

1 **Radiocarbon isotopic evidence for assimilation of**
2 **atmospheric CO₂ by the seagrass *Zostera marina***

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9
10 **Abstract**

11 Submerged aquatic vegetation takes up water-column dissolved inorganic carbon (DIC) as a
12 carbon source across its thin cuticle layer. It is expected that marine macrophytes also use
13 atmospheric CO₂ when exposed to air during low tide, although assimilation of atmospheric
14 CO₂ has never been quantitatively evaluated. Using the radiocarbon isotopic signatures ($\Delta^{14}\text{C}$)
15 of the seagrass *Zostera marina*, DIC and POC, we show quantitatively that *Z. marina* takes up
16 and assimilates atmospheric modern CO₂ in a shallow coastal ecosystem. The $\Delta^{14}\text{C}$ values of
17 the seagrass ($-40\text{\textperthousand}$ to $-10\text{\textperthousand}$) were significantly higher than those of aquatic DIC ($-46\text{\textperthousand}$ to
18 $-18\text{\textperthousand}$), indicating that the seagrass uses a ^{14}C -rich carbon source (atmospheric CO₂, $+17\text{\textperthousand}$).
19 A carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean, 17%) of
20 its inorganic carbon as atmospheric CO₂. CO₂ exchange between the air and the seagrass
21 might be enhanced by the presence of a very thin film of water over the air-exposed leaves
22 during low tide. Our radiocarbon isotope analysis, showing assimilation of atmospheric
23 modern CO₂ as an inorganic carbon source, improves our understanding of the role of
24 seagrass meadows in coastal carbon dynamics.

25
26 **1 Introduction**

27 Submerged aquatic vegetation assimilates dissolved inorganic carbon (DIC) from the water
28 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 CO₂ (C_{air}), cannot directly reach
3 seagrasses when they are completely submerged; however, seagrasses can take up C_{air} 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 CO₂ [CO₂(aq)] as a carbon
6 source for photosynthesis in nature (Beer and Koch, 1996). Some seagrass species, however,
7 can use bicarbonate ions (HCO₃⁻) as a major carbon source (Beer et al., 2002; Beer and
8 Rehnberg, 1997), although there is considerable interspecific variation in HCO₃⁻ utilization
9 (Campbell and Fourqurean, 2013). As CO₂(aq) is in limited supply under normal seawater
10 conditions (pH ≈ 8), comprising only 1% (roughly 10–15 μmol L⁻¹) 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 (HCO₃⁻) 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 HCO₃⁻ under low-CO₂(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 HCO₃⁻ into CO₂(aq) via membrane-bound enzymes (Beer and Rehnberg 1997); or (2)
19 electrogenic proton (H⁺) extrusion into an boundary layer on the leaf surface, facilitating
20 HCO₃⁻/H⁺ cotransport (Hellblom et al. 2001).

21 Diffusion of CO₂ 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_{air}, in contrast to high tide, when there is a thick water layer
24 inhibiting the uptake of C_{air} (Ji and Tanaka, 2002). Previous studies have shown the
25 possibility of C_{air} 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 C_{air}) (Clavier et al., 2011; Cooper
2 and McRoy, 1988; Raven et al., 2002). However, the ^{13}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 HCO_3^-] and the primary
5 production rate. The chemical species in the carbonate system ($\text{CO}_2(\text{aq})$, HCO_3^- , and
6 carbonate ion [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 HCO_3^- (0‰) is isotopically distinct from that of both $\text{CO}_2(\text{aq})$ (−9‰) and
9 C_{air} (−8‰) under normal seawater conditions ($\text{pH} \approx 8$), high $\delta^{13}\text{C}$ (>−10‰) in seagrasses
10 shows that they use HCO_3^- as a carbon source because isotopic discrimination during 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}C -
13 depleted $\text{CO}_2(\text{aq})$ and C_{air} , quantification of the contribution of C_{air} is impossible because of
14 the overlap between their $\delta^{13}\text{C}$ values.

15 The natural abundance of radiocarbon (^{14}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}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 C_{air} . The
19 $\Delta^{14}\text{C}$ of DIC generally differs from that of atmospheric 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 C_{air} , based on the $\Delta^{14}\text{C}$ values of the seagrass and two carbon sources.

