duarte and Chiscano, 1999), although the reported aboveground/belowground production ratio of *T. hemprichii* and *E. acoroides* varies among studies (Duarte et al., 1998; Duarte and Chiscano, 1999; Erftemeijer, Osinga, and Mars, 1993). Higher recalcitrance is also possible; Holmer and Olsen (2002) reported that during a 43 day decomposition experiment, *E. acoroides* rhizomes did not lose weight, whereas buried leaves lost  $80.3 \pm 4.2\%$  of their weight. Also, Fourqurean and Schrlau (2003) showed that only  $5 \pm 2\%$  of *Thalassia testudinum* leaves, but  $49 \pm 6\%$  of *T. testudinum* rhizomes, remained after 348 days of decomposition.

## 4.2 Mechanism of the OC supply to sediment

OC<sub>sed</sub> was significantly and positively correlated with aboveground biomass at both sites (Fig. 7b) and to belowground biomass at the back-reef site (Fig. 7c). This result is contrary to the finding of most previous studies that there is no relationship between biomass and %OC or OC mass (Kennedy et al., 2004, 2010; Howard et al., 2017; [Rozaimi et al. 2017](#); but cf. Samper-Villarreal et al., 2016). This contrary result may be due to our data collection strategy of (1) sympatric sampling of all stock components (live and dead above- and belowground biomass and sediments) in intact cores, and (2) selection of sampling points aiming at controlling for variables other than seagrass biomass (i.e., mud content, wave height, and the amount of allochthonous OC inputs were relatively homogenous among points), although we could not exclude the possibility that our sites may have specific sedimentary OC storage characteristics different from those of other Indo-Pacific seagrass meadows. Several mechanisms can plausibly explain the positive relationship between seagrass biomass and OC<sub>sed</sub>, including (1) trapping of suspended OC (both allochthonous and autochthonous OC) by seagrass leaves, and (2) the direct supply of belowground seagrass-derived autochthonous OC. If it is assumed that the suspended OC settling on the sediment surface is spatially homogeneous in nature (quality) and that the contribution of trapped OC is larger than that of directly supplied OC, then  $\delta^{13}\text{C}_{\text{sed}}$  should be constant regardless of the aboveground biomass and its associated trapping capacity. However, OC<sub>sed</sub> was significantly and positively correlated with  $\delta^{13}\text{C}_{\text{sed}}$  (Fig. 7e), and the average  $\delta^{13}\text{C}$  of OC<sub>sed</sub> was significantly higher than  $\delta^{13}\text{C}$  values of allochthonous OC (algae and corals, suspended POM, and terrestrial POM) at the back-reef site (Fig. 6). Furthermore, OC<sub>sed</sub> was positively correlated with OC<sub>dead</sub> at both sites (Fig. 7d), and the main component of OC<sub>dead</sub> was belowground detritus (Fig. 5d). Taken together, these results suggest that directly supplied seagrass-derived OC was mainly from the belowground detritus. The positive correlations between  $\delta^{13}\text{C}_{\text{sed}}$  and belowground biomass (Fig. 7f) and between  $\delta^{13}\text{C}_{\text{sed}}$  and OC<sub>dead</sub> (Fig. 7g) at the back-reef site also support this mechanism. From these lines of evidence, we conclude that the direct supply of recalcitrant belowground seagrass detritus is a major mechanism of OC<sub>sed</sub> enrichment/accumulation at the back-reef site (Fig. 8). Although we inferred that a direct autochthonous OC supply from belowground biomass is the major mechanism of OC<sub>sed</sub> enrichment/accumulation, suspended allochthonous OC may also have been supplied from the water column at the back-reef site, as has been reported elsewhere (Kennedy et al., 2010), because the average  $\delta^{13}\text{C}_{\text{sed}}$  at the back-reef site was significantly lower than  $\delta^{13}\text{C}$  of seagrass (Fig. 6).

The seagrass-derived OC increase according to the development of the seagrass meadows at the back-reef site (Fig. 7a and Fig. 7e) suggests that seagrass meadows are autotrophic and the time since seagrass colonization is longer. This inference is consistent with a previous report that net primary production (NPP) at the back-reef site is higher where the seagrass cover is high (cover 91.7%; NPP 68.14 mmol C m<sup>-2</sup> d<sup>-1</sup>) than where the seagrass cover is low (cover 55.1%; NPP 34.20 mmol C m<sup>-2</sup> d<sup>-1</sup>) (Nakamura and Nakamori, 2009). It is also possible that seagrass mortality increases with time since colonization, leading to an increase in dead plant structures (Cebrian et al., 2000).

At the estuarine site, OC<sub>sed</sub> increased with increasing aboveground seagrass biomass (Fig. 7b), but it did not increase with increasing belowground seagrass biomass (Fig. 7c), indicating that trapping of suspended OC by seagrass leaves surpassed



the direct supply of belowground seagrass-derived OC (Fig. 8). However,  $OC_{dead}$  was significantly and positively correlated with  $OC_{sed}$  (Fig. 7d), indicating that direct supply also contributed to  $OC_{sed}$  enrichmentaccumulation at the site. A plausible mechanism explaining the hypothesized dominance of suspended OC trapping is a lower belowground turnover rate (i.e., the production/biomass ratio) at the estuarine site than at the back-reef site. Because  $OC_{sed}$  was not significantly different between the sites and directly supplied seagrass-derived OC was the major component of  $OC_{sed}$  at the back-reef site and only a minor component at the estuarine site, the capacity of the estuarine site to directly supply belowground seagrass-derived OC to the sediment was lower than that of the back-reef site (Fig. 8). Moreover, given that the directly supplied amount is determined by two factors, the belowground biomass and its turnover rate, and that the belowground biomass was not significantly different between the sites (Fig. 5a), we anticipate that a difference in the belowground turnover rate was responsible for the difference in the direct supply contribution between the sites. Another possible explanation for the inferred difference is that the absolute input of allochthonous OC was higher at the estuarine site than at the back-reef site. The slope of the regression between aboveground biomass and  $OC_{sed}$  was not significantly different between the sites (Fig. 7b), which suggests that the trapping ability for autochthonous and allochthonous OC was not different between the sites. However, the fact that  $OC_{sed}$  at vegetated points was not significantly different between the sites (Fig. 4b and Fig. 4d) together with the apparently minor direct belowground supply at the estuarine site implies that the contribution of OC from the water column to  $OC_{sed}$  was larger at the estuarine site. Moreover, the fact that average  $\delta^{13}C_{sed}$  was significantly lower at the estuarine site than at the back-reef site (Fig. 6) would support a major role of allochthonous OC from the water column in  $OC_{sed}$  enrichmentaccumulation. The effect of particle trapping by seagrasses is reported to be enhanced particularly in particle-poor waters (Duarte et al., 1999). Thus, trapping is likely to be an important mechanism especially at sites with particle-poor water such as coral reef sites.

