# Radiocarbon isotopic evidence for assimilation of atmospheric CO$_2$ by the seagrass *Zostera marina*

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## Abstract

Submerged aquatic vegetation takes up water-column dissolved inorganic carbon (DIC) as a carbon source across its thin cuticle layer. It is expected that marine macrophytes also use atmospheric CO$_2$ when exposed to air during low tide, although assimilation of atmospheric CO$_2$ has never been quantitatively evaluated. Using the radiocarbon isotopic signatures ($\Delta^{14}$C) of the seagrass *Zostera marina*, DIC and POC, we show quantitatively that *Z. marina* takes up and assimilates atmospheric modern CO$_2$ in a shallow coastal ecosystem. The $\Delta^{14}$C values of the seagrass (−40‰ to −10‰) were significantly higher than those of aquatic DIC (−46‰ to −18‰), indicating that the seagrass uses a $^{14}$C-rich carbon source (atmospheric CO$_2$, +17‰). A carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean, 17%) of its inorganic carbon as atmospheric CO$_2$. CO$_2$ exchange between the air and the seagrass might be enhanced by the presence of a very thin film of water over the air-exposed leaves during low tide. Our radiocarbon isotope analysis, showing assimilation of atmospheric modern CO$_2$ as an inorganic carbon source, improves our understanding of the role of seagrass meadows in coastal carbon dynamics.

## 1   Introduction

Submerged aquatic vegetation assimilates dissolved inorganic carbon (DIC) from the water column as a carbon source. Seagrasses take up DIC across their thin cuticle layer (Hemminga

and Duarte, 2000), as their leaves lack stomata despite being angiosperms (Larkum and Den Hartog, 1989). An alternative carbon source, atmospheric $CO_2$ ($C_{air}$), cannot directly reach seagrasses when they are completely submerged; however, seagrasses can take up $C_{air}$ when their leaves are exposed to air during low tide (Leuschner and Rees, 1993; Clavier et al., 2011; Jiang et al., 2014). Seagrasses rely largely on aqueous $CO_2$ [$CO_2(aq)$] as a carbon source for photosynthesis in nature (Beer and Koch, 1996). Some seagrass species, however, can use bicarbonate ions ($HCO_3^-$) as a major carbon source (Beer et al., 2002; Beer and Rehnberg, 1997), although there is considerable interspecific variation in $HCO_3^-$ utilization (Campbell and Fourqurean, 2013). As $CO_2(aq)$ is in limited supply under normal seawater conditions (pH ≈ 8), comprising only 1% (roughly 10–15 μmol $L^{-1}$) of the DIC pool, photosynthesis in seagrasses under high light conditions is frequently limited by carbon availability (Zimmerman et al., 1995; Invers et al., 2001; Campbell and Fourqurean, 2013). Under normal seawater pH conditions, the bicarbonate ion ($HCO_3^-$) is the most abundant inorganic carbon species, accounting for nearly 90% of the DIC pool (Plummer and Busenberg, 1982; Zeebe and Wolf-Gladrow, 2001). Some seagrass species indirectly use $HCO_3^-$ under low-$CO_2(aq)$ conditions (Beer et al., 2002; Campbell and Fourqurean, 2013), using one or both of the following suggested mechanisms: (1) extracellular dehydration of $HCO_3^-$ into $CO_2(aq)$ via membrane-bound enzymes (Beer and Rehnberg 1997); or (2) electrogenic proton ($H^+$) extrusion into an boundary layer on the leaf surface, facilitating $HCO_3^-/H^+$ cotransport (Hellblom et al. 2001).

Diffusion of $CO_2$ in water is much slower than that in air. During low tide, air-exposed aquatic macrophytes have a thin film of water between the air and their leaves, which promotes the uptake of $C_{air}$, in contrast to high tide, when there is a thick water layer inhibiting the uptake of $C_{air}$ (Ji and Tanaka, 2002). Previous studies have shown the possibility of $C_{air}$ uptake by seagrasses by using evidence from stable carbon isotope ratios

($\delta^{13}C$) in seagrasses and the two carbon sources (DIC and $C_{air}$) (Clavier et al., 2011; Cooper and McRoy, 1988; Raven et al., 2002). However, the $^{13}C$ method has considerable uncertainty because in addition to the source of carbon, the $\delta^{13}C$ values of seagrasses are also determined by other factors such as the chemical species of DIC [$CO_2(aq)$ or $HCO_3^-$] and the primary production rate. The chemical species in the carbonate system ($CO_2(aq)$, $HCO_3^-$, and carbonate ion [$CO_3^{2-}$]) have distinct $\delta^{13}C$ values, and isotopic fractionations change depending on pH and temperature (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995). Because the $\delta^{13}C$ of $HCO_3^-$ (0‰) is isotopically distinct from that of both $CO_2(aq)$ (−9‰) and $C_{air}$ (−8‰) under normal seawater conditions (pH ≈ 8), high $\delta^{13}C$ (>−10‰) in seagrasses shows that they use $HCO_3^-$ as a carbon source because isotopic discrimination during $CO_2$ assimilation results in $\delta^{13}C$ values that are always higher than those of the carbon sources. Although low $\delta^{13}C$ (<−10‰) in seagrasses could be explained by the assimilation of both $^{13}C$-depleted $CO_2(aq)$ and $C_{air}$, quantification of the contribution of $C_{air}$ is impossible because of the overlap between their $\delta^{13}C$ values.

