

# Radiocarbon isotopic evidence for assimilation of atmospheric CO<sub>2</sub> 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<sub>2</sub> when exposed to air during low tide, although assimilation of atmospheric CO<sub>2</sub> has never been quantitatively evaluated. Using the radiocarbon isotopic signatures ( $\Delta^{14}\text{C}$ ) of the seagrass *Zostera marina*, DIC and POC, we show quantitatively that *Z. marina* takes up and assimilates atmospheric modern CO<sub>2</sub> in a shallow coastal ecosystem. The  $\Delta^{14}\text{C}$  values of the seagrass (−40‰ to −10‰) were significantly higher than those of aquatic DIC (−46‰ to −18‰), indicating that the seagrass uses a <sup>14</sup>C-rich carbon source (atmospheric CO<sub>2</sub>, +17‰). A carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean, 17%) of its inorganic carbon as atmospheric CO<sub>2</sub>. CO<sub>2</sub> 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<sub>2</sub> 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

1 and Duarte, 2000), as their leaves lack stomata despite being angiosperms (Larkum and Den  
2 Hartog, 1989). An alternative carbon source, atmospheric CO<sub>2</sub> (C<sub>air</sub>), cannot directly reach  
3 seagrasses when they are completely submerged; however, seagrasses can take up C<sub>air</sub> when  
4 their leaves are exposed to air during low tide (Leuschner and Rees, 1993; Clavier et al.,  
5 2011; Jiang et al., 2014). Seagrasses rely largely on aqueous CO<sub>2</sub> [CO<sub>2</sub>(aq)] as a carbon  
6 source for photosynthesis in nature (Beer and Koch, 1996). Some seagrass species, however,  
7 can use bicarbonate ions (HCO<sub>3</sub><sup>-</sup>) as a major carbon source (Beer et al., 2002; Beer and  
8 Rehnberg, 1997), although there is considerable interspecific variation in HCO<sub>3</sub><sup>-</sup> utilization  
9 (Campbell and Fourqurean, 2013). As CO<sub>2</sub>(aq) is in limited supply under normal seawater  
10 conditions (pH ≈ 8), comprising only 1% (roughly 10–15 μmol L<sup>-1</sup>) of the DIC pool,  
11 photosynthesis in seagrasses under high light conditions is frequently limited by carbon  
12 availability (Zimmerman et al., 1995; Invers et al., 2001; Campbell and Fourqurean, 2013).  
13 Under normal seawater pH conditions, the bicarbonate ion (HCO<sub>3</sub><sup>-</sup>) is the most abundant  
14 inorganic carbon species, accounting for nearly 90% of the DIC pool (Plummer and  
15 Busenberg, 1982; Zeebe and Wolf-Gladrow, 2001). Some seagrass species indirectly use  
16 HCO<sub>3</sub><sup>-</sup> under low-CO<sub>2</sub>(aq) conditions (Beer et al., 2002; Campbell and Fourqurean, 2013),  
17 using one or both of the following suggested mechanisms: (1) extracellular dehydration of  
18 HCO<sub>3</sub><sup>-</sup> into CO<sub>2</sub>(aq) via membrane-bound enzymes (Beer and Rehnberg 1997); or (2)  
19 electrogenic proton (H<sup>+</sup>) extrusion into an boundary layer on the leaf surface, facilitating  
20 HCO<sub>3</sub><sup>-</sup>/H<sup>+</sup> cotransport (Hellblom et al. 2001).

21 Diffusion of CO<sub>2</sub> in water is much slower than that in air. During low tide, air-exposed  
22 aquatic macrophytes have a thin film of water between the air and their leaves, which  
23 promotes the uptake of C<sub>air</sub>, in contrast to high tide, when there is a thick water layer  
24 inhibiting the uptake of C<sub>air</sub> (Ji and Tanaka, 2002). Previous studies have shown the  
25 possibility of C<sub>air</sub> uptake by seagrasses by using evidence from stable carbon isotope ratios