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## 5 Conclusion

Using our data collection strategy, namely, sympatric sampling in intact cores of live and dead seagrass es-bodies and sediments and analyses of the organic carbon mass and stable carbon isotope composition of all components of the cores, we successfully demonstrated the pathways of sedimentary OC enrichmentaccumulation in seagrass meadows and showed that the contributions of both a direct supply of seagrass-derived OC by belowground production and particle trapping are important, although the latter is generally assumed as the main mechanism of OC enrichmentaccumulation in seagrass meadows compared with the bare sediment sites. Our results indicate that it is critical to consider both below- and aboveground biomass productivity in addition to the morphological complexity of seagrass meadows as factors controlling OC, and that identifying the mechanism of enrichmentaccumulation is important for improving OC stock estimation and reducing the uncertainty in global blue-carbon estimates.

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## Authors contribution

Toko Tanaya, Hajime Kayanne, and Tomohiro Kuwae conceived the idea; Toko Tanaya, Kenta Watanabe, and Tomohiro Kuwae designed the methodology; Toko Tanaya, Kenta Watanabe, Shoji Yamamoto, and Chuki Hongo collected the samples and data; Toko Tanaya and Kenta Watanabe performed sample analyses; Toko Tanaya and Tomohiro Kuwae led the writing  
5 of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## Competing interests

The authors declare that they have no conflict of interest.

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**Table 1: Organic carbon contents and dry weights of each component of living biomass at the back-reef and estuarine sites.**

	Back reef		Estuary	
	%OC (%DW)	Dry weight (g m <sup>-2</sup> )	%OC (%DW)	Dry weight (g m <sup>-2</sup> )
	mean ± SD ( <i>n</i> )	mean ± SD ( <i>n</i> )	mean ± SD ( <i>n</i> )	mean ± SD ( <i>n</i> )
Aboveground biomass	38.47 ± 3.06 (39)	74 ± 45 (16)	35.95 ± 2.28 (20)	70 ± 34 (5)
Belowground biomass	31.35 ± 2.93 (20)	675 ± 450 (16)	30.38 ± 2.55 (13)	1354 ± 847 (5)

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**Table 2: Organic carbon content,  $\delta^{13}\text{C}$ , and dry density of each of sediment and dead plant component at the back-reef and estuarine sites.**

	Back reef			Estuary		
	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )	Organic carbon		Dry density ( $\text{mg cm}^{-3}$ ) mean $\pm$ SD ( <i>n</i> )
	%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )		%OC (% DW) mean $\pm$ SD ( <i>n</i> )	$\delta^{13}\text{C}$ (‰ vs. VPDB) mean $\pm$ SD ( <i>n</i> )	
Fine sediment	0.37 $\pm$ 0.13 (60)	-12.8 $\pm$ 0.8 (60)	<del>893 <math>\pm</math> 303 0.89</del> <del><math>\pm</math>0.30(60)</del>	0.42 $\pm$ 0.20 (24)	-17.4 $\pm$ 3.6 (24)	<del>760 <math>\pm</math> 294 0.76</del> <del><math>\pm</math>0.29(24)</del>
Coarse sediment	0.32 $\pm$ 0.13 (20)	-12.8 $\pm$ 1.1 (20)	<del>292 <math>\pm</math> 152 0.29</del> <del><math>\pm</math>0.15(20)</del>	0.26 $\pm$ 0.08 (8)	-15.9 $\pm$ 1.5 (8)	<del>475 <math>\pm</math> 142 0.48</del> <del><math>\pm</math>0.14(8)</del>
Dead leaf	24.80 $\pm$ 3.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	<del>0.05 <math>\pm</math> 0.04 0.00</del> <del><math>\pm</math>0.00(20)</del>	23.31 $\pm$ 3.86 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	<del>0.03 <math>\pm</math> 0.04 0.00</del> <del><math>\pm</math>0.00(8)</del>
Dead sheath and rhizome	21.29 $\pm$ 4.07 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	<del>0.55 <math>\pm</math> 0.63 0.00</del> <del><math>\pm</math>0.00(20)</del>	27.52 $\pm$ 1.75 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	<del>1.44 <math>\pm</math> 1.86 0.00</del> <del><math>\pm</math>0.00(8)</del>
Dead root	19.25 $\pm$ 1.67 (3)	-8.9 $\pm$ 0.6 <sup>a</sup> (5)	<del>0.26 <math>\pm</math> 0.25 0.00</del> <del><math>\pm</math>0.00(20)</del>	19.94 $\pm$ 5.89 <sup>b</sup> (3)	-9.3 $\pm$ 0.2 <sup>a</sup> (5)	<del>0.31 <math>\pm</math> 0.35 0.00</del> <del><math>\pm</math>0.00(8)</del>

<sup>a</sup>Total of sheath and rhizomes, and root.

<sup>b</sup>At one sampling point (FS1) where the dominant species was different, the values were dead leaf, 25.77%; dead sheath and rhizome, 19.05%; and dead root, 19.21%.