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 m²), 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 gloves and washed off using ultrapure water
4 (Milli-Q water; Millipore, Billerica, MA, USA). To estimate the $\Delta^{14}\text{C}$ 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 ($\delta^{13}\text{C}$) 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 H₃PO₄ and sparged using ultra-high
18 purity mixed N₂/H₂ 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 CO₂ evolved was collected cryogenically and purified in a vacuum line. The
22 purified CO₂ was then reduced to graphite using hydrogen and an iron catalyst at 650 °C for
23 10 h. The ¹³C and ¹⁴C 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
$$\Delta^{14}\text{C} (\text{\textperthousand}) = \delta^{14}\text{C} - 2(\delta^{13}\text{C} + 25)(1 + \delta^{14}\text{C}/1000). \quad (1)$$

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

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

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

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

1

2 where $[\text{CO}_2(\text{aq})]$, $[\text{CO}_3^{2-}]$, and $[\text{DIC}]$ are the concentrations of $\text{CO}_2(\text{aq})$, CO_3^{2-} and DIC ,
3 respectively; T is water temperature (K); and ε_{db} and ε_{cb} are factors for the isotopic
4 fractionation between $\text{CO}_2(\text{aq})$ 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 $\Delta^{14}\text{C}$ 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 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 \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 C_{air} to assimilated seagrass carbon was calculated by a two-
20 carbon-source mixing model using the $\Delta^{14}\text{C}$ values of DIC ($\Delta^{14}\text{C}_{\text{DIC}}$), C_{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
$$\text{C}_{\text{air}} (\% \text{ contribution}) = (\Delta^{14}\text{C}_{\text{seagrass}} - \Delta^{14}\text{C}_{\text{DIC}}) / (\Delta^{14}\text{C}_{\text{air}} - \Delta^{14}\text{C}_{\text{DIC}}) \times 100. \quad (6)$$

1 $\Delta^{14}\text{C}_{\text{air}}$ was estimated from the $\Delta^{14}\text{C}$ value of the sampled terrestrial plants ($\Delta^{14}\text{C} = +17.2\text{\textperthousand}$).

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

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

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}C -rich C_{air} ($\Delta^{14}\text{C}$

21 around $17\text{\textperthousand}$). 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 C_{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 C_{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_{air}
3 and air exposure time; however, the exposure time could mediate the assimilation of C_{air}
4 (Clavier et al., 2011).

5 As $\Delta^{14}C_{DIC}$ was significantly lower than $\Delta^{14}C_{air}$, the contribution of C_{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}C -depleted ($\Delta^{14}C_{DIC} < 0\text{\textperthousand}$)
8 (Talley, 2007). In contrast, the $\Delta^{14}C_{DIC}$ in surface seawater is generally higher than $\Delta^{14}C_{air}$ in
9 other regions of the Pacific Ocean because of bomb-derived ^{14}C (Talley, 2007).

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

23 Our $\Delta^{14}C$ analysis considerably reduces the limitations and uncertainties of conventional
24 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 $\Delta^{14}\text{C}$ 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 ($-14.0 \pm 2.4\text{\textperthousand}$) in the low-salinity zone
4 (salinity, 12–15) and high ($-8.8 \pm 1.9\text{\textperthousand}$) in the high-salinity zone (salinity, 25–29) (Fig. 2c).
5 There were significant correlations between salinity and $\delta^{13}\text{C}$ of DIC, 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 HCO_3^-
7 was isotopically distinct from $\delta^{13}\text{C}$ of both $\text{CO}_2(\text{aq})$ and C_{air} (Fig. 2d) and as *Z. marina* also
8 uses 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 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 $\delta^{13}\text{C}$ of C_{air}
12 overlapped those of both HCO_3^- and $\text{CO}_2(\text{aq})$ (Fig. 2d). Furthermore, $\delta^{13}\text{C}$ of both 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 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