1 ( $\delta^{13}\text{C}$ ) in seagrasses and the two carbon sources (DIC and  $\text{C}_{\text{air}}$ ) (Clavier et al., 2011; Cooper  
2 and McRoy, 1988; Raven et al., 2002). However, the  $^{13}\text{C}$  method has considerable uncertainty  
3 because in addition to the source of carbon, the  $\delta^{13}\text{C}$  values of seagrasses are also determined  
4 by other factors such as the chemical species of DIC [ $\text{CO}_2(\text{aq})$  or  $\text{HCO}_3^-$ ] and the primary  
5 production rate. The chemical species in the carbonate system ( $\text{CO}_2(\text{aq})$ ,  $\text{HCO}_3^-$ , and  
6 carbonate ion [ $\text{CO}_3^{2-}$ ]) have distinct  $\delta^{13}\text{C}$  values, and isotopic fractionations change  
7 depending on pH and temperature (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995).  
8 Because the  $\delta^{13}\text{C}$  of  $\text{HCO}_3^-$  (0‰) is isotopically distinct from that of both  $\text{CO}_2(\text{aq})$  (-9‰) and  
9  $\text{C}_{\text{air}}$  (-8‰) under normal seawater conditions (pH  $\approx$  8), high  $\delta^{13}\text{C}$  ( $>$ -10‰) in seagrasses  
10 shows that they use  $\text{HCO}_3^-$  as a carbon source because isotopic discrimination during  $\text{CO}_2$   
11 assimilation results in  $\delta^{13}\text{C}$  values that are always higher than those of the carbon sources.  
12 Although low  $\delta^{13}\text{C}$  ( $<$ -10‰) in seagrasses could be explained by the assimilation of both  $^{13}\text{C}$ -  
13 depleted  $\text{CO}_2(\text{aq})$  and  $\text{C}_{\text{air}}$ , quantification of the contribution of  $\text{C}_{\text{air}}$  is impossible because of  
14 the overlap between their  $\delta^{13}\text{C}$  values.

15 The natural abundance of radiocarbon ( $^{14}\text{C}$ ) has recently been used to assess food web  
16 structures (Ishikawa et al., 2014) and the origin and components of organic-matter pools  
17 (Goñi et al., 2013), as carbon sources have specific  $^{14}\text{C}$  concentrations ( $\Delta^{14}\text{C}$ ). The  $\Delta^{14}\text{C}$  of  
18 inorganic carbon also has specific values depending on the source, such as DIC or  $\text{C}_{\text{air}}$ . The  
19  $\Delta^{14}\text{C}$  of DIC generally differs from that of atmospheric  $\text{CO}_2$  because of the longer residence  
20 time of carbon in aquatic ecosystems than in the atmosphere (Ishikawa et al., 2014; Stuiver  
21 and Braziunas, 1993). The calculation of  $\Delta^{14}\text{C}$  by internal correction using  $\delta^{13}\text{C}$  values  
22 eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977), so the  $\Delta^{14}\text{C}$   
23 values of seagrasses are determined only by the two inorganic carbon sources. This study is  
24 the first to show quantitative evidence that the seagrass *Zostera marina* assimilates modern  
25  $\text{C}_{\text{air}}$ , based on the  $\Delta^{14}\text{C}$  values of the seagrass and two carbon sources.