**Table 3: Values of seagrass biomass organic carbon and sedimentary organic carbon mass in globally compiled data (Fourqurean *et al.*, 2012) and this study (mean  $\pm$  SD, *n*).**

	<u>Vegetated</u>		<u>No-vegetation</u>	
	<u>Seagrass biomass OC</u>	<u>Sedimentary OC</u>	<u>Seagrass biomass OC</u>	<u>Sedimentary OC</u>
	<u>(g C m<sup>-2</sup>)</u>	<u>(g C L<sup>-1</sup>)</u>	<u>(g C m<sup>-2</sup>)</u>	<u>(g C L<sup>-1</sup>)</u>
	<u>mean <math>\pm</math> SD (<i>n</i>)</u>	<u>mean <math>\pm</math> SD (<i>n</i>)</u>	<u>mean <math>\pm</math> SD (<i>n</i>)</u>	<u>mean <math>\pm</math> SD (<i>n</i>)</u>
<u>Fourqurean</u>	<u>251.4 <math>\pm</math> 395.6 (251)</u>	<u>12.32 <math>\pm</math> 8.04 (410)</u>	=	<u>8.08 <math>\pm</math> 5.90 (43)</u>
<u><i>et al.</i>, 2012</u>				
<u>This study</u>	<u>283.0 <math>\pm</math> 200.8 (21)</u>	<u>5.03 <math>\pm</math> 1.32 (21)</u>	=	<u>2.93 <math>\pm</math> 0.73 (7)</u>

## Figure Captions

Figure 1: Study sites. (a) (b) Study site location on Ishigaki Island, Japan. Sampling points at (c) the back-reef and (de) the estuarine site. At the back-reef site, the circle indicating the southernmost vegetated sampling point actually represents a cluster of six sampling points.

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Figure 2: The newly developed box corer and a sampled core. Schematic diagrams of (a) a cross section of a core and (b) the design of the corer. (c) Photograph of a core from the back-reef site. The dominant seagrass species is *Thalassia hemprichi*.

10 Figure 3: Calculation of total OC mass ( $OC_{total}$ ;  $g\ C\ m^{-2}$ ) in the top 0.15-m layer.  $OC_{sed}$  is sedimentary OC.  $OC_{bio}$  is OC in live seagrass ~~bodies~~,  $OC_{dead}$  is OC in dead plant structures (>2 mm in size: dead seagrass leaves, sheaths, rhizomes, and roots detached from live structures),  $OC_{csed}$  is OC in the coarse (>1 mm diameter) sediments (excluding dead plant structures >2 mm in size), and  $OC_{fsed}$  is OC in the fine (<1 mm diameter) sediments (including dead plant structures <1 mm in size).

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Figure 4: OC mass ( $OC_{bio}$ ,  $OC_{dead}$ ,  $OC_{csed}$ ,  $OC_{fsed}$ ,  $OC_{sed}$ , and  $OC_{total}$ ) at (a) no-vegetation (bare and unvegetated) points at the back-reef site, (b) vegetated points at the back-reef site, (c) no-vegetation points

at the estuarine site, and (d) vegetated points at the estuarine site. Boxes show the 25% and 75% quantiles; horizontal bands inside the boxes are median values; whiskers show maximum and minimum values; and the open circles are outliers.

OC mass ( $OC_{bio}$ ,  $OC_{dead}$ ,  $OC_{esed}$ ,  $OC_{fsed}$ ,  $OC_{sed}$ , and  $OC_{total}$ ) at (a) the back reef site and (b) the estuarine site.

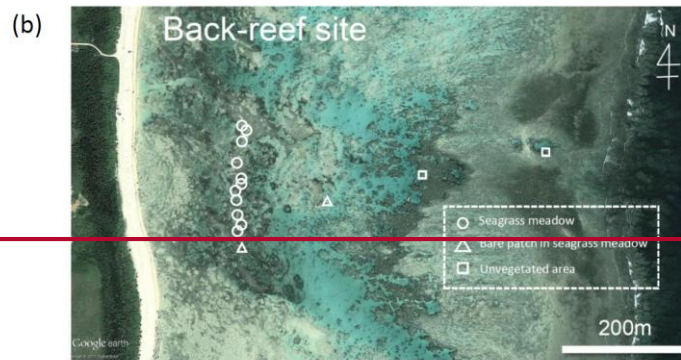
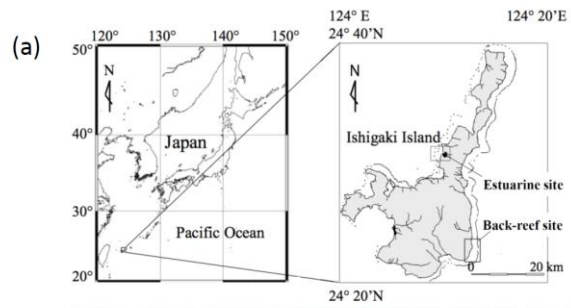
5 ~~Boxes show the 25% and 75% quantiles; horizontal bands inside the box are median values; whiskers show maximum and minimum values; and the open circle is an outlier.~~