The natural abundance of radiocarbon ($^{14}C$) has recently been used to assess food web structures (Ishikawa et al., 2014) and the origin and components of organic-matter pools (Goñi et al., 2013), as carbon sources have specific $^{14}C$ concentrations ($\Delta^{14}C$). The $\Delta^{14}C$ of inorganic carbon also has specific values depending on the source, such as DIC or $C_{air}$. The $\Delta^{14}C$ of DIC generally differs from that of atmospheric $CO_2$ because of the longer residence time of carbon in aquatic ecosystems than in the atmosphere (Ishikawa et al., 2014; Stuiver and Braziunas, 1993). The calculation of $\Delta^{14}C$ by internal correction using $\delta^{13}C$ values eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977), so the $\Delta^{14}C$ values of seagrasses are determined only by the two inorganic carbon sources. This study is the first to show quantitative evidence that the seagrass *Zostera marina* assimilates modern $C_{air}$, based on the $\Delta^{14}C$ values of the seagrass and two carbon sources.

## 2    Material and methods

### 2.1    Field surveys

Field surveys were conducted in 2014 during the growing season of *Z. marina* (May, July, September and November) in Furen Lagoon, Japan (Fig. 1; 43°19′46.5″N, 145°15′27.8″E). The lagoon is covered by ice from December to April. Furen Lagoon is brackish (salinity, ~30) and the northern part of the lagoon receives freshwater from the Furen, Yausubetsu, and Pon-Yausubetsu Rivers. The lagoon is covered by large seagrass meadows (67% of the total area) dominated by *Z. marina*. The offshore of the lagoon (Sea of Okhotsk) is influenced by the dynamics of both the Oyashio and the Soya warm current. Surface water samples (depth, 0.1 m) for DIC (concentration and isotopic signatures) and total alkalinity (TA) in the water column were collected from a research vessel along the salinity gradient at seven stations in the lagoon (Fig. 1; stations F1–F7). At each station, one water sample was collected for measuring DIC and TA and the salinity of the surface water was recorded with a conductivity-temperature sensor (COMPACT-CT; JFE Advantech, Nishinomiya, Japan). The samples for isotopic analysis of DIC were collected into 500-mL hermetically-sealed glass bottles (Duran bottle; SCHOTT AG, Mainz, Germany), which were poisoned by adding saturated mercuric chloride solution (400 µL per bottle) to prevent changes in DIC due to biological activity. The samples for measuring DIC concentration and TA were collected into 250-mL Duran bottles (SCHOTT AG), which were poisoned with saturated mercuric chloride solution (200 µL per bottle). Seagrass (*Z. marina*) leaves were collected at four stations covered by *Z. marina* meadows (Fig. 1; stations F3, F4, F8 and F9) along the salinity gradient. The stations were located in subtidal zones (mean water depth, 0.83–1.12 m). The aboveground wet-weight biomass of the seagrass, estimated from randomly thrown quadrats

1  (0.0625 m$^2$), ranged from 400 to 4300 g m$^{-2}$. Three or four independent samples of seagrass

2  leaves were collected at each station. Both the biofilm and epiphytes covering the leaves were

3  gently removed by hands with powder-free groves and washed off using ultrapure water

4  (Milli-Q water; Millipore, Billerica, MA, USA). To estimate the $\Delta^{14}$C of C$_{air}$, leaves of a

5  terrestrial plant (giant reed, *Phragmites australis*) were collected near the lagoon. Plant

6  samples were freeze-dried and subsamples were homogenized. To remove carbonate, the

7  plant samples were acidified with 1 N HCl and dried again.

8      Water samples for the isotopic analysis of terrestrial particulate organic carbon (POC) were

9  collected at three riverine stations (Fig. 1; stations R1–R3). Samples for POC were obtained

10  by filtration (approximately 1 L) onto pre-combusted (450 °C for 2 h) glass-fiber filters (GF/F,

11  Whatman, Maidstone, Kent, UK).

12

13  **2.2  Carbon isotope analysis**

14  We determined the stable carbon isotope ratios ($\delta^{13}$C) and radiocarbon concentrations ($\Delta^{14}$C)

15  of seagrass leaves, terrestrial plant leaves, DIC samples and POC samples. Prior to $\Delta^{14}$C and

16  $\delta^{13}$C measurements, samples were subjected to graphite purification as follows. DIC samples

17  for $\Delta^{14}$C and $\delta^{13}$C analysis were acidified (pH < 2) with H$_3$PO$_4$ and sparged using ultra-high

18  purity mixed N$_2$/H$_2$ gas. The powdered plant leaves and POC samples for $\Delta^{14}$C and $\delta^{13}$C

19  analysis were combusted in an elemental analyzer (either a Euro EA3000, EuroVector, Milan,

20  Italy; or a Flash 2000, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). For

21  each process, the CO$_2$ evolved was collected cryogenically and purified in a vacuum line. The

22  purified CO$_2$ was then reduced to graphite using hydrogen and an iron catalyst at 650 °C for

23  10 h. The $^{13}$C and $^{14}$C concentrations were measured using an accelerator mass spectrometer

1  (AMS). The AMS results are reported as $\Delta^{14}C$ (‰) values (Stuiver and Polach, 1977) as

2  follows:

3

4  $$\Delta^{14}C\ (‰) = \delta^{14}C - 2(\delta^{13}C + 25)(1 + \delta^{14}C/1000). \qquad (1)$$

5

6  The $\Delta^{14}C$ values were corrected by the radioactive decay of an international standard (oxalic

7  acid) since AD 1950 (Stuiver and Polach, 1977). The $\delta^{13}C$ values are reported relative to

8  Vienna Pee Dee Belemnite. $\delta^{13}C$ data were corrected using an internal standard. The

9  analytical precision of the AMS was within 0.7‰ for $\delta^{13}C$ and 3‰ for $\Delta^{14}C$.