1

## 2 **2 Material and methods**

### 3 **2.1 Field surveys**

4 Field surveys were conducted in 2014 during the growing season of *Z. marina* (May, July,  
5 September and November) in Furen Lagoon, Japan (Fig. 1; 43°19'46.5"N, 145°15'27.8"E).  
6 The lagoon is covered by ice from December to April. Furen Lagoon is brackish (salinity,  
7 ~30) and the northern part of the lagoon receives freshwater from the Furen, Yausubetsu, and  
8 Pon-Yausubetsu Rivers. The lagoon is covered by large seagrass meadows (67% of the total  
9 area) dominated by *Z. marina*. The offshore of the lagoon (Sea of Okhotsk) is influenced by  
10 the dynamics of both the Oyashio and the Soya warm current. Surface water samples (depth,  
11 0.1 m) for DIC (concentration and isotopic signatures) and total alkalinity (TA) in the water  
12 column were collected from a research vessel along the salinity gradient at seven stations in  
13 the lagoon (Fig. 1; stations F1–F7). At each station, one water sample was collected for  
14 measuring DIC and TA and the salinity of the surface water was recorded with a  
15 conductivity-temperature sensor (COMPACT-CT; JFE Advantech, Nishinomiya, Japan). The  
16 samples for isotopic analysis of DIC were collected into 500-mL hermetically-sealed glass  
17 bottles (Duran bottle; SCHOTT AG, Mainz, Germany), which were poisoned by adding  
18 saturated mercuric chloride solution (400 µL per bottle) to prevent changes in DIC due to  
19 biological activity. The samples for measuring DIC concentration and TA were collected into  
20 250-mL Duran bottles (SCHOTT AG), which were poisoned with saturated mercuric chloride  
21 solution (200 µL per bottle). Seagrass (*Z. marina*) leaves were collected at four stations  
22 covered by *Z. marina* meadows (Fig. 1; stations F3, F4, F8 and F9) along the salinity gradient.  
23 The stations were located in subtidal zones (mean water depth, 0.83–1.12 m). The  
24 aboveground wet-weight biomass of the seagrass, estimated from randomly thrown quadrats

1 (0.0625 m<sup>2</sup>), ranged from 400 to 4300 g m<sup>-2</sup>. 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 gloves and washed off using ultrapure water  
4 (Milli-Q water; Millipore, Billerica, MA, USA). To estimate the  $\Delta^{14}\text{C}$  of  $\text{C}_{\text{air}}$ , 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 ( $\delta^{13}\text{C}$ ) and radiocarbon concentrations ( $\Delta^{14}\text{C}$ )  
15 of seagrass leaves, terrestrial plant leaves, DIC samples and POC samples. Prior to  $\Delta^{14}\text{C}$  and  
16  $\delta^{13}\text{C}$  measurements, samples were subjected to graphite purification as follows. DIC samples  
17 for  $\Delta^{14}\text{C}$  and  $\delta^{13}\text{C}$  analysis were acidified (pH < 2) with  $\text{H}_3\text{PO}_4$  and sparged using ultra-high  
18 purity mixed  $\text{N}_2/\text{H}_2$  gas. The powdered plant leaves and POC samples for  $\Delta^{14}\text{C}$  and  $\delta^{13}\text{C}$   
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  $\text{CO}_2$  evolved was collected cryogenically and purified in a vacuum line. The  
22 purified  $\text{CO}_2$  was then reduced to graphite using hydrogen and an iron catalyst at 650 °C for  
23 10 h. The  $^{13}\text{C}$  and  $^{14}\text{C}$  concentrations were measured using an accelerator mass spectrometer

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

3

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

5

6 The  $\Delta^{14}\text{C}$  values were corrected by the radioactive decay of an international standard (oxalic  
7 acid) since AD 1950 (Stuiver and Polach, 1977). The  $\delta^{13}\text{C}$  values are reported relative to  
8 Vienna Pee Dee Belemnite.  $\delta^{13}\text{C}$  data were corrected using an internal standard. The  
9 analytical precision of the AMS was within 0.7‰ for  $\delta^{13}\text{C}$  and 3‰ for  $\Delta^{14}\text{C}$ .

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  $4 \mu\text{mol L}^{-1}$  for DIC and  $3 \mu\text{mol L}^{-1}$   
14 for TA. The concentrations of  $\text{CO}_2(\text{aq})$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$  were estimated using chemical  
15 equilibrium relationships and the TA and DIC concentrations of the water samples (Zeebe and  
16 Wolf-Gladrow, 2001). The  $\delta^{13}\text{C}$  values of  $\text{CO}_2(\text{aq})$  ( $\delta^{13}\text{C}_{\text{CO}_2(\text{aq})}$ ) and  $\text{HCO}_3^-$  ( $\delta^{13}\text{C}_{\text{HCO}_3^-}$ ) were  
17 calculated as follows (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995):