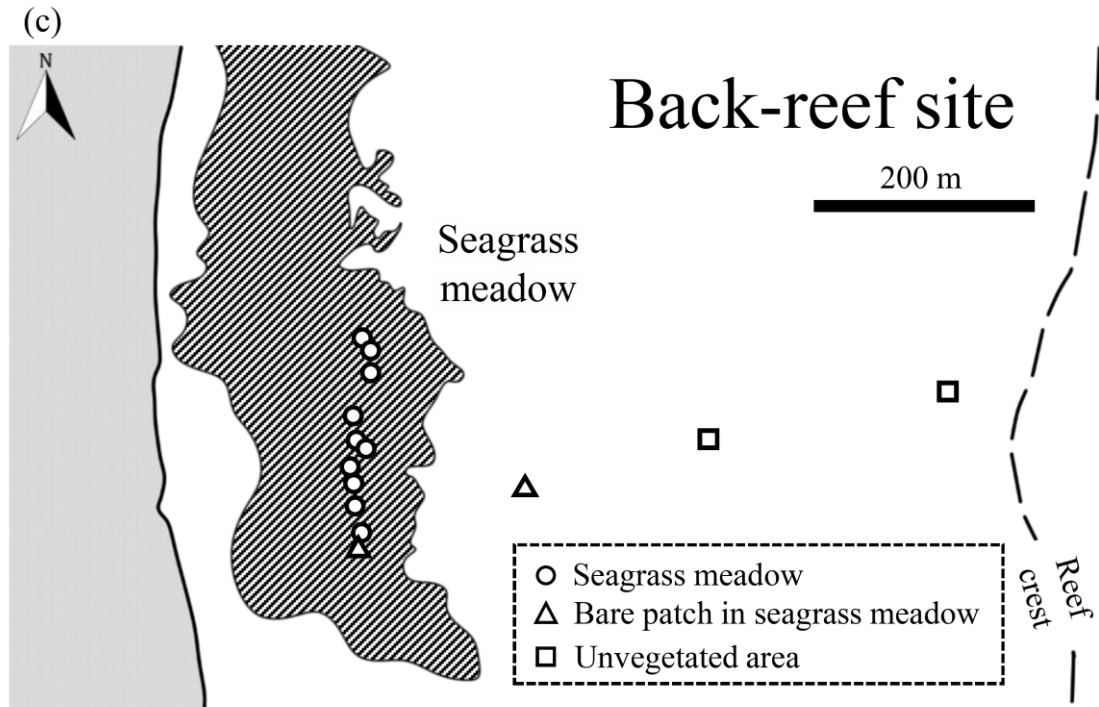
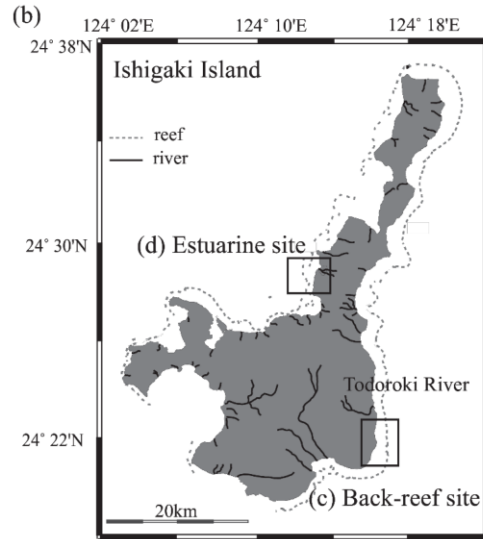
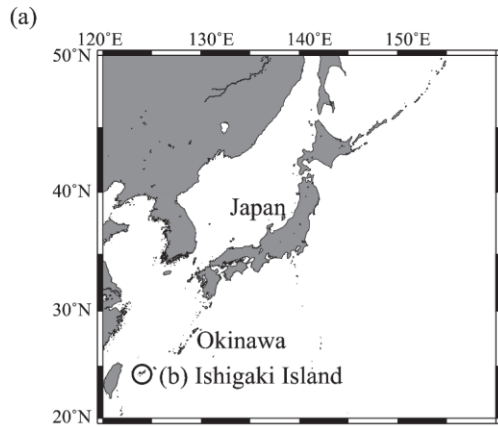
Figure 5: (a)  $OC_{bio}$  (sum of aboveground and belowground biomass) ( $g\ C\ m^{-2}$ ); (b) contribution of belowground biomass to  $OC_{bio}$  (%); (c)  $OC_{dead}$  (sum of above- and belowground detritus ( $g\ C\ m^{-2}$ ); and (d) contribution of belowground detritus to  $OC_{dead}$  (%). Boxes show the 25% and 75% quantiles; horizontal bands inside the box are median values; whiskers show maximum and minimum values; and open circles show outliers. (a) and (b) show the data of vegetated sampling points and (c) and (d) show the data of vegetated and bare sampling points.

15 Figure 6:  $\delta^{13}C_{sed}$  at each site and the  $\delta^{13}C$  values of potential sources of OC of  $\delta^{13}C_{sed}$  (means  $\pm$  SE).

Figure 7: Relationships at the back-reef (blue) and estuarine (orange) sites between  $OC_{sed}$  and (a) biomass ( $g\ C\ m^{-2}$ ), (b) aboveground biomass ( $g\ C\ m^{-2}$ ), (c) belowground biomass ( $g\ C\ m^{-2}$ ), and (d)  $OC_{dead}$  ( $g\ C\ m^{-2}$ ), and between (e)  $OC_{sed}$  and  $\delta^{13}C_{sed}$ , (f)  $\delta^{13}C_{sed}$  and belowground biomass, and (g)  $\delta^{13}C_{sed}$  and  $OC_{dead}$ .

- 5 Figure 8: Proposed mechanisms of OC ~~stock enrichment~~accumulation at our study sites. At the back-reef site dominated by *Thalassia hemprichii*, direct supply of recalcitrant belowground seagrass detritus is a major pathway of  $OC_{sed}$  ~~enrichment~~accumulation. At the estuarine site dominated by *Enhalus acoroides*, trapping of suspended autochthonous and allochthonous OC is the major pathway of OC ~~enrichment~~accumulation. A difference in the turnover rate of belowground biomass likely caused the major
- 10 mechanism of OC ~~stock enrichment~~accumulation to differ between the sites.