10

11  **2.3   Carbonate system analysis**

12  DIC concentration and TA were determined on a batch-sample analyzer (ATT-05; Kimoto

13  Electric, Osaka, Japan). The precision of the analyses was 4 μmol L$^{-1}$ for DIC and 3 μmol L$^{-1}$

14  for TA. The concentrations of $CO_2(aq)$, $HCO_3^-$, and $CO_3^{2-}$ were estimated using chemical

15  equilibrium relationships and the TA and DIC concentrations of the water samples (Zeebe and

16  Wolf-Gladrow, 2001). The $\delta^{13}C$ values of $CO_2(aq)$ ($\delta^{13}C_{CO_2(aq)}$) and $HCO_3^-$ ($\delta^{13}C_{HCO_3^-}$) were

17  calculated as follows (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995):

18

19  $$\delta^{13}C_{HCO_3^-} = \delta^{13}C_{DIC} - ([\varepsilon_{db} \times [CO_2(aq)] + \varepsilon_{cb} \times [CO_3^{2-}])/[DIC], \qquad (2)$$

20  $$\delta^{13}C_{CO_2(aq)} = \delta^{13}C_{HCO_3^-} + \varepsilon_{db}, \qquad (3)$$

21  $$\varepsilon_{db} = \varepsilon(CO_2(aq) - HCO_3^-) = -9866/T + 24.12\ (‰), \qquad (4)$$

22  $$\varepsilon_{cb} = \varepsilon(CO_3^{2-} - HCO_3^-) = -867/T + 2.52\ (‰), \qquad (5)$$

1

2 where $[CO_2(aq)]$, $[CO_3^{2-}]$, and $[DIC]$ are the concentrations of $CO_2(aq)$, $CO_3^{2-}$ and $DIC$,

3 respectively; $T$ is water temperature (K); and $\varepsilon_{db}$ and $\varepsilon_{cb}$ are factors for the isotopic

4 fractionation between $CO_2(aq)$ and $HCO_3^-$, and between $CO_3^{2-}$ and $HCO_3^-$, respectively.

5

6 **2.4 Data analysis**

7 Because DIC taken up by seagrasses is a mixture of DIC from two sources (terrestrial and

8 oceanic) each having distinct $\Delta^{14}C$ values, it is reasonable to use salinity as a proxy for the

9 extent of mixing of these two sources as well as for the salinity gradient-based comparison

10 between $\Delta^{14}C$ of DIC and seagrass (Table 1). This comparison was therefore possible even

11 though DIC and *Z. marina* samples were not necessarily collected from the same stations (Fig.

12 1). A general linear model (GLM) was used to examine the differences between the $\Delta^{14}C$

13 values of the seagrass leaves and those of DIC in May and July 2014. These differences

14 provide evidence that the seagrasses assimilate $C_{air}$. A GLM was suitable for this study

15 because both continuous (salinity) and categorical variables (seagrass leaves or DIC) were

16 used as explanatory variables. We selected salinity, category (seagrass leaves or DIC), and

17 their interaction (salinity × category) as the explanatory variables (Table 1). We used

18 Akaike's Information Criterion (AIC) to select the most parsimonious model.

19     The relative contribution of $C_{air}$ to assimilated seagrass carbon was calculated by a two-

20 carbon-source mixing model using the $\Delta^{14}C$ values of DIC ($\Delta^{14}C_{DIC}$), $C_{air}$ ($\Delta^{14}C_{air}$), and the

21 seagrass ($\Delta^{14}C_{seagrass}$) at each of four stations as follows:

22

23         $C_{air}$ (% contribution) $= (\Delta^{14}C_{seagrass} - \Delta^{14}C_{DIC})/(\Delta^{14}C_{air} - \Delta^{14}C_{DIC}) \times 100.$     (6)

2    $\Delta^{14}C_{air}$ was estimated from the $\Delta^{14}C$ value of the sampled terrestrial plants ($\Delta^{14}C$ = +17.2‰).

3    The $\Delta^{14}C$ values of DIC as the carbon source for *Z. marina* in the mixing model were

4    estimated from the best GLM (Table 1).

5

6    ## 3   Results and discussion

7    Our radiocarbon isotopic analysis shows quantitatively that the seagrass *Z. marina* uses $C_{air}$ in

8    a shallow lagoon (Fig. 2a and Table 1). The GLM strongly highlights the effect of salinity on

9    the $\Delta^{14}C$ of DIC and the seagrass in May and July 2014 (Table 1; GLM, $P < 0.001$). Our

10   results indicate that the changes in $\Delta^{14}C_{DIC}$ are regulated mostly by mixing between high-$\Delta^{14}C$

11   river water and low-$\Delta^{14}C$ seawater: the seagrass uses aquatic DIC as the main carbon source,

12   as expected from previous studies (Hemminga and Duarte, 2000; Invers et al., 2001;

13   Campbell and Fourqurean, 2013). The $\Delta^{14}C_{seagrass}$ could reflect $\Delta^{14}C_{DIC}$ from May to July

14   because *Z. marina* leaves start to grow in early May at the study site, with the turnover time of

15   leaves being 30–90 days (mean, 60 days; Hosokawa et al., 2009). Furthermore, the negative

16   relationship between salinity and $\Delta^{14}C_{seagrass}$ cannot be explained by any residual

17   contamination from terrestrial organic carbon on the leaves because the terrestrial POC was

18   $^{14}C$-depleted (mean $\Delta^{14}C$ of terrestrial POC, −74.7 ± 23.4‰).

