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

K. Watanabe¹ and T. Kuwae¹

[¹]{Coastal and Estuarine Environment Research Group, Port and Airport Research Institute, 3-1-1 Nagase, Yokosuka 239-0826, Japan}

Correspondence to: K. Watanabe (watanabe-ke@ipc.pari.go.jp)

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 CO₂ when exposed to air during low tide, although assimilation of atmospheric CO₂ has never been quantitatively evaluated. Using the radiocarbon isotopic signatures (Δ¹⁴C) of the seagrass *Zostera marina*, DIC and POC, we show quantitatively that *Z. marina* takes up and assimilates atmospheric modern CO₂ in a shallow coastal ecosystem. The Δ¹⁴C values of the seagrass (−40‰ to −10‰) were significantly higher than those of aquatic DIC (−46‰ to −18‰), indicating that the seagrass uses a ¹⁴C-rich carbon source (atmospheric CO₂, +17‰). A carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean, 17%) of its inorganic carbon as atmospheric CO₂. CO₂ 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 CO₂ 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 (Hemming...
and Duarte, 2000), as their leaves lack stomata despite being angiosperms (Larkum and Hartog, 1989). An alternative carbon source, atmospheric CO\(_2\) (C\(_{\text{air}}\)), cannot directly reach seagrasses when they are completely submerged; however, seagrasses can take up C\(_{\text{air}}\) 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 \(\approx\) 8), comprising only 1% (roughly 10–15 \(\mu\)mol 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\(_{\text{air}}\), in contrast to high tide, when there is a thick water layer inhibiting the uptake of C\(_{\text{air}}\) (Ji and Tanaka, 2002). Previous studies have shown the possibility of C\(_{\text{air}}\) uptake by seagrasses by using evidence from stable carbon isotope ratios.
(δ^{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 δ\(^{13}C\) values of seagrasses are also determined by other factors such as the chemical species of DIC [CO\(_2\)(aq) or HCO\(_3\)\(^{-}\) and photosynthetic carbon demand. The chemical species in the carbonate system (CO\(_2\)(aq), HCO\(_3\)\(^{-}\), and carbonate ion [CO\(_3\)\(^{2-}\)] have distinct δ\(^{13}C\) values, and isotopic fractionations change depending on pH and temperature (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995). Because the δ\(^{13}C\) 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), δ\(^{13}C\) values in seagrasses become higher with increasing of HCO\(_3\)\(^{-}\) use (Campbell and Fourqurean, 2009; Hemminga and Mateo, 1996; Raven et al., 2002). However, quantification of the contribution of C_{air} is impossible because of the δ\(^{13}C\) value overlap between CO\(_2\)(aq) and C_{air} although low δ\(^{13}C\) in seagrasses could be explained by the assimilation of either \(^{13}C\)-depleted CO\(_2\)(aq) or C_{air}. Also, changes in the photosynthetic carbon demand driven by irradiance fluctuations affect the isotopic fractionation factor (Hemminga and Mateo, 1996; Raven et al., 2002).

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 (Δ\(^{14}C\)). The Δ\(^{14}C\) of inorganic carbon also has specific values depending on the source, such as DIC or C_{air}. The Δ\(^{14}C\) 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). Moreover, the calculation of Δ\(^{14}C\) by internal correction using δ\(^{13}C\) values eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977), overcoming one of the major uncertainties in the conventional δ\(^{13}C\) approach. This study is
the first to show quantitative evidence that the seagrass *Zostera marina* assimilates modern 
$^{13}C_{\text{air}}$, 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
(0.0625 m²), ranged from 400 to 4300 g m⁻². Three or four independent samples of seagrass
leaves were collected at each station. Both the biofilm and epiphytes covering the leaves were
gently removed by hands with powder-free gloves and washed off using ultrapure water
(Milli-Q water; Millipore, Billerica, MA, USA). To estimate the Δ¹⁴C of C_air, leaves of a
terrestrial plant (giant reed, Phragmites australis) were collected near the lagoon. Plant
samples were freeze-dried and subsamples were homogenized. To remove carbonate, the
plant samples were acidified with 1 N HCl and dried again.

