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 CO₂ (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 CO₂ [CO₂(aq)] as a carbon source for photosynthesis in nature (Beer and Koch, 1996). Some seagrass species, however, can use bicarbonate ions (HCO₃⁻) as a major carbon source (Beer et al., 2002; Beer and Rehnberg, 1997), although there is considerable interspecific variation in HCO₃⁻ utilization (Campbell and Fourqurean, 2013). As CO₂(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 (HCO₃⁻) 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 HCO₃⁻ under low-CO₂(aq) conditions (Beer et al., 2002; Campbell and Fourqurean, 2013), using one or both of the following suggested mechanisms: (1) extracellular dehydration of HCO₃⁻ into CO₂(aq) via membrane-bound enzymes (Beer and Rehnberg 1997); or (2) electrogenic proton (H⁺) extrusion into an boundary layer on the leaf surface, facilitating HCO₃⁻/H⁺ cotransport (Hellblom et al. 2001).

Diffusion of CO₂ 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.
(δ^{13}C) in seagrasses and the two carbon sources (DIC and C\textsubscript{air}) (Clavier et al., 2011; Cooper and McRoy, 1988; Raven et al., 2002). However, the δ^{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\textsubscript{2}(aq) or HCO\textsubscript{3}^{-}] and the primary production rate. **The chemical species in the carbonate system (CO\textsubscript{2}(aq), HCO\textsubscript{3}^{-}, and carbonate ion [CO\textsubscript{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\textsubscript{3}^{-} (0‰) is isotopically distinct from that of both CO\textsubscript{2}(aq) (−9‰) and C\textsubscript{air} (−8‰) under normal seawater conditions (pH ≈ 8), high δ^{13}C (>−10‰) in seagrasses shows that they use HCO\textsubscript{3}^{-} as a carbon source because isotopic discrimination during CO\textsubscript{2} assimilation results in δ^{13}C values that are always higher than those of the carbon sources. Although low δ^{13}C (<−10‰) in seagrasses could be explained by the assimilation of both 13C-depleted CO\textsubscript{2}(aq) and C\textsubscript{air}, quantification of the contribution of C\textsubscript{air} is impossible because of the overlap between their δ^{13}C values.

The natural abundance of radiocarbon (14C) 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 14C concentrations (Δ\textsuperscript{14}C). The Δ\textsuperscript{14}C of inorganic carbon also has specific values depending on the source, such as DIC or C\textsubscript{air}. **The Δ\textsuperscript{14}C of DIC generally differs from that of atmospheric CO\textsubscript{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 Δ\textsuperscript{14}C by internal correction using δ^{13}C values eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977), so the Δ\textsuperscript{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\textsubscript{air}, based on the Δ\textsuperscript{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), ranging 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 groves and washed off using ultrapure water (Milli-Q water; Millipore, Billerica, MA, USA). To estimate the Δ¹⁴C of C₄ 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. The¹³C and¹⁴C 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:

$$\Delta^{14}$C ($\%$) = $\delta^{14}$C $- 2(\delta^{13}$C + 25)(1 + $\delta^{14}$C/1000).$$  \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; Kîimoto 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) ($\delta^{13}$CCO$_2$(aq)) and HCO$_3^-$ ($\delta^{13}$CHCO$_3^-$) were calculated as follows (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995):

$$\delta^{13}$CHCO$_3^-$ = $\delta^{13}$CDIC $- (\varepsilon_{db} \times [CO_2(aq)] + \varepsilon_{cb} \times [CO_3^{2-}])/[DIC],$$ \hspace{1cm} (2)

$$\delta^{13}$CCO$_2$(aq) = $\delta^{13}$CHCO$_3^-$ + $\varepsilon_{db}$, \hspace{1cm} (3)

$$\varepsilon_{db} = \varepsilon (CO_2(aq) - HCO_3^-) = -9866/T + 24.12 (\%o),$$ \hspace{1cm} (4)

$$\varepsilon_{cb} = \varepsilon (CO_3^{2-} - HCO_3^-) = -867/T + 2.52 (\%o),$$ \hspace{1cm} (5)
where $[\text{CO}_2(\text{aq})]$, $[\text{CO}_3^{2-}]$, and $[\text{DIC}]$ 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 (Table 1). This comparison was therefore possible even though DIC and *Z. marina* samples were not necessarily collected from the same stations (Fig. 1). A general linear model (GLM) was used to examine the differences between the $\Delta^{14}C$ values of the seagrass leaves and those of DIC in May and July 2014. These differences provide evidence that the seagrasses assimilate C$_{\text{air}}$. A GLM was suitable for this study because both continuous (salinity) and categorical variables (seagrass leaves or DIC) were used as explanatory variables. We selected salinity, category (seagrass leaves or DIC), and their interaction (salinity $\times$ category) as the explanatory variables (Table 1). We used Akaike’s Information Criterion (AIC) to select the most parsimonious model.

