We thank to referees' critical review and constructive comments regarding our paper. The following list includes the alterations we have made to address the referees' feedback. We have added figure 4 and removed table 1. We believe that the manuscript has been substantially improved following adoption of the valuable suggestions.

Authors' replies to the comments of Referee #1

1) The study has been improved but there is still insufficient quantification of variation in end-members, especially the oceanic DIC source, and the fact that the seagrass leaves reflect a one-three month growing period prior to sampling. Together these issues mean that the approach cannot support the conclusion regarding atmospheric carbon uptake for half the sampling events (no $\Delta^{14}C$ gradient), and may be confounded for the other two sampling events when a gradient in $\Delta^{14}C$ was evident. It is difficult to have confidence in the approach and conclusions without adequate quantification and assessment of end-member variability.

I am still not convinced the oceanographic variation in $\Delta^{14}C$ has been adequately quantified and excluded as an explanation for the $\Delta^{14}C$ of the seagrass leaves in addition to atmospheric carbon uptake. I think better quantification of the oceanic end-member is needed, e.g. a time series of $\Delta^{14}C$ of offshore water entering the lagoon; this may already have been measured but the seasonal variability in end members should be explicitly presented in a figure. The authors state that the endmembers were sufficiently distinct during point sampling in May and July, but what about other times? The same processes that meant the approach was invalid based on end-member estimates in September and November could apply at any time, e.g. due to sporadic upwelling. This issue is especially important given the highly seasonal impact on the validity of the approach and capacity to draw conclusions about atmospheric carbon uptake.

1) Author's reply: Based on the evidence of the seasonal analysis of oceanic end-member $\Delta^{14}C$ below, we reconfirmed that the inference of the C_{air} assimilation by the seagrass leaves was robust from May to July, 2014 even though considering the seagrass leaves reflecting a one-three month growing period prior to sampling. We analyzed daily distributions of SST derived from the MODIS image data and confirmed that the oceanic boundary of Furen Lagoon was the Oyashio (low-$\Delta^{14}C_{DIC}$) current from January to August 2014 and that the Soya warm current (high-$\Delta^{14}C_{DIC}$) did not intrude into the boundary at that period. This pattern had also supported by our data (bimonthly oceanic end-member $\Delta^{14}C$). Therefore, the oceanic end-member $\Delta^{14}C$ would not overlap

with $\Delta^{14}C_{air}$ during January to August 2014, which clearly including the whole period of a one-three month seagrass leaves growing prior to sampling, i.e., February to July. A series of evidence shown here clearly indicates that the ^{14}C approach is able to evaluate the C_{air} assimilation adequately from May to July when the oceanic end-member $\Delta^{14}C$ was distinct from the C_{air} end-member $\Delta^{14}C$. In turn, as we had pointed out in the previous manuscript, the ^{14}C approach is not applicable to September and November, 2014 when the oceanic end-member $\Delta^{14}C$ was not distinct. We have added the following sentences to the text: "According to the distribution of sea surface temperature derived from the Moderate Resolution Imaging Spectroradiometers (MODIS) images in 2014 (Fig. 4; 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 $\Delta^{14}C_{DIC}$ 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 $\Delta^{14}C_{DIC}$ would, therefore, not overlap with $\Delta^{14}C_{air}$ 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_{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_{air} contribution here would be underestimated because the $\Delta^{14}C_{DIC}$ 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. (P9L19-P10L9)" We have replaced "could" to "would" (P9L10).

2) The short growing season in this location seems like it should constrain the issue of turnover times somewhat, but as above, the $\Delta^{14}C$ of the seagrass leaves likely reflects an integration of the variation in in end-members over the one to three month prior to sampling the leaves – this variation has not been adequately quantified.

2) Author's reply: We have revised the sentences as you see reply-1. And we have changed the following sentence: "The $\Delta^{14}C_{seagrass}$ reflects $\Delta^{14}C_{DIC}$ 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)."

3) The location map has been vastly improved, but how about showing the $\Delta^{14}C$ of the

end members here (and their seasonal range, e.g. with the currents).

3) Author’s reply: We have added the Figure 4.