19     The model also reinforced our observations that $\Delta^{14}C_{seagrass}$ was higher than $\Delta^{14}C_{DIC}$ (Fig.

20   2a and Table 1; GLM, $P < 0.001$). This shows that the seagrass assimilates $^{14}C$-rich $C_{air}$ ($\Delta^{14}C$

21   around 17‰). The two-carbon-source mixing model indicated that the seagrass assimilated 0–

22   40% (mean ± SD, 17 ± 12%) of its inorganic carbon as $C_{air}$; the contribution was 20 ± 12% in

23   the low-salinity zone (salinity, 12–15) and 13 ± 12% in the high-salinity zone (salinity, 25–

24   29) (Fig. 2b). The contribution of $C_{air}$ as a carbon source varied greatly even between samples

from the same station (Fig. 2b). Because we did not determine the exposure time of each shoot in this study, we are unable to quantify any relationship between the contribution of $C_{air}$ and air exposure time; however, the exposure time could mediate the assimilation of $C_{air}$ (Clavier et al., 2011).

As $\Delta^{14}C_{DIC}$ was significantly lower than $\Delta^{14}C_{air}$, the contribution of $C_{air}$ can be determined for May and July 2014 (Fig. 2a). This radiocarbon isotopic approach would be useful in the high latitudes of the Pacific Ocean where surface seawater is $^{14}C$-depleted ($\Delta^{14}C_{DIC} < 0‰$) (Talley, 2007). In contrast, the $\Delta^{14}C_{DIC}$ in surface seawater is generally higher than $\Delta^{14}C_{air}$ in other regions of the Pacific Ocean because of bomb-derived $^{14}C$ (Talley, 2007).

In any case, the $\Delta^{14}C$ approach is potentially applicable to other regions by using the $\Delta^{14}C$ gradient. However, the seasonal dynamics of $\Delta^{14}C_{DIC}$ could affect the application of this approach because it is only applicable when the $\Delta^{14}C$ values for endmembers (seawater DIC, freshwater DIC, and $C_{air}$) are distinct (not overlapping) as they were in May and July 2014 during this study. We could not use the $\Delta^{14}C$ approach to quantify the $C_{air}$ contribution in September or November 2014 in Furen Lagoon because the $\Delta^{14}C_{DIC}$ of seawater increased to near $\Delta^{14}C_{air}$ and there was overlap between the two (Fig. 3). The overlapping in the range of values, induced by variations in the $\Delta^{14}C_{DIC}$ of seawater, likely caused by the dynamics of the Oyashio (mean $\Delta^{14}C_{DIC}$, $-41‰$; Aramaki et al., 2001) and the Soya warm current ($\Delta^{14}C_{DIC} > 50‰$; Aramaki et al., 2007) (Fig. 1). The variation in $\Delta^{14}C_{DIC}$ of seawater could also be affected by seasonal stratification via regulation of the upwelling of low-$\Delta^{14}C$ bottom water. The applicability of the $\Delta^{14}C$ technique to other areas will depend on the $\Delta^{14}C$ dynamics of endmembers.

Our $\Delta^{14}C$ analysis considerably reduces the limitations and uncertainties of conventional methods such as that using only $\delta^{13}C$ (Clavier et al., 2011; Cooper and McRoy, 1988; Raven

1 et al., 2002). In particular, the use of $\Delta^{14}C$ has the advantage of avoiding effects of isotopic

2 fractionation (Stuiver and Polach, 1977); the use of $\delta^{13}C$ does not and therefore generates

3 large uncertainties. The $\delta^{13}C$ of the seagrass was low ($-14.0 \pm 2.4$‰) in the low-salinity zone

4 (salinity, 12–15) and high ($-8.8 \pm 1.9$‰) in the high-salinity zone (salinity, 25–29) (Fig. 2c).

5 There were significant correlations between salinity and $\delta^{13}C$ of DIC, $HCO_3^-$, $CO_2(aq)$ and

6 the seagrass (Pearson's correlation coefficient: $P < 0.001$; Fig. 2c, d). As the $\delta^{13}C$ of $HCO_3^-$

7 was isotopically distinct from $\delta^{13}C$ of both $CO_2(aq)$ and $C_{air}$ (Fig. 2d) and as *Z. marina* also

8 uses $HCO_3^-$ as a carbon source under low-$CO_2(aq)$ conditions (Beer and Rehnberg, 1997), the

9 $\delta^{13}C$ of the seagrass should change depending on the contribution of $HCO_3^-$ as a carbon

10 source (Campbell and Fourqurean, 2009; Raven et al., 2002). However, it is not possible to

11 distinguish the contribution of $C_{air}$ from that of other carbon sources because the $\delta^{13}C$ of $C_{air}$

12 overlapped those of both $HCO_3^-$ and $CO_2(aq)$ (Fig. 2d). Furthermore, $\delta^{13}C$ of both $HCO_3^-$ and

13 $CO_2(aq)$ change through mixing between low-$\delta^{13}C$ river water and high-$\delta^{13}C$ seawater in

14 brackish areas (Fig. 2d).