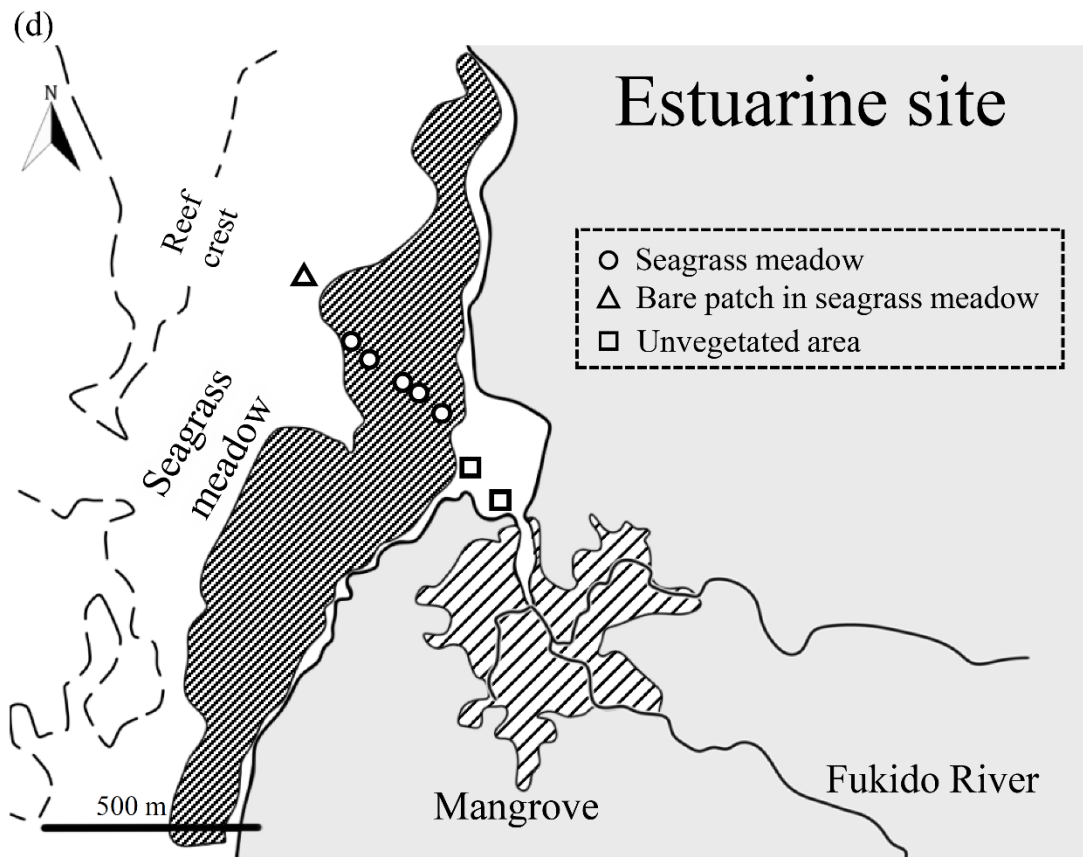
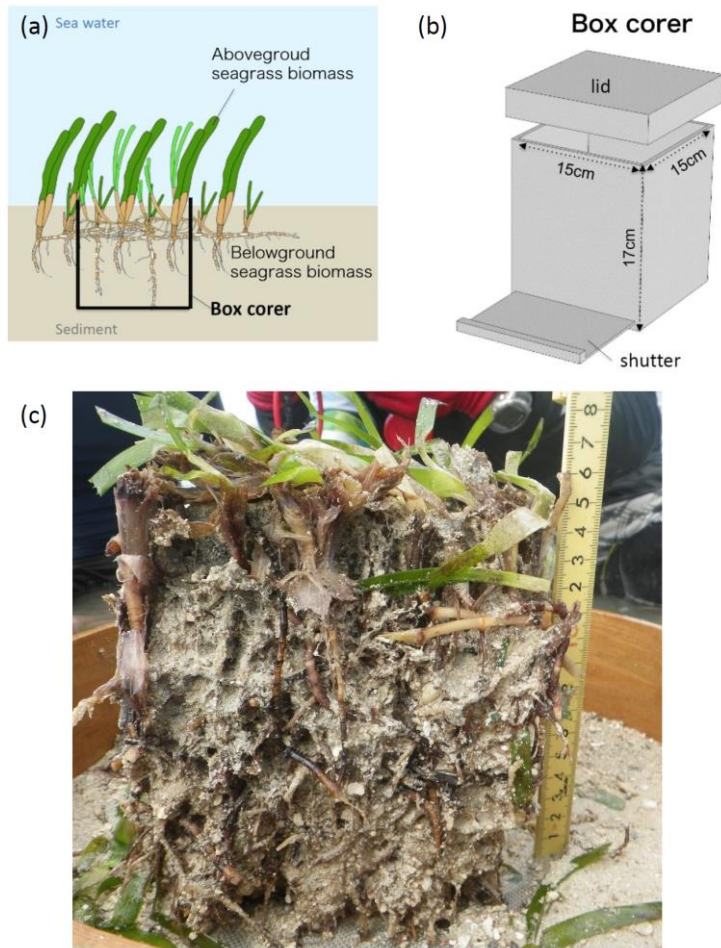


Figure 1: Study sites. (a) (b) Study site location on Ishigaki Island, Japan. Sampling points at (c) (b) the back-reef and (d) (e) the estuarine site. At the back-reef site, the circle indicating the southernmost vegetated sampling point actually represents a cluster of six sampling points.





**Figure 2: The newly developed box corer and a sampled core. Schematic diagrams of (a) a cross section of a core and (b) the design of the corer. (c) Photograph of a core from the back-reef site. The dominant seagrass species is *Thalassia hemprichi*.**