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_{air} (Maher and Eyre, 2012; Tokoro et al., 2014; Watanabe and
7 Kuwae, 2015). Previously, however, such an exchange of 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_{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 $\Delta^{14}C$ 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 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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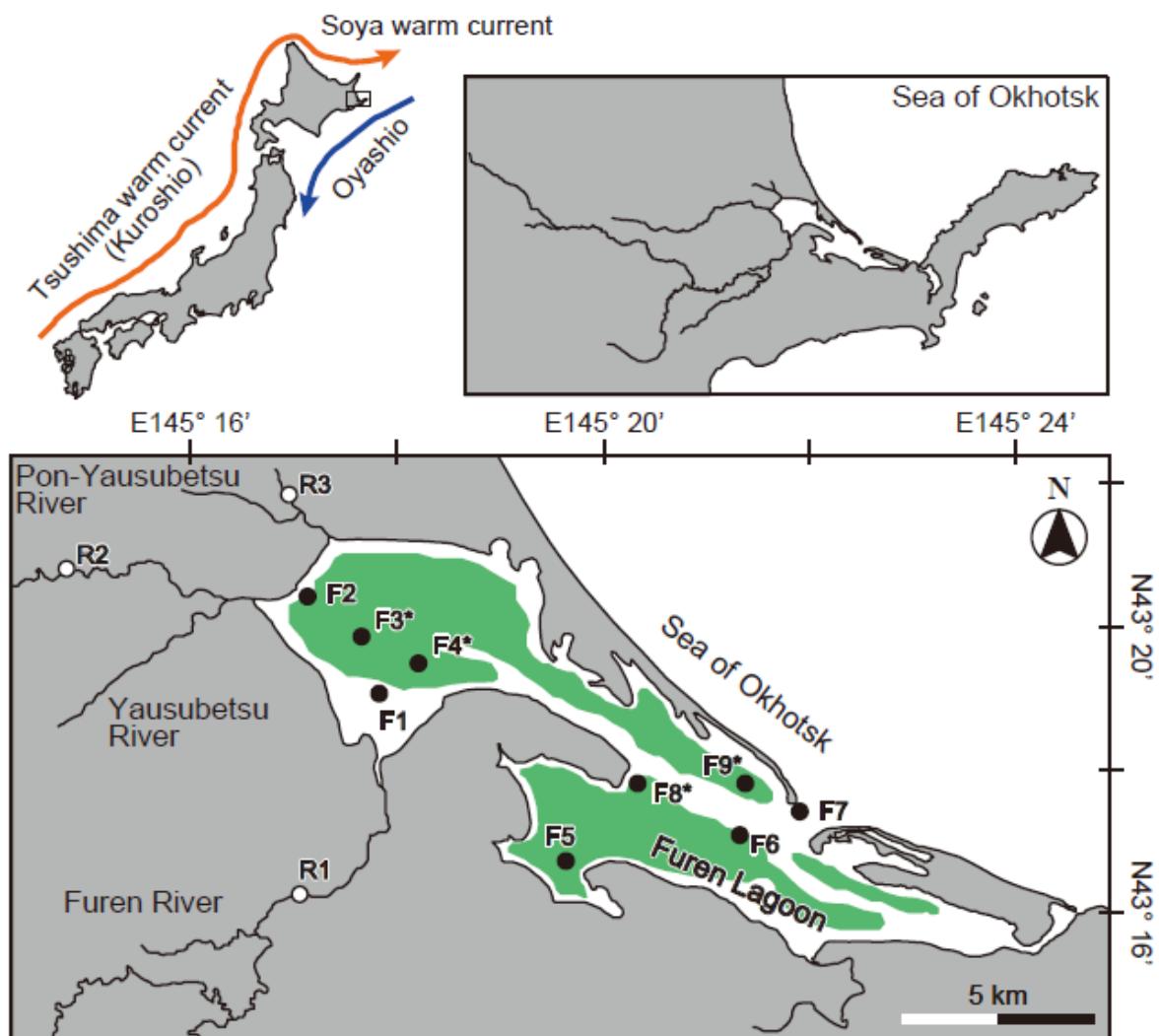
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30 Physiol.*, 108, 1665–1671, 1995.

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.

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 \times category			-0.05 ± 0.25	n.s.
(Intercept)	4.40 ± 2.59	n.s.	3.97 ± 3.36	n.s.