15     In any case, there are large uncertainties when using $\delta^{13}C$ to quantitatively estimate the

16 contribution of $C_{air}$ as a carbon source because the isotopic fractionation that occurs in the

17 steps between the carbon source and organic plant compounds changes depending on the

18 photosynthetic rate (Raven et al., 2002). The radiocarbon isotopic approach can avoid the

19 uncertainties derived from both the contribution of $HCO_3^-$ as a carbon source and isotopic

20 fractionation in carbon assimilation.

21     The seagrass leaves assimilated $C_{air}$ when exposed to air during low tide (Fig. 4). $CO_2$

22 exchange between the air and water would occur at the very thin film of water on the air-

23 exposed seagrass leaves (Fig. 4c), likely enhancing the passive uptake of $C_{air}$ by diffusion.

24 Our high estimate of the $C_{air}$ contribution (mean, 17%) was unexpected because prior works

suggest that photosynthetic rates of seagrasses in intertidal zones decrease during air exposure (Clavier, 2011), particularly in cases of desiccation (Leuschner et al., 1998). However, the leaves of subtidal seagrass are never desiccated because of the presence of the thin film of water, which reduces the negative effects of air exposure (i.e., desiccation).

The net ecosystem production of seagrass meadows is a key factor determining whether they are sinks or sources of $C_{air}$ (Maher and Eyre, 2012; Tokoro et al., 2014; Watanabe and Kuwae, 2015). Previously, however, such an exchange of $CO_2$ has been thought to occur only via the air–water interface with subsequent exchange with seagrasses as DIC. This study using radiocarbon isotope analysis demonstrates the assimilation of modern $C_{air}$ by seagrass. Moreover, our radiocarbon isotopic approach has potential for application to other photoautotrophs living near the air–water interface, such as intertidal macroalgae and amphibious macrophytes. Other applications may include determining the origin of the DIC source (e.g., terrestrial or oceanic) in deeper seagrass systems. However, adequate determinations will require separation and stability in the endmember values (e.g., in oceanographic contexts and in the dynamics of $\Delta^{14}C$ in coastal waters). The relative contribution of gas exchange via the air–seagrass water film to the total exchange is still unknown. To understand the role of seagrass meadows in the global carbon cycle, it will be necessary in future studies to precisely measure $CO_2$ exchanges at both the air–water and air–seagrass water-film interfaces.

**Author contribution**

K.W. and T.K. designed this study, K.W. carried out the field surveys and analyzed the data, and K.W. and T.K. wrote the manuscript.

1 References

2 Aramaki, T., Watanabe, S., Kuji, T., and Wakatsuchi, M.: The Okhotsk-Pacific seawater

3 exchange in the viewpoint of vertical profiles of radiocarbon around the Bussol' Strait,

4 Geophys. Res. Lett., 28, 3971–3974, 2001.

5 Aramaki, T., Senjyu, T., Togawa, O., Otosaka, S., Suzuki, T., Kitamura, T., Amano, H., and

6 Volkov, Y. N.: Circulation in the northern Japan Sea studied chiefly with radiocarbon,

7 Radiocarbon, 49, 915–924, 2007.

8 Beer, S. and Koch, E.: Photosynthesis of marine macroalgae and seagrasses in globally

9 changing $CO_2$ environments, Mar. Ecol. Progr. Ser., 141, 199–204, 1996.

10 Beer, S. and Rehnberg, J.: The acquisition of inorganic carbon by the seagrass *Zostera marina*,

11 Aquat. Bot., 56, 277–283, 1997.

12 Beer, S., Björk, M., Hellblom, F., and Axelsson, L.: Inorganic carbon utilization in marine

13 angiosperms (seagrasses), Funct. Plant. Biol., 29, 349–354, 2002.

14 Campbell, J. E. and Fourqurean, J. W.: Interspecific variation in the elemental and stable

15 isotopic content of seagrasses in South Florida, Mar. Ecol. Prog. Ser., 387, 109–123, 2009.

16 Campbell, J. E. and Fourqurean, J. W.: Mechanisms of bicarbonate use influence the

17 photosynthetic carbon dioxide sensitivity of tropical seagrasses, Limnol. Oceanogr., 58, 839–

18 848, 2013.

19 Clavier, J., Chauvaud, L., Carlier, A., Amice, E., Van der Geest, M., Labrosse, P., Diagne, A.,

20 and Hily, C.: Aerial and underwater carbon metabolism of a *Zostera noltii* seagrass bed in the

21 Banc d'Arguin, Mauritania, Aquat. Bot., 95, 24–30, 2011.

22 Cooper, L. W. and McRoy, C. P.: Stable carbon isotope ratio variations in marine

23 macrophytes along intertidal gradients, Oecologia, 77, 238–241, 1988.

Goñi, M. A., O'Connor, A. E., Kuzyk, Z. Z., Yunker, M. B., Gobeil, C., and Macdonald, R. W.: Distribution and sources of organic matter in surface marine sediments across the North American Arctic margin, J. Geophys. Res. Oceans, 118, 4017–4035, 2013.

Hellblom, F., Beer, S., Björk, M., and Axelsson, L.: A buffer sensitive inorganic carbon utilisation system in *Zostera marina*, Aquat. Bot., 69, 55–62, 2001.

Hemminga, M. A. and Duarte, C. M.: Seagrass architectural features, in: Seagrass Ecology, edited by: Hemminga, M. A. and Duarte, C. M., Cambridge University Press, Cambridge, 27–64, 2000.