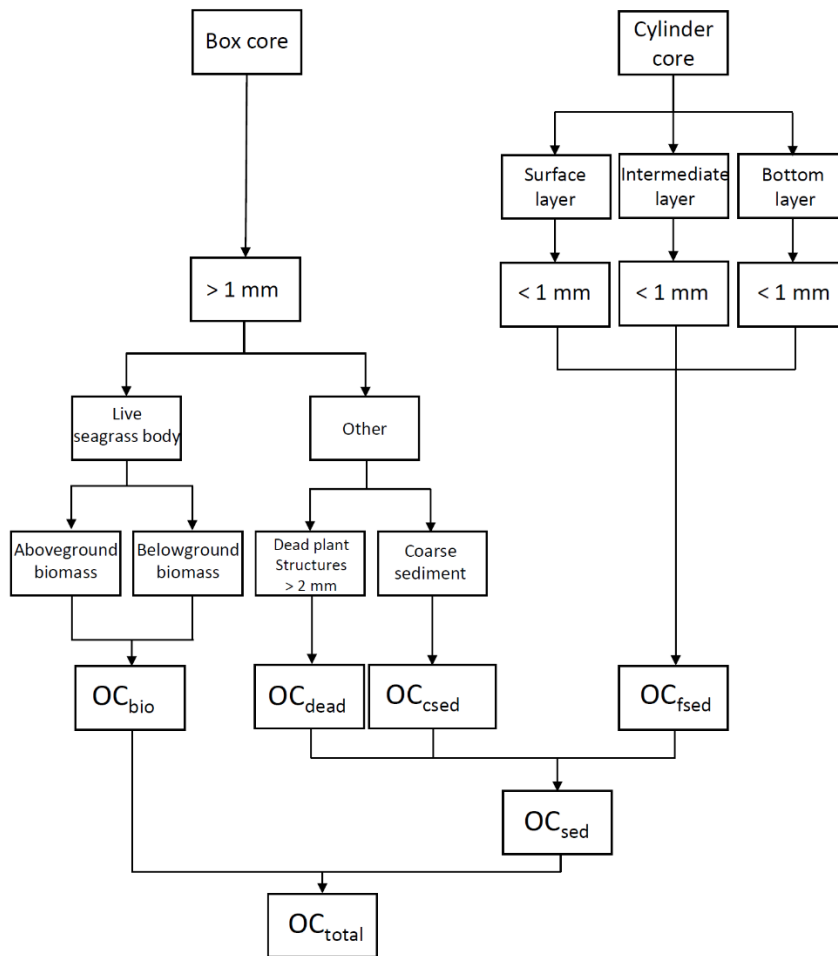
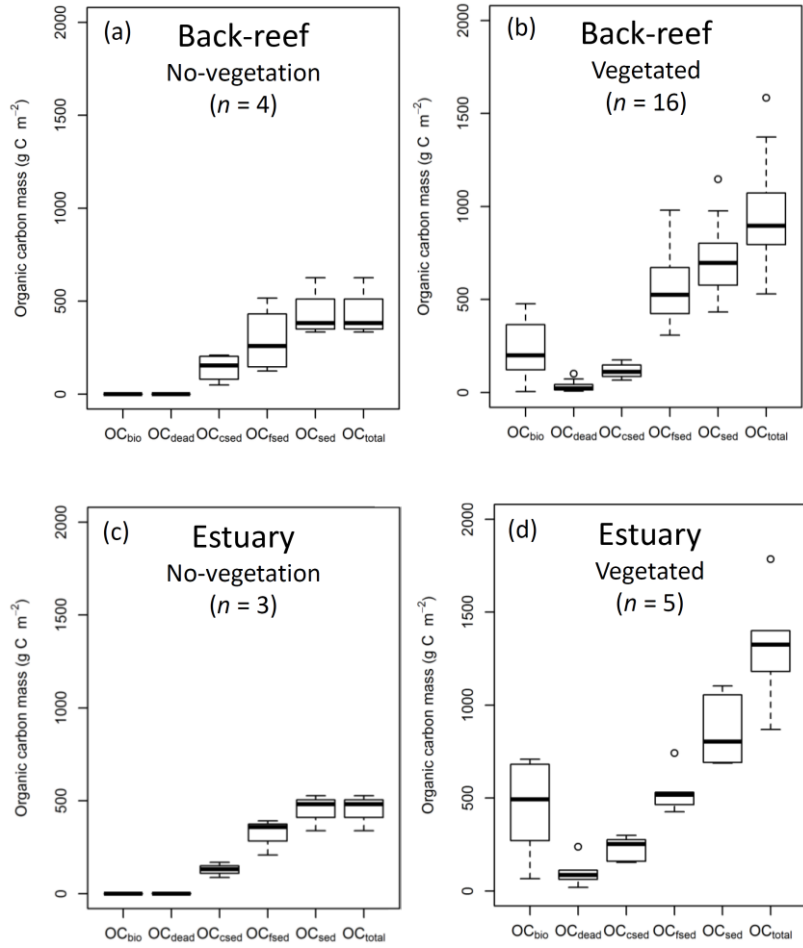
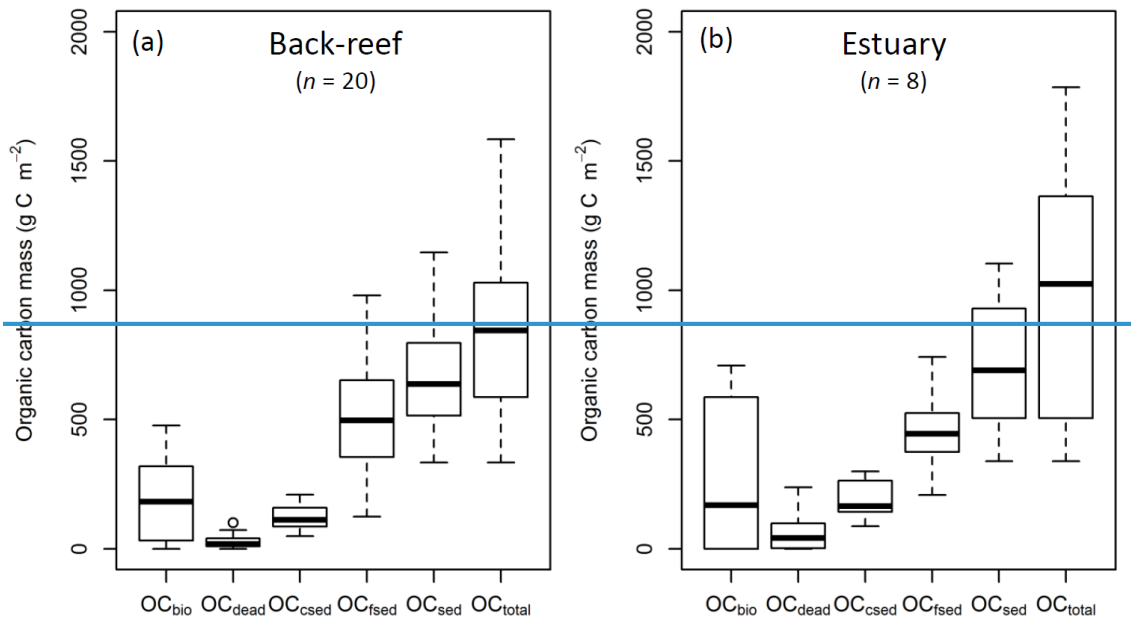


Figure 3: Calculation of total OC mass ( $OC_{total}$ ;  $g\ C\ m^{-2}$ ) in the top 0.15-m layer.  $OC_{sed}$  is sedimentary OC,  $OC_{bio}$  is OC in live seagrass bodies,  $OC_{dead}$  is OC in dead plant structures (>2 mm in size: dead seagrass leaves, sheaths, rhizomes, and roots detached from live structures),  $OC_{csed}$  is OC in the coarse (>1 mm diameter) sediments (excluding dead plant structures >2 mm in size), and  $OC_{fsed}$  is OC in the fine (<1 mm diameter) sediments (including dead plant structures <1 mm in size).