18

$$19 \quad \delta^{13}\text{C}_{\text{HCO}_3^-} = \delta^{13}\text{C}_{\text{DIC}} - ([\varepsilon_{db} \times [\text{CO}_2(\text{aq})] + \varepsilon_{cb} \times [\text{CO}_3^{2-}]]/[\text{DIC}]), \quad (2)$$

$$20 \quad \delta^{13}\text{C}_{\text{CO}_2(\text{aq})} = \delta^{13}\text{C}_{\text{HCO}_3^-} + \varepsilon_{db}, \quad (3)$$

$$21 \quad \varepsilon_{db} = \varepsilon(\text{CO}_2(\text{aq}) - \text{HCO}_3^-) = -9866/T + 24.12 (\text{‰}), \quad (4)$$

$$22 \quad \varepsilon_{cb} = \varepsilon(\text{CO}_3^{2-} - \text{HCO}_3^-) = -867/T + 2.52 (\text{‰}), \quad (5)$$

1

2 where  $[\text{CO}_2(\text{aq})]$ ,  $[\text{CO}_3^{2-}]$ , and  $[\text{DIC}]$  are the concentrations of  $\text{CO}_2(\text{aq})$ ,  $\text{CO}_3^{2-}$  and DIC,  
3 respectively;  $T$  is water temperature (K); and  $\varepsilon_{db}$  and  $\varepsilon_{cb}$  are factors for the isotopic  
4 fractionation between  $\text{CO}_2(\text{aq})$  and  $\text{HCO}_3^-$ , and between  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$ , 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  $\Delta^{14}\text{C}$  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  $\Delta^{14}\text{C}$  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  $\Delta^{14}\text{C}$   
13 values of the seagrass leaves and those of DIC in May and July 2014. These differences  
14 provide evidence that the seagrasses assimilate  $\text{C}_{\text{air}}$ . 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  $\times$  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  $\text{C}_{\text{air}}$  to assimilated seagrass carbon was calculated by a two-  
20 carbon-source mixing model using the  $\Delta^{14}\text{C}$  values of DIC ( $\Delta^{14}\text{C}_{\text{DIC}}$ ),  $\text{C}_{\text{air}}$  ( $\Delta^{14}\text{C}_{\text{air}}$ ), and the  
21 seagrass ( $\Delta^{14}\text{C}_{\text{seagrass}}$ ) at each of four stations as follows:

22

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

1

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

3 The  $\Delta^{14}\text{C}$  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  $\text{C}_{\text{air}}$  in

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

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

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

11 river water and low- $\Delta^{14}\text{C}$  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  $\Delta^{14}\text{C}_{\text{seagrass}}$  could reflect  $\Delta^{14}\text{C}_{\text{DIC}}$  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  $\Delta^{14}\text{C}_{\text{seagrass}}$  cannot be explained by any residual

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

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

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

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

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

22 40% (mean  $\pm$  SD,  $17 \pm 12\%$ ) of its inorganic carbon as  $\text{C}_{\text{air}}$ ; the contribution was  $20 \pm 12\%$  in

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

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



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

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

10 In any case, the  $\Delta^{14}\text{C}$  approach is potentially applicable to other regions by using the  $\Delta^{14}\text{C}$   
11 gradient. However, the seasonal dynamics of  $\Delta^{14}\text{C}_{\text{DIC}}$  could affect the application of this  
12 approach because it is only applicable when the  $\Delta^{14}\text{C}$  values for endmembers (seawater DIC,  
13 freshwater DIC, and  $C_{\text{air}}$ ) are distinct (not overlapping) as they were in May and July 2014  
14 during this study. We could not use the  $\Delta^{14}\text{C}$  approach to quantify the  $C_{\text{air}}$  contribution in  
15 September or November 2014 in Furen Lagoon because the  $\Delta^{14}\text{C}_{\text{DIC}}$  of seawater increased to  
16 near  $\Delta^{14}\text{C}_{\text{air}}$  and there was overlap between the two (Fig. 3). The overlapping in the range of  
17 values, induced by variations in the  $\Delta^{14}\text{C}_{\text{DIC}}$  of seawater, likely caused by the dynamics of the  
18 Oyashio (mean  $\Delta^{14}\text{C}_{\text{DIC}}$ ,  $-41\text{‰}$ ; Aramaki et al., 2001) and the Soya warm current ( $\Delta^{14}\text{C}_{\text{DIC}} >$   
19  $50\text{‰}$ ; Aramaki et al., 2007) (Fig. 1). The variation in  $\Delta^{14}\text{C}_{\text{DIC}}$  of seawater could also be  
20 affected by seasonal stratification via regulation of the upwelling of low- $\Delta^{14}\text{C}$  bottom water.  
21 The applicability of the  $\Delta^{14}\text{C}$  technique to other areas will depend on the  $\Delta^{14}\text{C}$  dynamics of  
22 endmembers.