Hosokawa, S., Nakamura, Y., and Kuwae, T.: Temperature induces shorter leaf life span in an aquatic plant, Oikos, 118, 1158–1163, 2009.

Invers, O., Zimmerman, R. C., Alberte, R. S., Perez, M., and Romero, J.: Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters, J. Exp. Mar. Biol. Ecol., 265, 203–217, 2001.

Ishikawa, N. F., Uchida, M., Shibata, Y., and Tayasu, I.: Carbon storage reservoirs in watersheds support stream food webs via periphyton production, Ecology, 95, 1264–1271, 2014.

Ji, Y. and Tanaka, J.: Effect of desiccation on the photosynthesis of seaweeds from the intertidal zone in Honshu, Japan, Phycol. Res., 50, 145–153, 2002.

Jiang, Z., Huang, X., Zhang, J., Zhou, C., Lian, Z., and Ni, Z.: The effects of air exposure on the desiccation rate and photosynthetic activity of *Thalassia hemprichii* and *Enhalus acoroides*, Mar. Biol., 161, 1051–1061, 2014.

Larkum, A. W. D. and Den Hartog, C.: Evolution and biogeography of seagrasses, in: Biology of seagrasses. Aquatic Plant Studies, edited by: Larkum, A. W. D., McComb, A. J., and Shepherd, S. A., Elsevier, Amsterdam, 112–156, 1989.

Leuschner, C., Landwehr, S., and Mehlig, U.: Limitation of carbon assimilation of intertidal *Zostera noltii* and *Z. marina* by desiccation at low tide, Aquat. Bot., 62, 171−176, 1998.

Leuschner, C. and Rees, U.: $CO_2$ gas exchange of two intertidal seagrass species, *Zostera marina* L. and *Zostera noltii* Hornem., during emersion, Aquat. Bot., 45, 53−62, 1993.

Maher, D. T. and Eyre, B. D.: Carbon budgets for three autotrophic Australian estuaries: Implications for global estimates of the coastal air-water $CO_2$ flux, Global Biogeochem. Cy., 26, GB1032, 2012.

Plummer, L. N. and Busenberg, E.: The solubilities of calcite, aragonite and vaterite in $CO_2$-$H_2O$ solutions between 0 and 90°C, and an evaluation of the aqueous model for the system $CaCO_3$-$CO_2$-$H_2O$, Geochim. Cosmochim. Acta, 46, 1011−1040, 1982.

Raven, J. A., Johnston, A. M., Kübler, J. E., Korb, R., McInroy, S. G., Handley, L. L., Scrimgeour, C. M., Walker, D. I., Beardall, J., Vanderklift, M., Fredriksen, S., and Dunton, K. H.: Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses, Funct. Plant Biol., 29, 355–378, 2002.

Stuiver, M. and Braziunas, T. F.: Modeling atmospheric ^{14}C influences and ^{14}C ages of marine samples to 10,000 BC, Radiocarbon, 35, 137–189, 1993.

Stuiver, M. and Polach, H. A.: Discussion: reporting of ^{14}C data, Radiocarbon, 19, 355–363, 1977.

Talley, L. D.: Hydrographic Atlas of the World Ocean Circulation Experiment (WOCE), Volume 2: Pacific Ocean, edited by: Sparrow, M., Chapman, P., and Gould, J., International WOCE Project Office, Southampton, 2007.

Tokoro, T., Hosokawa, S., Miyoshi, E., Tada, K., Watanabe, K., Montani, S., Kayanne, H., and Kuwae, T.: Net uptake of atmospheric $CO_2$ by coastal submerged aquatic vegetation, Global Change Biol., 20, 1873–1884, 2014.

Watanabe, K. and Kuwae, T.: How organic carbon derived from multiple sources contributes to carbon sequestration processes in a shallow coastal system?, Global Change Biol., 21, 2612–2623, 2015.

Zeebe, R. E. and Wolf-Gladrow, D.: $CO_2$ in seawater: equilibrium, kinetics, and isotopes, in: Elsevier Oceanography Series 65, edited by: Halpern, D., Elsevier, Amsterdam, p.346, 2001.

Zhang, J., Quay, P. D., and Wilbur, D. O.: Carbon isotope fractionation during gas-water exchange and dissolution of $CO_2$, Geochim. Cosmochim. Acta, 59, 107–114, 1995.

Zimmerman, R. C., Kohrs, D. G., Steller, D. L., and Alberte, R. S.: Carbon partitioning in eelgrass: regulation by photosynthesis and the response to daily light–dark cycles, Plant Physiol., 108, 1665–1671, 1995.