Authors' replies to the comments of Referee #2

1) P3L8-10: I would provide a more comprehensive discussion of other environmental factors that drive $\delta^{13}C$ variation in submerged macrophytes (e.g. the influence of light on photosynthetic carbon demand and isotope discrimination) Hemminga & Mateo (1996).

1) Author's reply: We have revised the sentences as follows: "Because the $\delta^{13}C$ of HCO_3^- (0‰) is isotopically distinct from that of both $CO_2(aq)$ (−9‰) and C_{air} (−8‰) under normal seawater conditions (pH ≈ 8), $\delta^{13}C$ 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 $\delta^{13}C$ value overlap between $CO_2(aq)$ and C_{air} although low $\delta^{13}C$ 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). (P3L8-15)" Furthermore, we changed the following sentence: "The radiocarbon isotopic approach can avoid the uncertainties derived from both the chemical species of DIC and the isotopic fractionation factor in carbon assimilation. (P11L6-8)"

2) P3L8-13: Slightly confusing. Clearly resultant $\delta^{13}C$ values hinge upon a multitude of factors, such as carbon source, concentration, degree of HCO_3^- use, and photosynthetic demand (as driven by irradiance). I would revise this paragraph after reviewing Hemminga & Mateo 1996.

2) Author's reply: We have revised the sentences as you see reply-1.

3) P7L7: It appears that category (seagrass vs DIC) was not a significant explanatory variable of $\Delta^{14}C$ within the full model, whose AIC score was rather similar to the reduced model, which removed the interaction between salinity and category. Examining Fig 2, it appears that at two of the higher salinity stations (20-30), seagrass $\Delta^{14}C$ was similar to or lower than DIC $\Delta^{14}C$. Can you explain?

3) Author's reply: To avoid ambiguity in statistical inference of the model selection using AIC, we have adopted a more statistically robust test method (ANCOVA) after

confirming the prerequisite of ANCOVA. The results showed that the effects of both salinity and categorical data (seagrass vs DIC) were significant ($p<0.001$), but the interaction (salinity × category) was not (ANCOVA, $p>0.05$). Therefore, we selected the linear model fitted with ANCOVA using salinity and categorical data as the explanatory variables. We have changed the related sentences (P7L8-P8L3, P8L7-9, P8L19-20, P18L4-5) and removed table 1.

$\Delta^{14}C$ values of seagrass leaves which just start growing would be similar to that of DIC because the exposure time of the short-length leaves would be considerably short. We have changed the related sentence as follows: “the exposure time would mediate the assimilation of C_{air} (Clavier et al., 2011). (P9L2-3)”

4) P8L23-P9L3: It seems, in my opinion, that exposure time would be a rather large factor in regards to determining the resultant contribution of C_{air}. This point could be motivated by a stronger statement.

4) Author’s reply: As you pointed out, the exposure time is a rather large factor in regards to determining the resultant contribution of C_{air}. We have replaced the term “could” to “would” (P9L3).

Radiocarbon isotopic evidence for assimilation of atmospheric CO_2 by the seagrass *Zostera marina*

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Abstract

Submerged aquatic vegetation takes up water-column dissolved inorganic carbon (DIC) as a carbon source across its thin cuticle layer. It is expected that marine macrophytes also use atmospheric CO_2 when exposed to air during low tide, although assimilation of atmospheric CO_2 has never been quantitatively evaluated. Using the radiocarbon isotopic signatures ($\Delta^{14}C$) of the seagrass *Zostera marina*, DIC and POC, we show quantitatively that *Z. marina* takes up and assimilates atmospheric modern CO_2 in a shallow coastal ecosystem. The $\Delta^{14}C$ values of the seagrass (−40‰ to −10‰) were significantly higher than those of aquatic DIC (−46‰ to −18‰), indicating that the seagrass uses a ^{14}C-rich carbon source (atmospheric CO_2, +17‰). A carbon-source mixing model indicated that the seagrass assimilated 0–40% (mean, 17%) of its inorganic carbon as atmospheric CO_2. CO_2 exchange between the air and the seagrass might be enhanced by the presence of a very thin film of water over the air-exposed leaves during low tide. Our radiocarbon isotope analysis, showing assimilation of atmospheric modern CO_2 as an inorganic carbon source, improves our understanding of the role of seagrass meadows in coastal carbon dynamics.