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

1 et al., 2002). In particular, the use of  $\Delta^{14}\text{C}$  has the advantage of avoiding effects of isotopic  
2 fractionation (Stuiver and Polach, 1977); the use of  $\delta^{13}\text{C}$  does not and therefore generates  
3 large uncertainties. The  $\delta^{13}\text{C}$  of the seagrass was low ( $-14.0 \pm 2.4\text{‰}$ ) in the low-salinity zone  
4 (salinity, 12–15) and high ( $-8.8 \pm 1.9\text{‰}$ ) in the high-salinity zone (salinity, 25–29) (Fig. 2c).  
5 There were significant correlations between salinity and  $\delta^{13}\text{C}$  of DIC,  $\text{HCO}_3^-$ ,  $\text{CO}_2(\text{aq})$  and  
6 the seagrass (Pearson's correlation coefficient:  $P < 0.001$ ; Fig. 2c, d). As the  $\delta^{13}\text{C}$  of  $\text{HCO}_3^-$   
7 was isotopically distinct from  $\delta^{13}\text{C}$  of both  $\text{CO}_2(\text{aq})$  and  $\text{C}_{\text{air}}$  (Fig. 2d) and as *Z. marina* also  
8 uses  $\text{HCO}_3^-$  as a carbon source under low- $\text{CO}_2(\text{aq})$  conditions (Beer and Rehnberg, 1997), the  
9  $\delta^{13}\text{C}$  of the seagrass should change depending on the contribution of  $\text{HCO}_3^-$  as a carbon  
10 source (Campbell and Fourqurean, 2009; Raven et al., 2002). However, it is not possible to  
11 distinguish the contribution of  $\text{C}_{\text{air}}$  from that of other carbon sources because the  $\delta^{13}\text{C}$  of  $\text{C}_{\text{air}}$   
12 overlapped those of both  $\text{HCO}_3^-$  and  $\text{CO}_2(\text{aq})$  (Fig. 2d). Furthermore,  $\delta^{13}\text{C}$  of both  $\text{HCO}_3^-$  and  
13  $\text{CO}_2(\text{aq})$  change through mixing between low- $\delta^{13}\text{C}$  river water and high- $\delta^{13}\text{C}$  seawater in  
14 brackish areas (Fig. 2d).

15 In any case, there are large uncertainties when using  $\delta^{13}\text{C}$  to quantitatively estimate the  
16 contribution of  $\text{C}_{\text{air}}$  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  $\text{HCO}_3^-$  as a carbon source and isotopic  
20 fractionation in carbon assimilation.

21 The seagrass leaves assimilated  $\text{C}_{\text{air}}$  when exposed to air during low tide (Fig. 4).  $\text{CO}_2$   
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  $\text{C}_{\text{air}}$  by diffusion.  
24 Our high estimate of the  $\text{C}_{\text{air}}$  contribution (mean, 17%) was unexpected because prior works

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

5 The net ecosystem production of seagrass meadows is a key factor determining whether  
6 they are sinks or sources of  $C_{\text{air}}$  (Maher and Eyre, 2012; Tokoro et al., 2014; Watanabe and  
7 Kuwae, 2015). Previously, however, such an exchange of  $\text{CO}_2$  has been thought to occur only  
8 via the air–water interface with subsequent exchange with seagrasses as DIC. This study  
9 using radiocarbon isotope analysis demonstrates the assimilation of modern  $C_{\text{air}}$  by seagrass.  
10 Moreover, our radiocarbon isotopic approach has potential for application to other  
11 photoautotrophs living near the air–water interface, such as intertidal macroalgae and  
12 amphibious macrophytes. Other applications may include determining the origin of the DIC  
13 source (e.g., terrestrial or oceanic) in deeper seagrass systems. However, adequate  
14 determinations will require separation and stability in the endmember values (e.g., in  
15 oceanographic contexts and in the dynamics of  $\Delta^{14}\text{C}$  in coastal waters). The relative  
16 contribution of gas exchange via the air–seagrass water film to the total exchange is still  
17 unknown. To understand the role of seagrass meadows in the global carbon cycle, it will be  
18 necessary in future studies to precisely measure  $\text{CO}_2$  exchanges at both the air–water and air–  
19 seagrass water-film interfaces.