Water samples for the isotopic analysis of terrestrial particulate organic carbon (POC) were
collected at three riverine stations (Fig. 1; stations R1–R3). Samples for POC were obtained
by filtration (approximately 1 L) onto pre-combusted (450 °C for 2 h) glass-fiber filters (GF/F,
Whatman, Maidstone, Kent, UK).

2.2 Carbon isotope analysis

We determined the stable carbon isotope ratios (δ¹³C) and radiocarbon concentrations (Δ¹⁴C)
of seagrass leaves, terrestrial plant leaves, DIC samples and POC samples. Prior to Δ¹⁴C and
δ¹³C measurements, samples were subjected to graphite purification as follows. DIC samples
for Δ¹⁴C and δ¹³C analysis were acidified (pH < 2) with H₃PO₄ and sparged using ultra-high
purity mixed N₂/H₂ gas. The powdered plant leaves and POC samples for Δ¹⁴C and δ¹³C
analysis were combusted in an elemental analyzer (either a Euro EA3000, EuroVector, Milan,
Italy; or a Flash 2000, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). For
each process, the CO₂ evolved was collected cryogenically and purified in a vacuum line. The
purified CO₂ was then reduced to graphite using hydrogen and an iron catalyst at 650 °C for
10 h. The $^{13}$C and $^{14}$C concentrations were measured using an accelerator mass spectrometer (AMS). The AMS results are reported as $\Delta^{14}$C (‰) values (Stuiver and Polach, 1977) as follows:

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

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

### 2.3 Carbonate system analysis

DIC concentration and TA were determined on a batch-sample analyzer (ATT-05; Kimoto Electric, Osaka, Japan). The precision of the analyses was 4 µmol L$^{-1}$ for DIC and 3 µmol L$^{-1}$ for TA. The concentrations of CO$_2$(aq), HCO$_3^-$, and CO$_3^{2-}$ were estimated using chemical equilibrium relationships and the TA and DIC concentrations of the water samples (Zeebe and Wolf-Gladrow, 2001). The $\delta^{13}$C values of CO$_2$(aq) ($\delta^{13}$C$_{\text{CO}_2}$) and HCO$_3^-$ ($\delta^{13}$C$_{\text{HCO}_3}$) were calculated as follows (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995):

$$\delta^{13}\text{C}_{\text{HCO}_3} = \delta^{13}\text{C}_{\text{DIC}} - \left([\epsilon_{db} \times [\text{CO}_2(\text{aq})] + \epsilon_{cb} \times [\text{CO}_3^{2-}]]/[\text{DIC}]\right),$$  \hspace{1cm} (2)

$$\delta^{13}\text{C}_{\text{CO}_2(\text{aq})} = \delta^{13}\text{C}_{\text{HCO}_3} + \epsilon_{db},$$  \hspace{1cm} (3)

$$\epsilon_{db} = \epsilon(\text{CO}_2(\text{aq}) - \text{HCO}_3^-) = -9866/T + 24.12 \text{ (‰)},$$  \hspace{1cm} (4)
\[ \varepsilon_{cb} = \varepsilon(CO_3^{2-} - HCO_3^-) = -867/T + 2.52 \text{ (‰),} \quad (5) \]

where \([CO_2(aq)], [CO_3^{2-}],\) and \([DIC]\) are the concentrations of \(CO_2(aq), CO_3^{2-}\) and \(DIC\), respectively; \(T\) is water temperature (K); and \(\varepsilon_{db}\) and \(\varepsilon_{cb}\) are factors for the isotopic fractionation between \(CO_2(aq)\) and \(HCO_3^-\), and between \(CO_3^{2-}\) and \(HCO_3^-\), respectively.