4



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

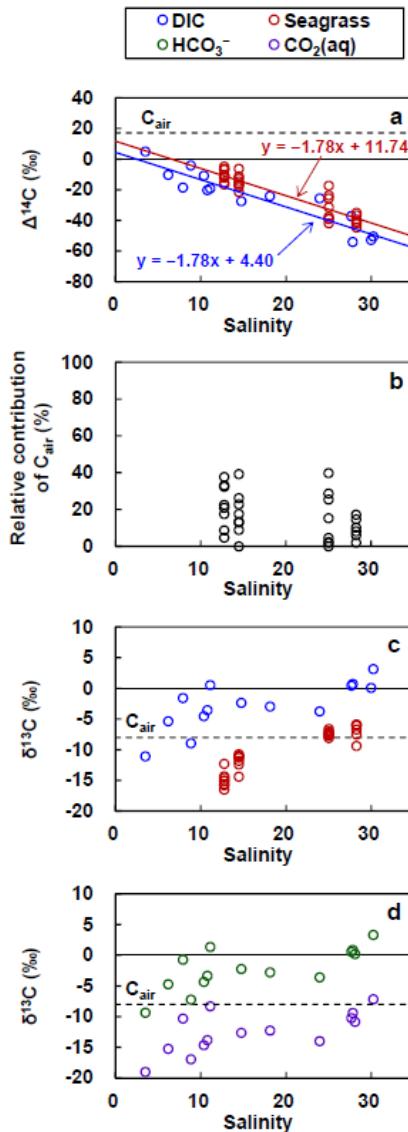
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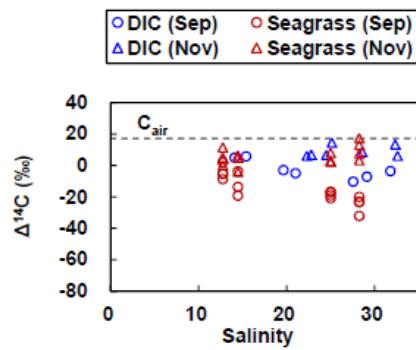
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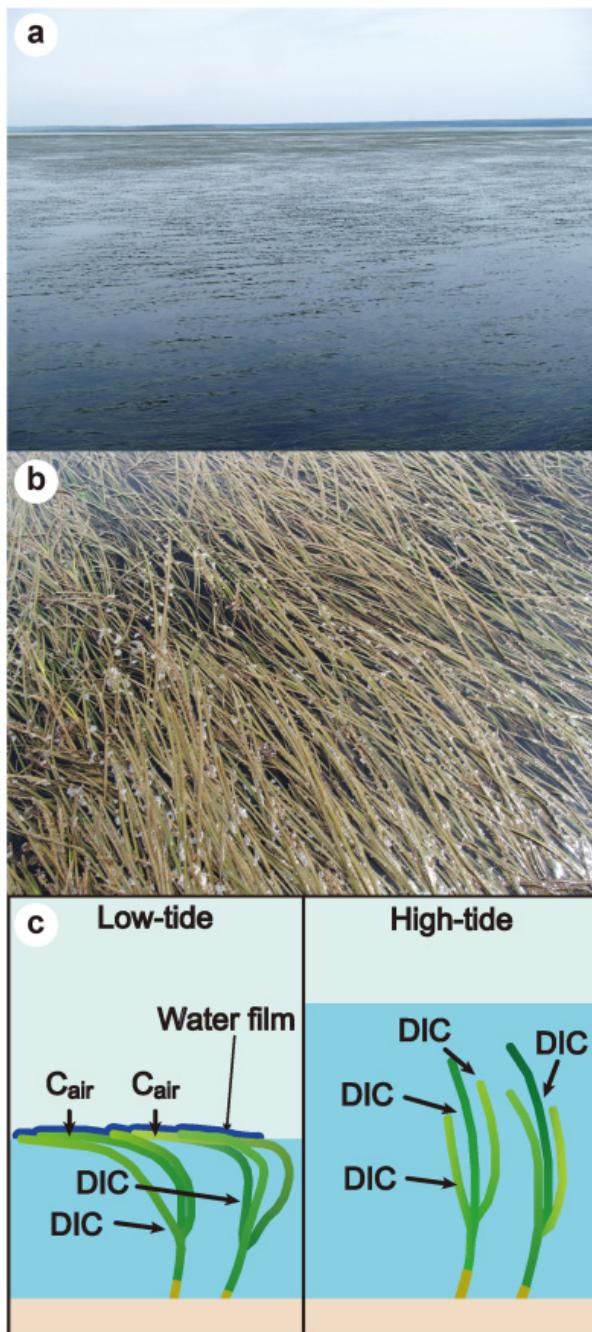
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2 Figure 2. **(a)** Spatial distribution of the $\Delta^{14}\text{C}$ values of dissolved inorganic carbon (DIC) (blue
3 open circles) and seagrass (red open circles) along the salinity gradient in May and July 2014
4 in Furen Lagoon, Japan. Blue and 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_{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 (HCO_3^-)
10 (green open circles) and aqueous CO_2 [$\text{CO}_2(\text{aq})$] (purple open circles) along the salinity
11 gradient. The dashed line indicates the isotopic signature of atmospheric CO_2 (C_{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 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).