20

## 21 **Author contribution**

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

24

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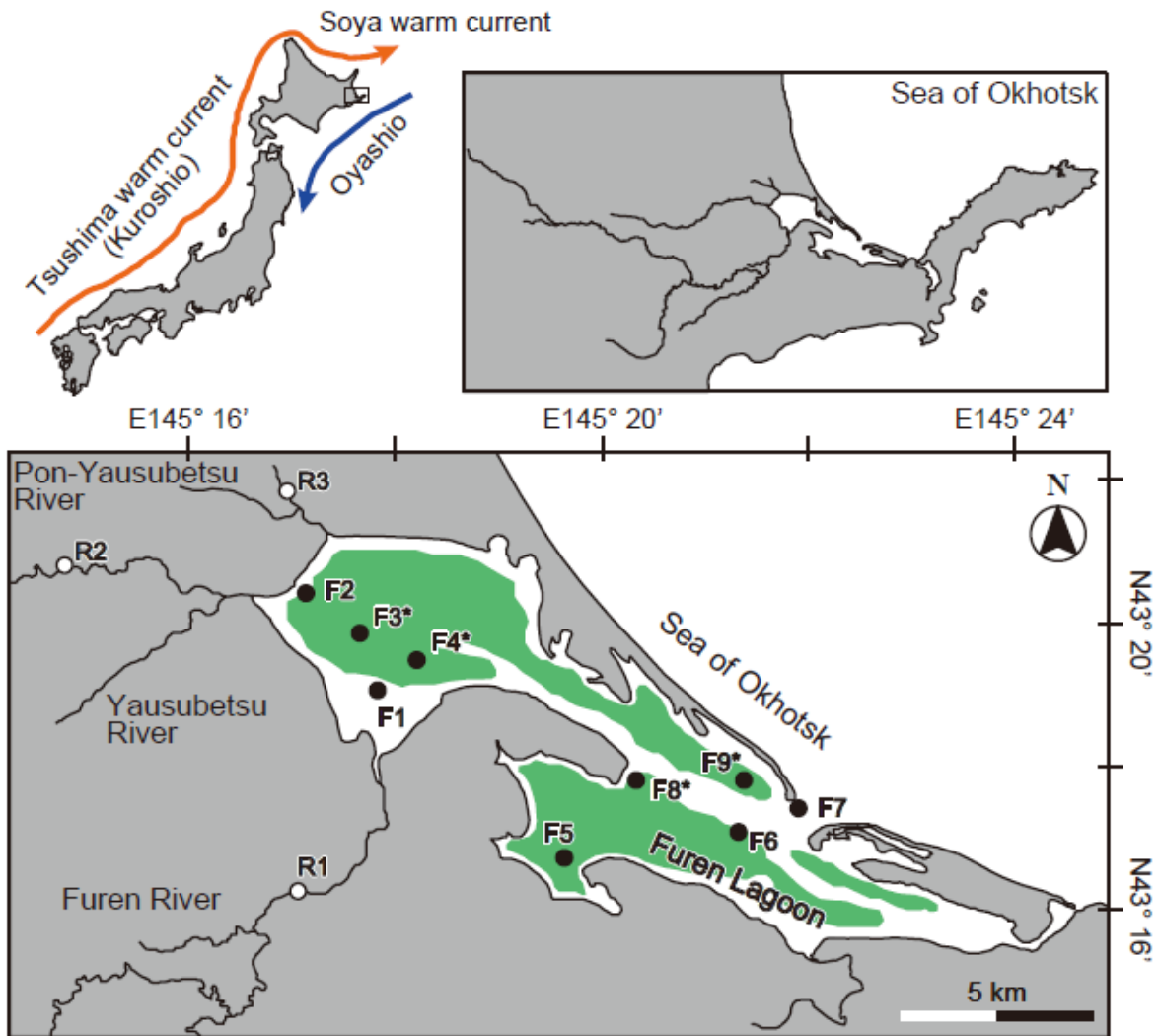
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1 Table 1. Coefficients (median  $\pm$  standard error) and significance levels for the general linear  
 2 models (GLMs) examined for samples collected in May and July 2014. AIC, Akaike  
 3 information criterion.