### 2.4 Data analysis

Because DIC taken up by seagrasses is a mixture of DIC from two sources (terrestrial and oceanic) each having distinct \(\Delta^{14}C\) values, it is reasonable to use salinity as a proxy for the extent of mixing of these two sources as well as for the salinity gradient-based comparison between \(\Delta^{14}C\) of DIC and seagrass. This comparison was therefore possible even though DIC and \(Z. marina\) samples were not necessarily collected from the same stations (Fig. 1). Analyses of covariance (ANCOVA) were used to examine the difference in \(\Delta^{14}C\) value between seagrass leaves and DIC. These differences provide evidence that the seagrasses assimilate \(C_{air}\). We selected salinity, categorical data (seagrass leaves or DIC) and the interaction term as the explanatory variables.

The relative contribution of \(C_{air}\) to assimilated seagrass carbon was calculated by a two-carbon-source mixing model using the \(\Delta^{14}C\) values of DIC \((\Delta^{14}C_{DIC})\), \(C_{air} (\Delta^{14}C_{air})\), and the seagrass \((\Delta^{14}C_{seagrass})\) at each of four stations as follows:

\[ C_{air} \text{ (% contribution)} = (\Delta^{14}C_{seagrass} - \Delta^{14}C_{DIC})/(\Delta^{14}C_{air} - \Delta^{14}C_{DIC}) \times 100. \quad (6) \]
Δ¹⁴C_{air} was estimated from the Δ¹⁴C value of the sampled terrestrial plants (Δ¹⁴C = +17.2‰).

The Δ¹⁴C values of DIC as the carbon source for *Z. marina* in the mixing model were estimated from the linear model fitted with the ANCOVA.

### Results and discussion

Our radiocarbon isotopic analysis shows quantitatively that the seagrass *Z. marina* uses C_{air} in a shallow lagoon (Fig. 2a). In May and July 2014, Δ¹⁴C_{seagrass} was significantly higher than Δ¹⁴C_{DIC} even if the effects of salinity was considered (ANCOVA, *P* < 0.001), and the interaction term was not significant (ANCOVA, *P* > 0.05). Our results indicate that the changes in Δ¹⁴C_{DIC} are regulated mostly by mixing between high-Δ¹⁴C river water and low-Δ¹⁴C seawater: the seagrass uses aquatic DIC as the main carbon source, as expected from previous studies (Hemminga and Duarte, 2000; Invers et al., 2001; Campbell and Fourqurean, 2013). The Δ¹⁴C_{seagrass} reflects Δ¹⁴C_{DIC} from May to July because *Z. marina* leaves start to grow in early May when sea ice is thawing at the study site, with the turnover time of leaves being 30–90 days (mean, 60 days; Hosokawa et al., 2009). Furthermore, the negative relationship between salinity and Δ¹⁴C_{seagrass} cannot be explained by any residual contamination from terrestrial organic carbon on the leaves because the terrestrial POC was ¹⁴C-depleted (mean Δ¹⁴C of terrestrial POC, −74.7 ± 23.4‰).

The significantly higher values in Δ¹⁴C_{seagrass} than Δ¹⁴C_{DIC} shows that the seagrass assimilates ¹⁴C-rich C_{air} (Δ¹⁴C around 17‰) (Fig. 2a). The two-carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean ± SD, 17 ± 12%) of its inorganic carbon as C_{air}; the contribution was 20 ± 12% in the low-salinity zone (salinity, 12–15) and 13 ± 12% in the high-salinity zone (salinity, 25–29) (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 \( C_{\text{air}} \) and air exposure time; however, the exposure time would mediate the assimilation of \( C_{\text{air}} \) (Clavier et al., 2011).