1 Introduction

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

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

dehydration of HCO_3^- into CO_2(aq) via membrane-bound enzymes (Beer and Rehnberg 1997); or (2) electrogenic proton (H^+) extrusion into an boundary layer on the leaf surface, facilitating HCO_3^-/H^+ cotransport (Hellblom et al. 2001).

Diffusion of CO_2 in water is much slower than that in air. During low tide, air-exposed aquatic macrophytes have a thin film of water between the air and their leaves, which promotes the uptake of C_{air}, in contrast to high tide, when there is a thick water layer inhibiting the uptake of C_{air} (Ji and Tanaka, 2002). Previous studies have shown the possibility of C_{air} uptake by seagrasses by using evidence from stable carbon isotope ratios ($\delta^{13}C$) in seagrasses and the two carbon sources (DIC and C_{air}) (Clavier et al., 2011; Cooper and McRoy, 1988; Raven et al., 2002). However, the ^{13}C method has considerable uncertainty because in addition to the source of carbon, the $\delta^{13}C$ values of seagrasses are also determined by other factors such as the chemical species of DIC [CO_2(aq) or HCO_3^-] and photosynthetic carbon demand. The chemical species in the carbonate system (CO_2(aq), HCO_3^-, and carbonate ion [CO_3^{2-}]) have distinct $\delta^{13}C$ values, and isotopic fractionations change depending on pH and temperature (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995). Because the $\delta^{13}C$ of HCO_3^- (0‰) is isotopically distinct from that of both CO_2(aq) (−9‰) and C_{air} (−8‰) under normal seawater conditions (pH ≈ 8), $\delta^{13}C$ 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 $\delta^{13}C$ value overlap between $CO_2(aq)$ and C_{air} although low $\delta^{13}C$ 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 ($\Delta^{14}C$). The $\Delta^{14}C$ of inorganic carbon also has specific values depending on the source, such as DIC or C_{air}. The $\Delta^{14}C$ of DIC generally differs from that of atmospheric CO_2 because of the longer residence time of carbon in aquatic ecosystems than in the atmosphere (Ishikawa et al., 2014; Stuiver and Braziunas, 1993). Moreover, the calculation of $\Delta^{14}C$ by internal correction using $\delta^{13}C$ values eliminates any effects from isotopic fractionation (Stuiver and Polach, 1977), overcoming one of the major uncertainties in the conventional $\delta^{13}C$ approach. This study is the first to show quantitative evidence that the seagrass *Zostera marina* assimilates modern C_{air}, based on the $\Delta^{14}C$ values of the seagrass and two carbon sources.

2 Material and methods

2.1 Field surveys

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

mercuric chloride solution (400 μL per bottle) to prevent changes in DIC due to biological activity. The samples for measuring DIC concentration and TA were collected into 250-mL Duran bottles (SCHOTT AG), which were poisoned with saturated mercuric chloride solution (200 μL per bottle). Seagrass (*Z. marina*) leaves were collected at four stations covered by *Z. marina* meadows (Fig. 1; stations F3, F4, F8 and F9) along the salinity gradient. The stations were located in subtidal zones (mean water depth, 0.83–1.12 m). The aboveground wet-weight biomass of the seagrass, estimated from randomly thrown quadrats (0.0625 m^2), 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 groves and washed off using ultrapure water (Milli-Q water; Millipore, Billerica, MA, USA). To estimate the $\Delta^{14}C$ 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 ($\delta^{13}C$) and radiocarbon concentrations ($\Delta^{14}C$) of seagrass leaves, terrestrial plant leaves, DIC samples and POC samples. Prior to $\Delta^{14}C$ and $\delta^{13}C$ measurements, samples were subjected to graphite purification as follows. DIC samples for $\Delta^{14}C$ and $\delta^{13}C$ analysis were acidified (pH < 2) with H_3PO_4 and sparged using ultra-high purity mixed N_2/H_2 gas. The powdered plant leaves and POC samples for $\Delta^{14}C$ and $\delta^{13}C$ 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 CO_2 evolved was collected cryogenically and purified in a vacuum line. The purified CO_2 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:

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

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 $CO_2(aq)$ ($\delta^{13}C_{CO_2(aq)}$) and HCO_3^- ($\delta^{13}C_{HCO_3^-}$) were calculated as follows (Zeebe and Wolf-Gladrow, 2001; Zhang et al., 1995):

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

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

(3)

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

(4)

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

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 ε_{db} and ε_{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:

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

$\Delta^{14}C_{air}$ was estimated from the $\Delta^{14}C$ value of the sampled terrestrial plants ($\Delta^{14}C$ = +17.2‰). The $\Delta^{14}C$ values of DIC as the carbon source for *Z. marina* in the mixing model were estimated from the linear model fitted with the ANCOVA.

3 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, $\Delta^{14}C_{seagrass}$ was significantly higher than $\Delta^{14}C_{DIC}$ 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 $\Delta^{14}C_{DIC}$ are regulated mostly by mixing between high-$\Delta^{14}C$ river water and low-$\Delta^{14}C$ 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 $\Delta^{14}C_{seagrass}$ reflects $\Delta^{14}C_{DIC}$ 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 $\Delta^{14}C_{seagrass}$ cannot be explained by any residual contamination from terrestrial organic carbon on the leaves because the terrestrial POC was ^{14}C-depleted (mean $\Delta^{14}C$ of terrestrial POC, −74.7 ± 23.4‰).

The significantly higher values in $\Delta^{14}C_{seagrass}$ than $\Delta^{14}C_{DIC}$ shows that the seagrass assimilates ^{14}C-rich C_{air} ($\Delta^{14}C$ 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_{air} and air exposure time; however, the exposure time would mediate the assimilation of C_{air} (Clavier et al., 2011).