1 Table 1. Coefficients (median ± standard error) and significance levels for the general linear

2 models (GLMs) examined for samples collected in May and July 2014. AIC, Akaike

3 information criterion.

| Variable | Best model (AIC = 288.33) | | Full model (AIC = 290.29) | |
|---|---|---|---|---|
| | Coefficient | $P$ | Coefficient | $P$ |
| Salinity | −1.78 ± 0.12 | <0.001 | −1.76 ± 0.18 | <0.001 |
| Category (seagrass) | 7.34 ± 2.00 | <0.001 | 8.23 ± 4.89 | n.s. |
| Salinity × category | | | −0.05 ± 0.25 | n.s. |
| (Intercept) | 4.40 ± 2.59 | n.s. | 3.97 ± 3.36 | n.s. |

4
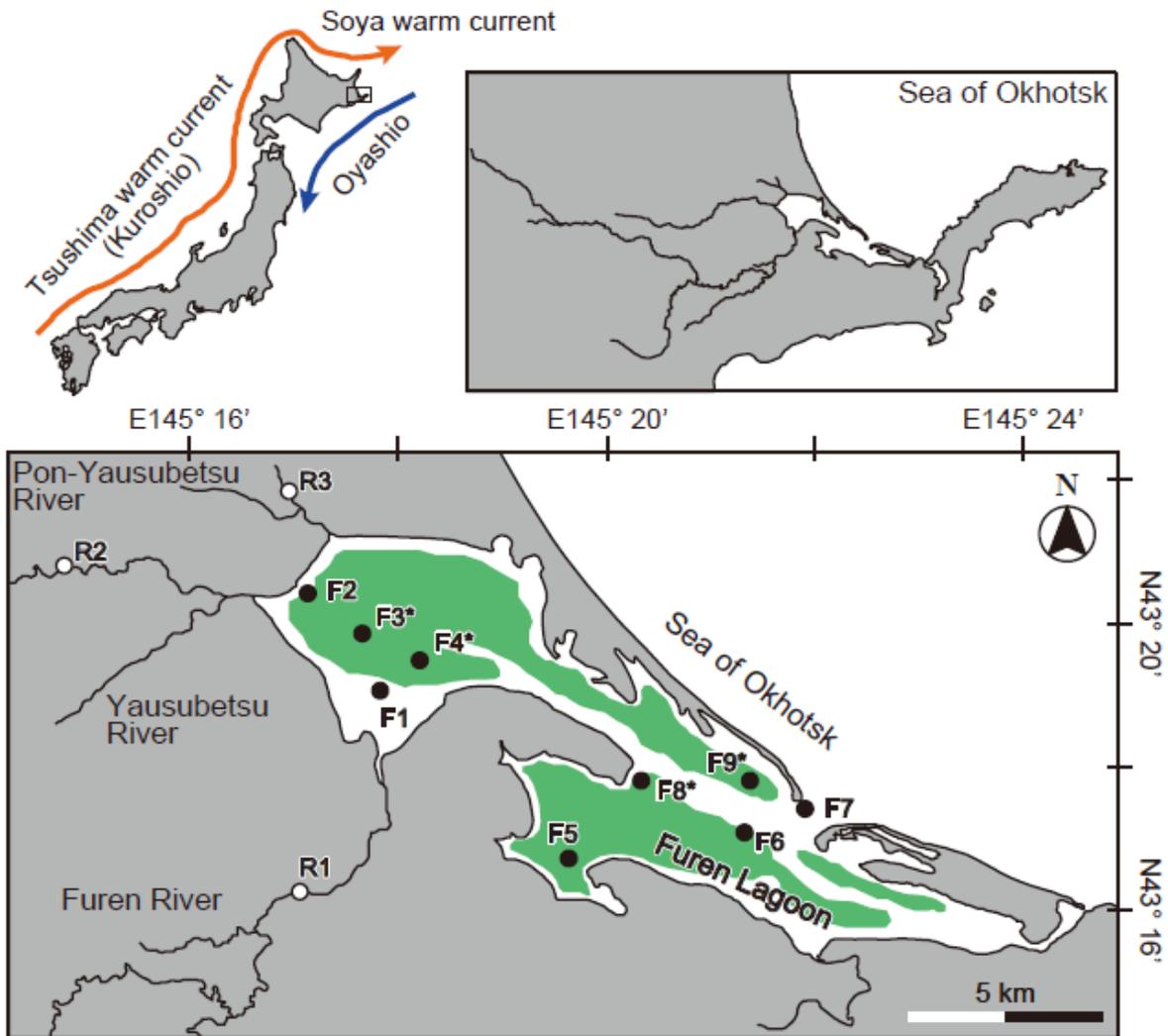
Figure 1. Location of Furen Lagoon and sampling stations. The area offshore of Furen Lagoon is affected by both the Oyashio and the Soya warm current. The northern part of the lagoon receives freshwater from the Furen, Yausubetsu, and Pon-Yausubetsu Rivers. Closed circles show lagoon stations. Water samples for DIC were collected at stations F1–F7. Seagrass samples were collected at stations F3, F4, F8 and F9 (marked with *). POC samples were collected at stations R1–R3. The green-shaded areas indicate seagrass meadows.
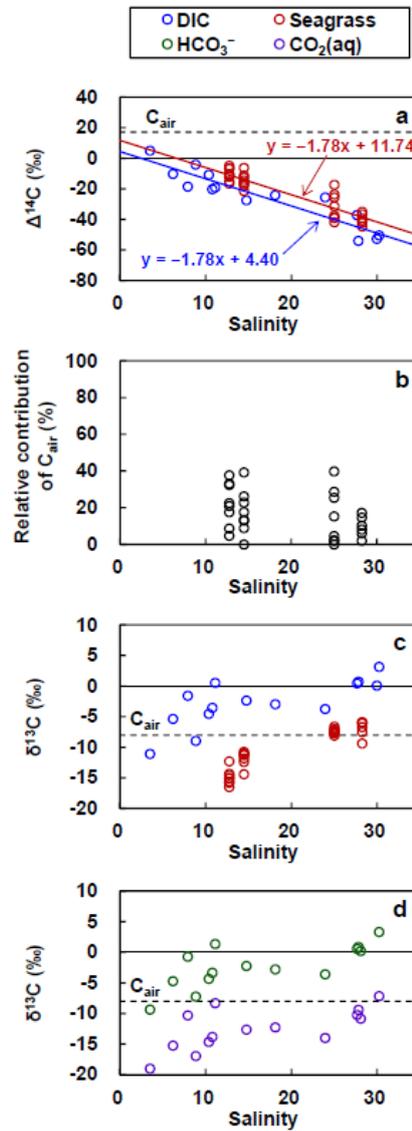
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2 Figure 2. **(a)** Spatial distribution of the $\Delta^{14}C$ values of dissolved inorganic carbon (DIC) (blue