As \( \Delta^{14}C_{\text{DIC}} \) was significantly lower than \( \Delta^{14}C_{\text{air}} \), the contribution of \( C_{\text{air}} \) 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 \( (\Delta^{14}C_{\text{DIC}} < 0\%) \) (Talley, 2007). In contrast, the \( \Delta^{14}C_{\text{DIC}} \) in surface seawater is generally higher than \( \Delta^{14}C_{\text{air}} \) 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 \( \Delta^{14}C_{\text{DIC}} \) would affect the application of this approach because it is only applicable when the \( \Delta^{14}C \) values for endmembers (seawater DIC, freshwater DIC, and \( C_{\text{air}} \)) 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 \( C_{\text{air}} \) contribution in September or November 2014 in Furen Lagoon because the \( \Delta^{14}C_{\text{DIC}} \) of seawater increased to near \( \Delta^{14}C_{\text{air}} \) and there was overlap between the two (Fig. 3). The overlapping in the range of values, induced by variations in the \( \Delta^{14}C_{\text{DIC}} \) of seawater, likely caused by the dynamics of the Oyashio (mean \( \Delta^{14}C_{\text{DIC}} \), \(-41\%\); Aramaki et al., 2001; Aramaki et al., 2007) and the Soya warm current (mean \( \Delta^{14}C_{\text{DIC}} \), \(52\%\); Aramaki et al., 2007; Kumamoto et al., 1998) (Figs. 1 and 4). According to the distribution of sea surface temperature derived from the Moderate Resolution Imaging Spectroradiometers (MODIS) images in 2014 (Fig. 4; \url{http://oceancolor.gsfc.nasa.gov/cms/}), the oceanic boundary of Furen Lagoon was the Oyashio throughout the year except from late summer to autumn when the Soya warm current intrudes into the boundary (Oguma et al., 2008; Takizawa, 1982). The oceanic end-member \( \Delta^{14}C_{\text{DIC}} \) would reflect the value of the Oyashio from January to August 2014, when the Soya
warm current did not reach the oceanic boundary of Furen Lagoon. The oceanic end-member
$\Delta^{14}C_{\text{DIC}}$ would, therefore, not overlap with $\Delta^{14}C_{\text{air}}$ during January to August 2014, which
includes the whole period of a one-three month seagrass leave growing prior to sampling, i.e.,
February to July, indicating that the uptake of $C_{\text{air}}$ by the seagrass is robust estimate during the
period. Even if the sporadic upwelling have occurred during the study period, our
determination of the $C_{\text{air}}$ contribution here would be underestimated because the $\Delta^{14}C_{\text{DIC}}$ of
the upwelling deep-sea water is lower than that of surface water (Aramaki et al., 2001;
Aramaki et al., 2007). Nevertheless, the applicability of the $\Delta^{14}C$ technique is dependent 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
et al., 2002). In particular, the use of $\Delta^{14}C$ has the advantage of avoiding effects of isotopic
fractionation (Stuiver and Polach, 1977); the use of $\delta^{13}C$ does not and therefore generates
large uncertainties. The $\delta^{13}C$ of the seagrass was low ($-14.0 \pm 2.4\%$) in the low-salinity zone
(salinity, 12–15) and high ($-8.8 \pm 1.9\%$) in the high-salinity zone (salinity, 25–29) (Fig. 2c).
There were significant correlations between salinity and $\delta^{13}C$ of DIC, $\text{HCO}_3^-$, $\text{CO}_2(aq)$ and
the seagrass (Pearson’s correlation coefficient: $P < 0.001$; Fig. 2c, d). As the $\delta^{13}C$ of $\text{HCO}_3^-$
was isotopically distinct from $\delta^{13}C$ of both $\text{CO}_2(aq)$ and $C_{\text{air}}$ (Fig. 2d) and as $Z. \text{marina}$ also
uses $\text{HCO}_3^-$ as a carbon source under low-$\text{CO}_2(aq)$ conditions (Beer and Rehnberg, 1997), the
$\delta^{13}C$ of the seagrass should change depending on the contribution of $\text{HCO}_3^-$ as a carbon
source (Campbell and Fourquean, 2009; Hemminga and Mateo, 1996; Raven et al., 2002).
However, it is not possible to distinguish the contribution of $C_{\text{air}}$ from that of other carbon
sources because the $\delta^{13}C$ of $C_{\text{air}}$ overlapped those of both $\text{HCO}_3^-$ and $\text{CO}_2(aq)$ (Fig. 2d).
Furthermore, $\delta^{13}C$ of both $\text{HCO}_3^-$ and $\text{CO}_2(aq)$ change through mixing between low-$\delta^{13}C$
river water and high-$\delta^{13}$C seawater in brackish areas (Fig. 2d; Hemminga and Mateo, 1996; Simenstad and Wissmar, 1985).