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

In any case, the $\Delta^{14}C$ approach is potentially applicable to other regions by using the $\Delta^{14}C$ gradient. However, the seasonal dynamics of $\Delta^{14}C_{DIC}$ 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_{air}) are distinct (not overlapping) as they were in May and July 2014 during this study. We could not use the $\Delta^{14}C$ approach to quantify the C_{air} contribution in September or November 2014 in Furen Lagoon because the $\Delta^{14}C_{DIC}$ of seawater increased to near $\Delta^{14}C_{air}$ and there was overlap between the two (Fig. 3). The overlapping in the range of values, induced by variations in the $\Delta^{14}C_{DIC}$ of seawater, likely caused by the dynamics of the Oyashio (mean $\Delta^{14}C_{DIC}$, −41‰; Aramaki et al., 2001; Aramaki et al., 2007) and the Soya warm current (mean $\Delta^{14}C_{DIC}$, 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; 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 $\Delta^{14}C_{DIC}$ 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 $\Delta^{14}C_{DIC}$ would, therefore, not overlap with $\Delta^{14}C_{air}$ 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_{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_{air} contribution here would be underestimated because the $\Delta^{14}C_{DIC}$ 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 ± 2.4‰) in the low-salinity zone (salinity, 12–15) and high (−8.8 ± 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; Hemminga and Mateo, 1996; 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; 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_{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_{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_{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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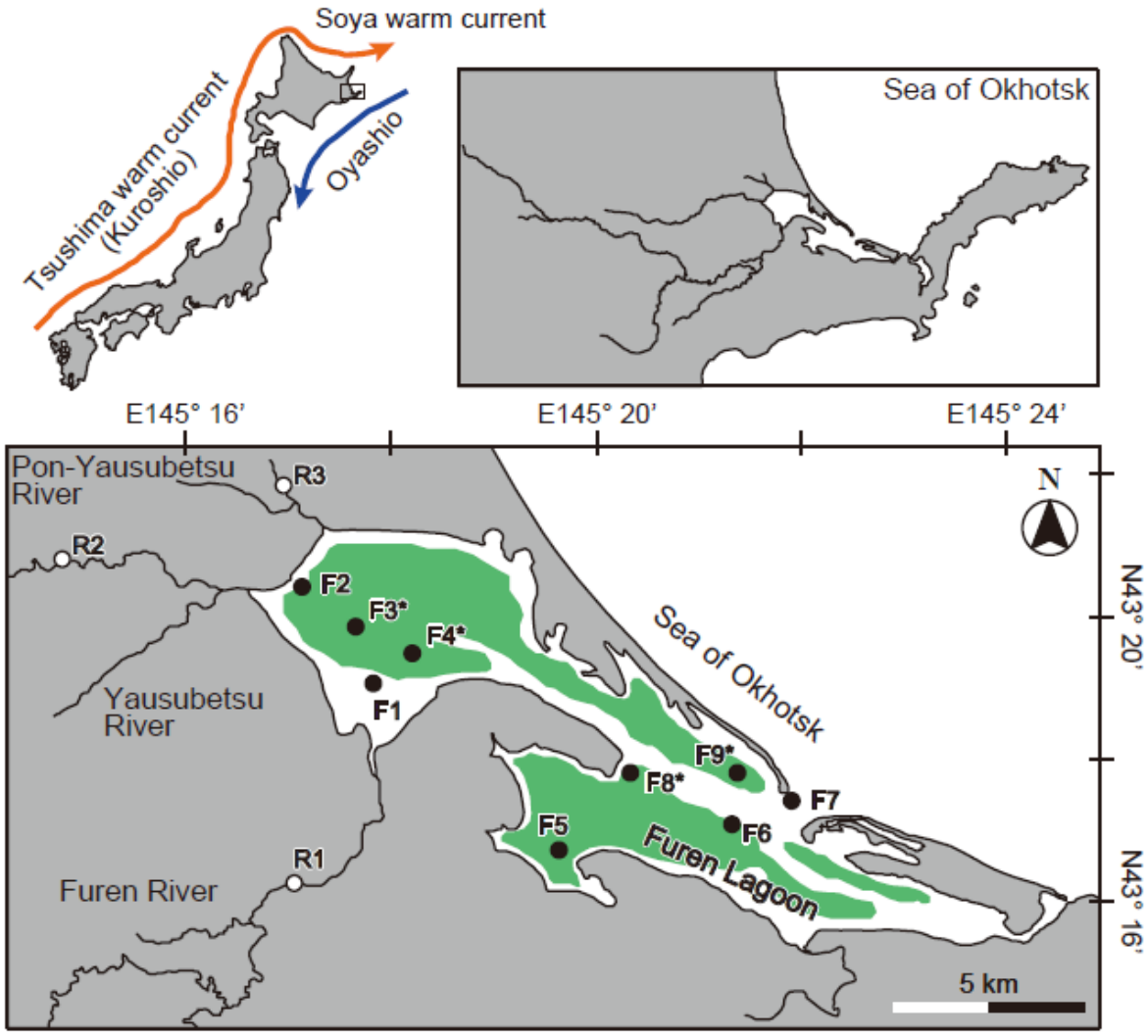

Figure 1. Location of Furen Lagoon and sampling stations. The area offshore of Furen Lagoon is affected by both the Oyashio and the Soya warm current. The northern part of the lagoon receives freshwater from the Furen, Yausubetsu, and Pon-Yausubetsu Rivers. Closed circles show lagoon stations. Water 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.