<b>Variable</b>	<b>Best model (AIC = 288.33)</b>		<b>Full model (AIC = 290.29)</b>	
	<b>Coefficient</b>	<b><i>P</i></b>	<b>Coefficient</b>	<b><i>P</i></b>
Salinity	-1.78 $\pm$ 0.12	<0.001	-1.76 $\pm$ 0.18	<0.001
Category (seagrass)	7.34 $\pm$ 2.00	<0.001	8.23 $\pm$ 4.89	n.s.
Salinity $\times$ category			-0.05 $\pm$ 0.25	n.s.
(Intercept)	4.40 $\pm$ 2.59	n.s.	3.97 $\pm$ 3.36	n.s.

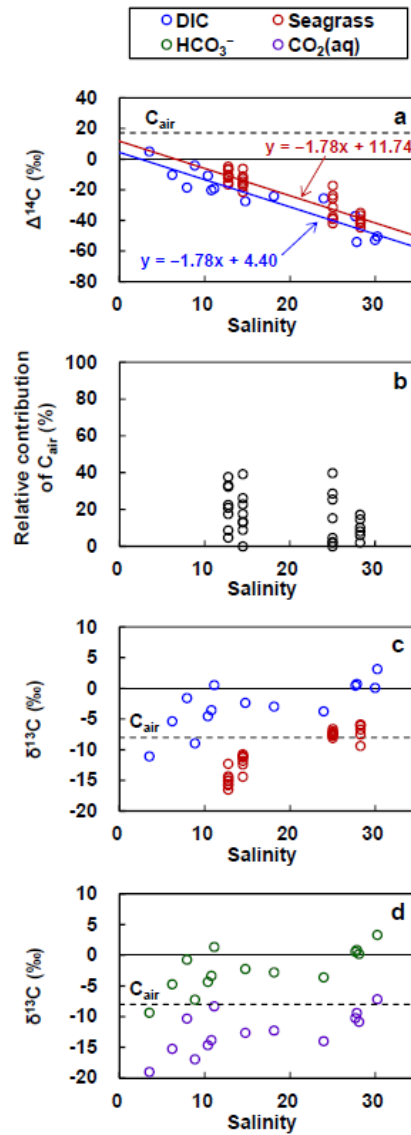
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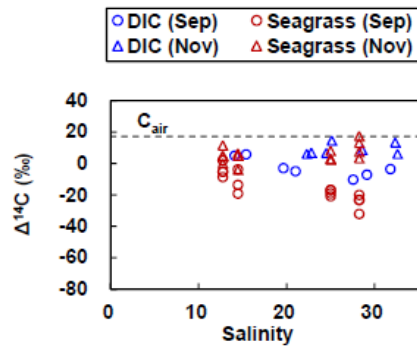


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 2 Figure 1. Location of Furen Lagoon and sampling stations. The area offshore of Furen Lagoon  
 3 is affected by both the Oyashio and the Soya warm current. The northern part of the lagoon  
 4 receives freshwater from the Furen, Yausubetsu, and Pon-Yausubetsu Rivers. Closed circles  
 5 show lagoon stations. Water samples for DIC were collected at stations F1–F7. Seagrass  
 6 samples were collected at stations F3, F4, F8 and F9 (marked with \*). POC samples were  
 7 collected at stations R1–R3. The green-shaded areas indicate seagrass meadows.