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

$$C_{\text{air}} \ (\% \ \text{contribution}) = \left( \frac{\Delta^{14}C_{\text{seagrass}} - \Delta^{14}C_{\text{DIC}}}{\Delta^{14}C_{\text{air}} - \Delta^{14}C_{\text{DIC}}} \right) \times 100. \quad (6)$$
Δ¹⁴Cₐir 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 best GLM (Table 1).

3 Results and discussion
Our radiocarbon isotopic analysis shows quantitatively that the seagrass Z. marina uses Cₐir in a shallow lagoon (Fig. 2a and Table 1). The GLM strongly highlights the effect of salinity on the Δ¹⁴C of DIC and the seagrass in May and July 2014 (Table 1; GLM, P < 0.001). 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 Fourquarean, 2013). The Δ¹⁴C seagrass could reflect Δ¹⁴C DIC from May to July because Z. marina leaves start to grow in early May 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 model also reinforced our observations that Δ¹⁴C seagrass was higher than Δ¹⁴C DIC (Fig. 2a and Table 1; GLM, P < 0.001). This shows that the seagrass assimilates ¹⁴C-rich Cₐir (Δ¹⁴C around 17‰). The two-carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean ± SD, 17 ± 12%) of its inorganic carbon as Cₐir; 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 Cₐir 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\textsubscript{air}
and air exposure time; however, the exposure time could mediate the assimilation of C\textsubscript{air}
(Clavier et al., 2011).

As \(\Delta^{14}\text{CDIC}\) was significantly lower than \(\Delta^{14}\text{Cair}\), the contribution of C\textsubscript{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 \(^{14}\text{C}\)-depleted (\(\Delta^{14}\text{CDIC} < 0\)‰)
(Talley, 2007). In contrast, the \(\Delta^{14}\text{CDIC}\) in surface seawater is generally higher than \(\Delta^{14}\text{Cair}\) in
other regions of the Pacific Ocean because of bomb-derived \(^{14}\text{C}\) (Talley, 2007).

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

Our \(\Delta^{14}\text{C}\) analysis considerably reduces the limitations and uncertainties of conventional
methods such as that using only \(\delta^{13}\text{C}\) (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, HCO$_3^-$, CO$_2$(aq) and the seagrass (Pearson’s correlation coefficient: $P < 0.001$; Fig. 2c, d). As the $\delta^{13}C$ of HCO$_3^-$ was isotopically distinct from $\delta^{13}C$ of both CO$_2$(aq) and C$_{air}$ (Fig. 2d) and as Z. marina also uses HCO$_3^-$ as a carbon source under low-CO$_2$(aq) conditions (Beer and Rehnberg, 1997), the $\delta^{13}C$ of the seagrass should change depending on the contribution of HCO$_3^-$ as a carbon source (Campbell and Fourqurean, 2009; Raven et al., 2002). However, it is not possible to distinguish the contribution of C$_{air}$ from that of other carbon sources because the $\delta^{13}C$ of C$_{air}$ overlapped those of both HCO$_3^-$ and CO$_2$(aq) (Fig. 2d). Furthermore, $\delta^{13}C$ of both HCO$_3^-$ and CO$_2$(aq) change through mixing between low-$\delta^{13}C$ river water and high-$\delta^{13}C$ seawater in brackish areas (Fig. 2d).

In any case, there are large uncertainties when using $\delta^{13}C$ to quantitatively estimate the contribution of C$_{air}$ 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 rate (Raven et al., 2002). The radiocarbon isotopic approach can avoid the uncertainties derived from both the contribution of HCO$_3^-$ as a carbon source and isotopic fractionation in carbon assimilation.

The seagrass leaves assimilated C$_{air}$ when exposed to air during low tide (Fig. 4). CO$_2$ exchange between the air and water would occur at the very thin film of water on the air-exposed seagrass leaves (Fig. 4c), likely enhancing the passive uptake of C$_{air}$ by diffusion. 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.
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References


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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Best model (AIC = 288.33)</th>
<th>Full model (AIC = 290.29)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>P</td>
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<tr>
<td>Salinity</td>
<td>-1.78 ± 0.12</td>
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<tr>
<td>Category (seagrass)</td>
<td>7.34 ± 2.00</td>
<td>&lt;0.001</td>
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<tr>
<td>Salinity × category</td>
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<tr>
<td>(Intercept)</td>
<td>4.40 ± 2.59</td>
<td>n.s.</td>
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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 best fitting model of all general linear models (GLMs) 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 ($HCO_3^-$) (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. (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$_2$ ($C_{\text{air}}$) across the surface-water film on the seagrass leaves during low tide (left), and the uptake of DIC during high tide (right).