3 open circles) and seagrass (red open circles) along the salinity gradient in May and July 2014

4 in Furen Lagoon, Japan. Blue and red solid lines represent the best fitting model of all general

5 linear models (GLMs) examined for DIC and seagrass, respectively. **(b)** Spatial distribution of

6 the relative contribution of $C_{air}$ to total inorganic carbon assimilated by seagrass along the

7 salinity gradient, as calculated by the two-carbon-source mixing model. **(c)** Spatial

8 distribution of the $\delta^{13}C$ values of DIC (blue open circles) and seagrass (red open circles) along

9 the salinity gradient. **(d)** Spatial distribution of the $\delta^{13}C$ values of bicarbonate ion ($HCO_3^-$)

10 (green open circles) and aqueous $CO_2$ [$CO_2(aq)$] (purple open circles) along the salinity

11 gradient. The dashed line indicates the isotopic signature of atmospheric $CO_2$ ($C_{air}$).
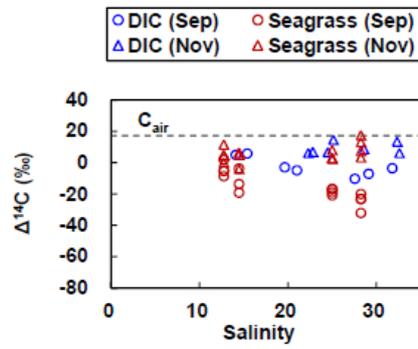
Figure 3. Spatial distribution of the $\Delta^{14}C$ values of dissolved inorganic carbon (DIC) (blue) and seagrass (red) along the salinity gradient in September (open circles) and November (open triangles) 2014 in Furen Lagoon, Japan. The dashed line indicates the $\Delta^{14}C$ of atmospheric $CO_2$ ($\Delta^{14}C_{air}$).
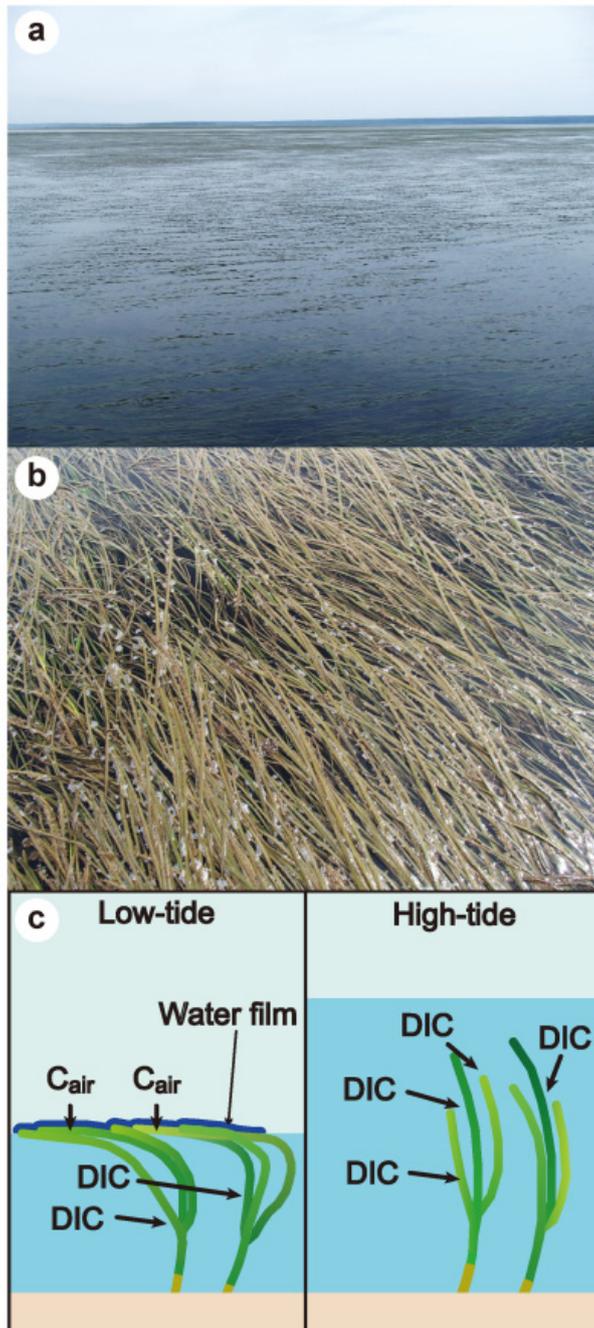
Figure 4. **(a)** Distant and **(b)** close-up views of the seagrass leaves exposed to the air during low tide in Furen Lagoon, Japan. **(c)** Conceptual diagram of the uptake of atmospheric $CO_2$ ($C_{air}$) across the surface-water film on the seagrass leaves during low tide (left), and the uptake of DIC during high tide (right).