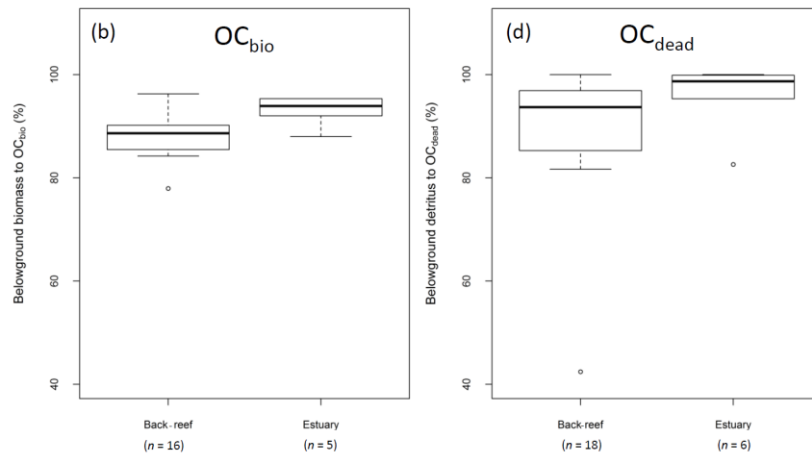
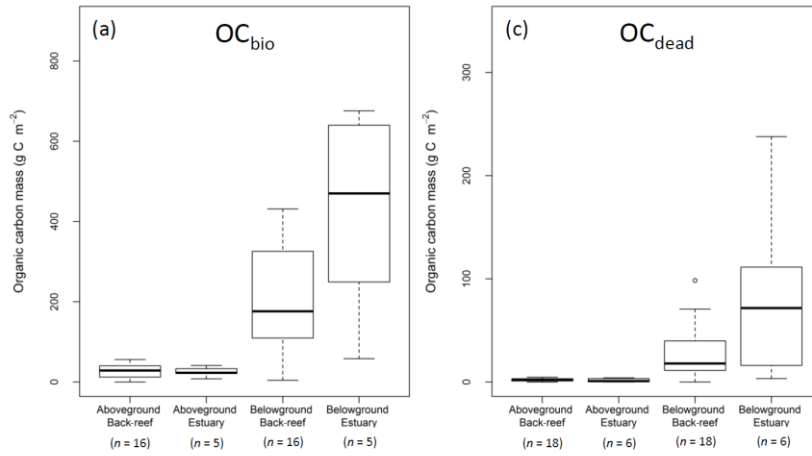
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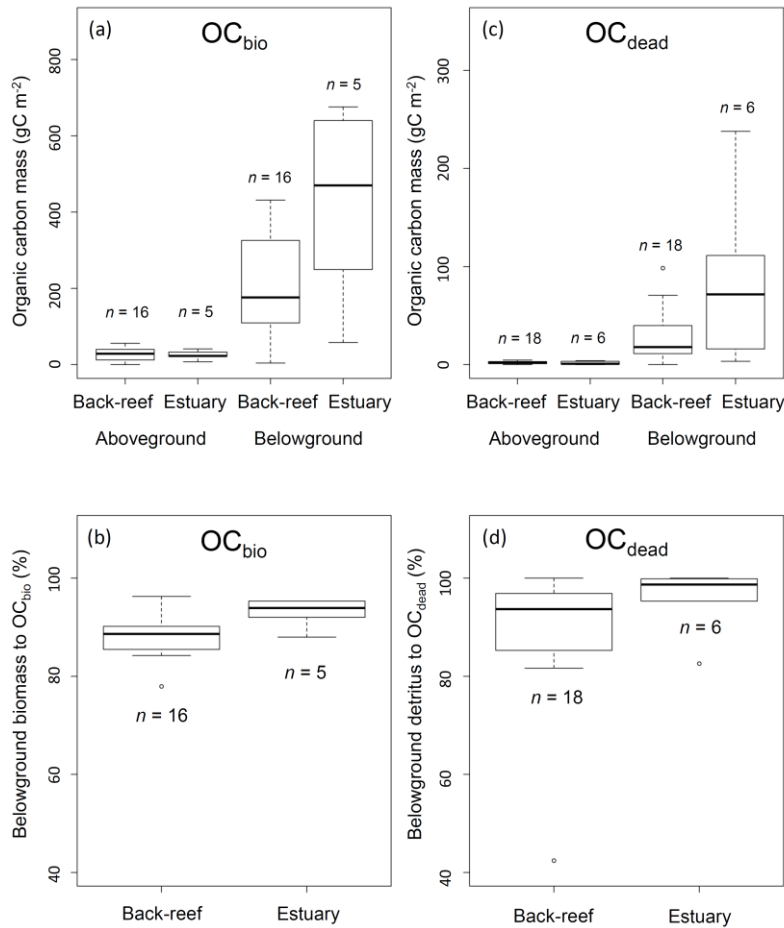




**Figure 4:** OC mass (OC<sub>bio</sub>, OC<sub>dead</sub>, OC<sub>csed</sub>, OC<sub>fsed</sub>, OC<sub>sed</sub>, and OC<sub>total</sub>) at (a) no-vegetation (bare and unvegetated) points at the back-reef site, (b) vegetated points at the back-reef site, (c) no-vegetation points at the estuarine site, and (d) vegetated points at the estuarine site. Boxes show the 25% and 75% quantiles; horizontal bands inside the boxes are median values; whiskers show maximum and minimum values; and the open circles are outliers.

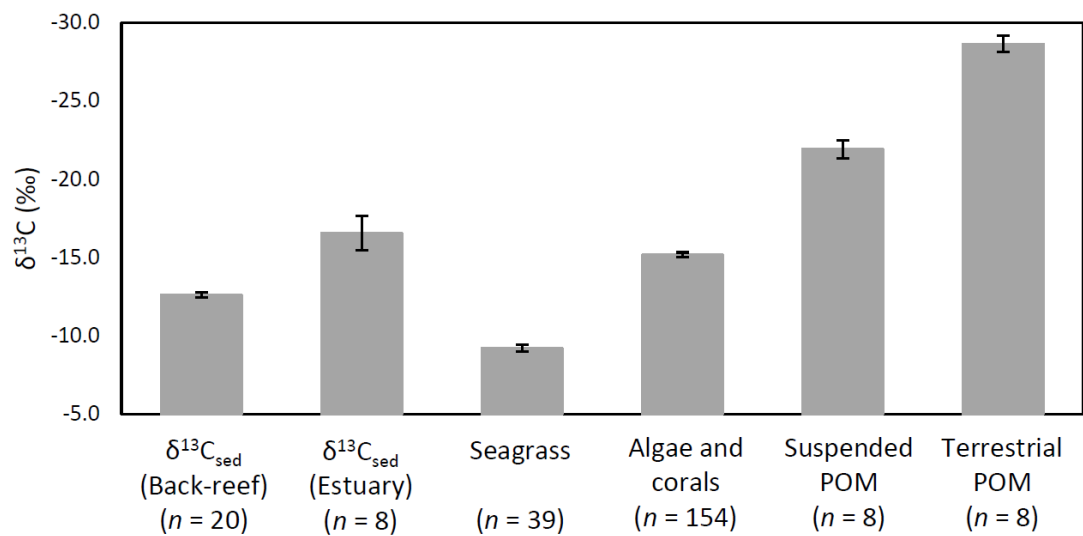
5 OC mass (OC<sub>bio</sub>, OC<sub>dead</sub>, OC<sub>csed</sub>, OC<sub>fsed</sub>, OC<sub>sed</sub>, and OC<sub>total</sub>) at (a) the back-reef site and (b) the estuarine site. Boxes show the 25% and 75% quantiles; horizontal bands inside the box are median values; whiskers show maximum and minimum values; and the open circle is an outlier.