In any case, there are large uncertainties when using $\delta^{13}$C to quantitatively estimate the contribution of C$_{\text{air}}$ as a carbon source because the isotopic fractionation that occurs in the steps between the carbon source and organic plant compounds changes depending on the photosynthetic carbon demand (Hemminga and Mateo, 1996; Raven et al., 2002). The radiocarbon isotopic approach can avoid the uncertainties derived from both the chemical species of DIC and the isotopic fractionation factor in carbon assimilation.

The seagrass leaves assimilated C$_{\text{air}}$ when exposed to air during low tide (Fig. 5). CO$_2$ exchange between the air and water would occur at the very thin film of water on the air-exposed seagrass leaves (Fig. 5c), likely enhancing the passive uptake of C$_{\text{air}}$ by diffusion. Our high estimate of the C$_{\text{air}}$ 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$_{\text{air}}$ (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$_{\text{air}}$ 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
collection 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.

Acknowledgements
We thank H. Moki, E. Miyoshi, S. Montani, and the members of Hokkaido University for
help in field surveys, T. Tokoro for helpful comments, and K. Sakihara and A. Okuno for
chemical analysis. This study was supported by a Canon Foundation grant, a Grant-in-Aid for
Challenging Exploratory Researches (No. 24656316 and 26630251) from the Japan Society
for the Promotion of Science, and a Core Research for Evolutional Science and Technology
(CREST) Program of the Japan Science and Technology Agency.
References


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.
Figure 2. (a) Spatial distribution of the $\Delta^{14}C$ values of dissolved inorganic carbon (DIC) (blue open circles) and seagrass (red open circles) along the salinity gradient in May and July 2014 in Furen Lagoon, Japan. Blue and red solid lines represent the linear models fitted with analyses of covariance (ANCOVA) examined for DIC and seagrass, respectively. (b) Spatial distribution of the relative contribution of $C_{\text{air}}$ to total inorganic carbon assimilated by seagrass along the salinity gradient, as calculated by the two-carbon-source mixing model. (c) Spatial distribution of the $\delta^{13}C$ values of DIC (blue open circles) and seagrass (red open circles) along the salinity gradient. (d) Spatial distribution of the $\delta^{13}C$ values of bicarbonate ion ($\text{HCO}_3^-$) (green open circles) and aqueous CO$_2$ [CO$_2$(aq)] (purple open circles) along the salinity gradient. The dashed line indicates the isotopic signature of atmospheric CO$_2$ ($C_{\text{air}}$).
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$_2$ ($\Delta^{14}C_{\text{air}}$).
Figure 4. Seasonal dynamics of ocean currents affecting the oceanic boundary of Furen Lagoon. Red circle shows the location of Furen Lagoon. (a) From mid-November to August, the oceanic boundary is the Oyashio ($\Delta^{14}$C$_{\text{DIC}}$, $-60--10\%$; Aramaki et al., 2001). (b) From September to early-November, the Soya warm current ($\Delta^{14}$C$_{\text{DIC}}$, 50–70‰; Aramaki et al., 2007) intruded into the boundary.
Figure 5. (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).