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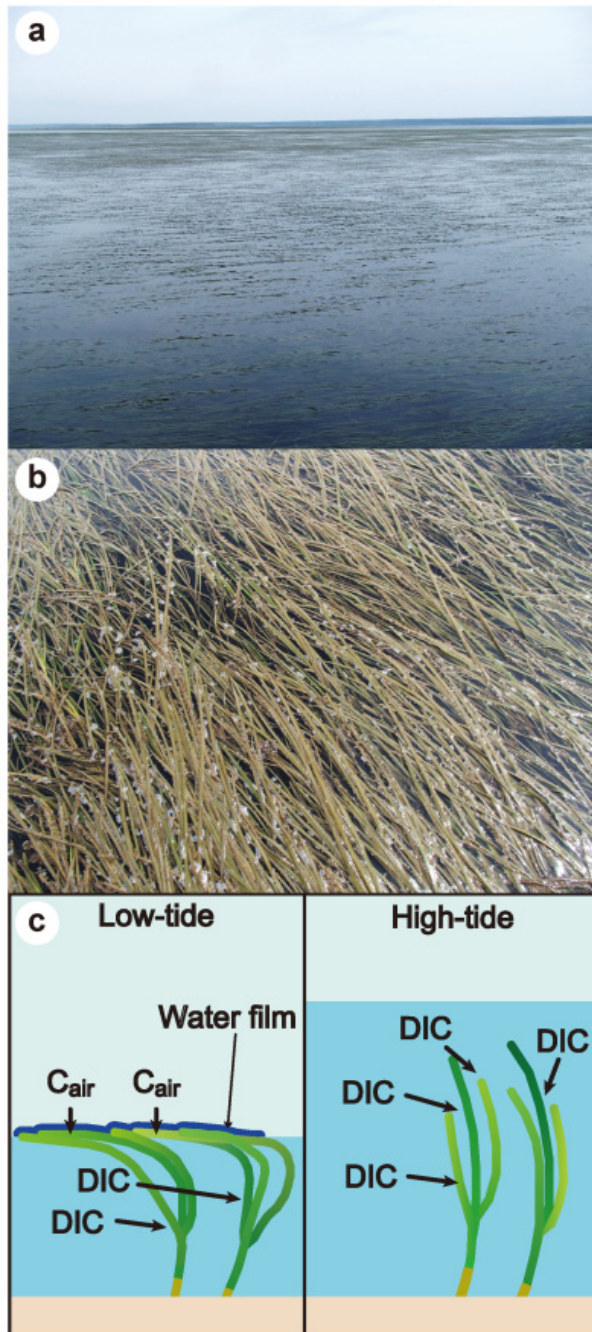
1  
 2 Figure 2. **(a)** Spatial distribution of the  $\Delta^{14}\text{C}$  values of dissolved inorganic carbon (DIC) (blue  
 3 open circles) and seagrass (red open circles) along the salinity gradient in May and July 2014  
 4 in Furen Lagoon, Japan. Blue and red solid lines represent the best fitting model of all general  
 5 linear models (GLMs) examined for DIC and seagrass, respectively. **(b)** Spatial distribution of  
 6 the relative contribution of  $C_{\text{air}}$  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  $\delta^{13}\text{C}$  values of DIC (blue open circles) and seagrass (red open circles) along  
 9 the salinity gradient. **(d)** Spatial distribution of the  $\delta^{13}\text{C}$  values of bicarbonate ion ( $\text{HCO}_3^-$ )  
 10 (green open circles) and aqueous  $\text{CO}_2$  [ $\text{CO}_2(\text{aq})$ ] (purple open circles) along the salinity  
 11 gradient. The dashed line indicates the isotopic signature of atmospheric  $\text{CO}_2$  ( $C_{\text{air}}$ ).



1

2 Figure 3. Spatial distribution of the  $\Delta^{14}\text{C}$  values of dissolved inorganic carbon (DIC) (blue)  
 3 and seagrass (red) along the salinity gradient in September (open circles) and November  
 4 (open triangles) 2014 in Furen Lagoon, Japan. The dashed line indicates the  $\Delta^{14}\text{C}$  of  
 5 atmospheric  $\text{CO}_2$  ( $\Delta^{14}\text{C}_{\text{air}}$ ).

6



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2 Figure 4. (a) Distant and (b) close-up views of the seagrass leaves exposed to the air during  
3 low tide in Furen Lagoon, Japan. (c) Conceptual diagram of the uptake of atmospheric  $CO_2$   
4 ( $C_{air}$ ) across the surface-water film on the seagrass leaves during low tide (left), and the  
5 uptake of DIC during high tide (right).