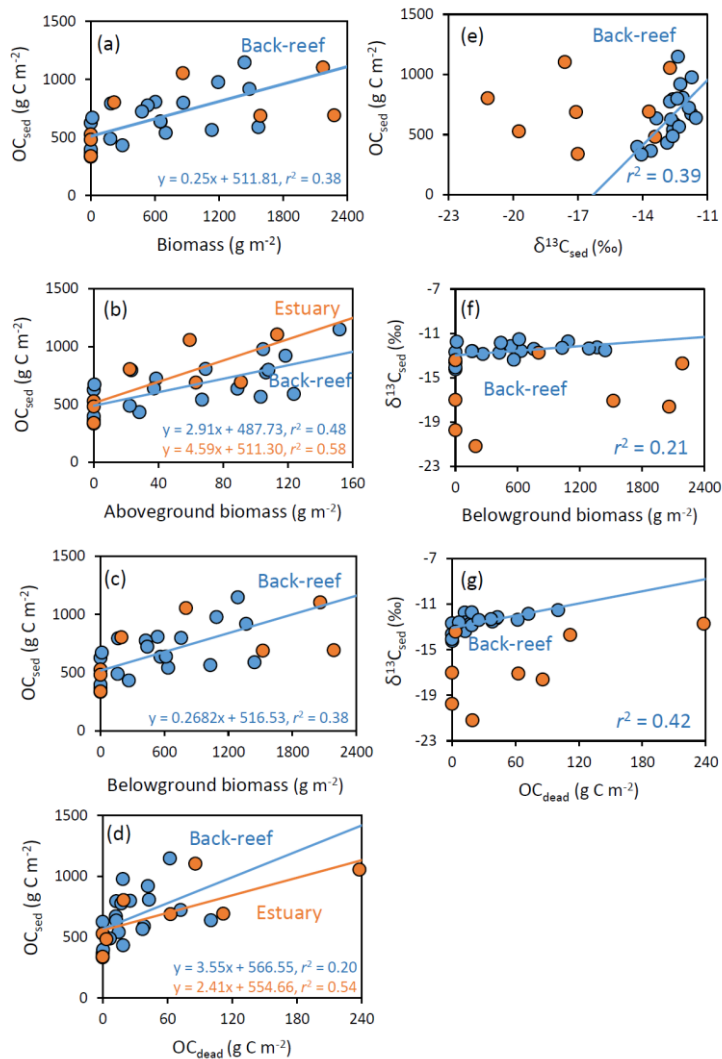


**Figure 5: (a)  $\text{OC}_{\text{bio}}$  (sum of aboveground and belowground biomass) ( $\text{g C m}^{-2}$ ); (b) contribution of belowground biomass to  $\text{OC}_{\text{bio}}$  (%); (c)  $\text{OC}_{\text{dead}}$  (sum of above- and belowground detritus ( $\text{g C m}^{-2}$ ); and (d) contribution of belowground detritus to  $\text{OC}_{\text{dead}}$  (%). Boxes show the 25% and 75% quantiles; horizontal bands inside the box are median values; whiskers show maximum and minimum values; and open circles show outliers. [\(a\) and \(b\)](#) show the data of vegetated sampling points and [\(c\) and \(d\)](#) show the data of vegetated and bare sampling points.**

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**Figure 6:**  $\delta^{13}\text{C}_{\text{sed}}$  at each site and the  $\delta^{13}\text{C}$  values of potential sources of OC of  $\delta^{13}\text{C}_{\text{sed}}$  (means  $\pm$  SE).



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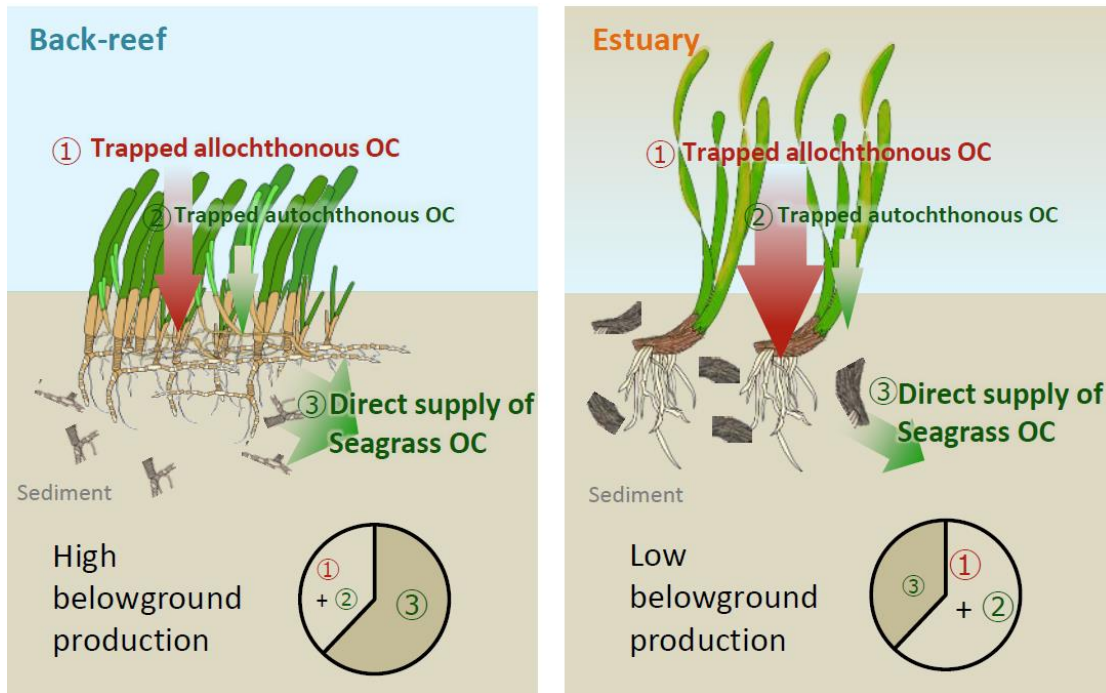


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