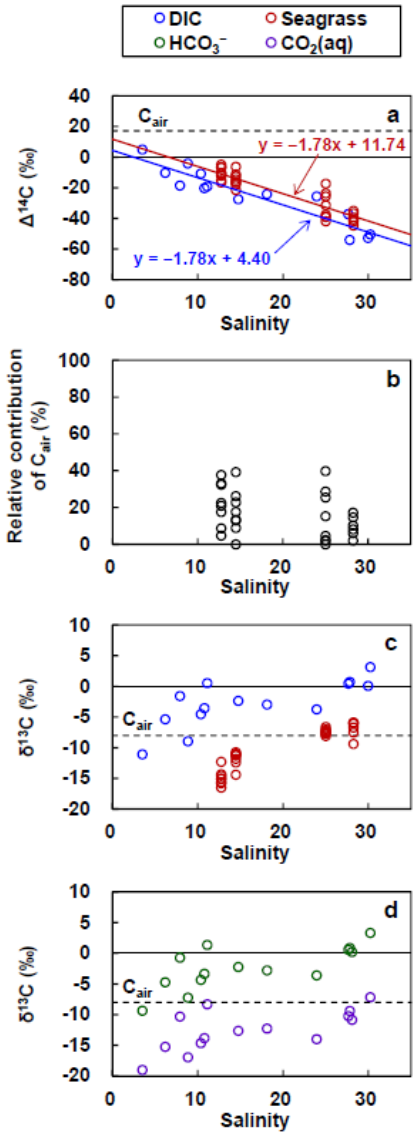

Figure 2. **(a)** Spatial distribution of the $\Delta^{14}C$ values of dissolved inorganic carbon (DIC) (blue open circles) and seagrass (red open circles) along the salinity gradient in May and July 2014 in Furen Lagoon, Japan. Blue and red solid lines represent the linear models fitted with analyses of covariance (ANCOVA) examined for DIC and seagrass, respectively. **(b)** Spatial distribution of the relative contribution of C_{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_{air}).

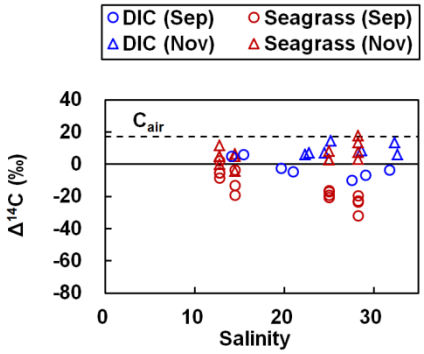

Figure 3. Spatial distribution of the $\Delta^{14}C$ values of dissolved inorganic carbon (DIC) (blue) and seagrass (red) along the salinity gradient in September (open circles) and November (open triangles) 2014 in Furen Lagoon, Japan. The dashed line indicates the $\Delta^{14}C$ of atmospheric CO_2 ($\Delta^{14}C_{air}$).

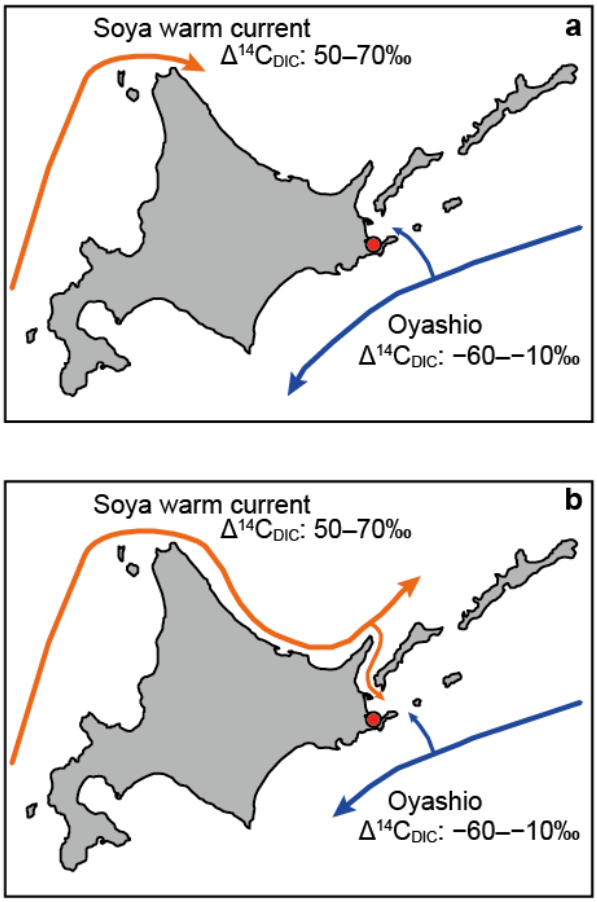

Figure 4. Seasonal dynamics of ocean currents affecting the oceanic boundary of Furen Lagoon. Red circle shows the location of Furen Lagoon. **(a)** From mid-November to August, the oceanic boundary is the Oyashio ($\Delta^{14}C_{DIC}$, −60–−10‰; Aramaki et al., 2001). **(b)** From September to early-November, the Soya warm current ($\Delta^{14}C_{DIC}$, 50–70‰; Aramaki et al., 2007) intruded into the boundary.

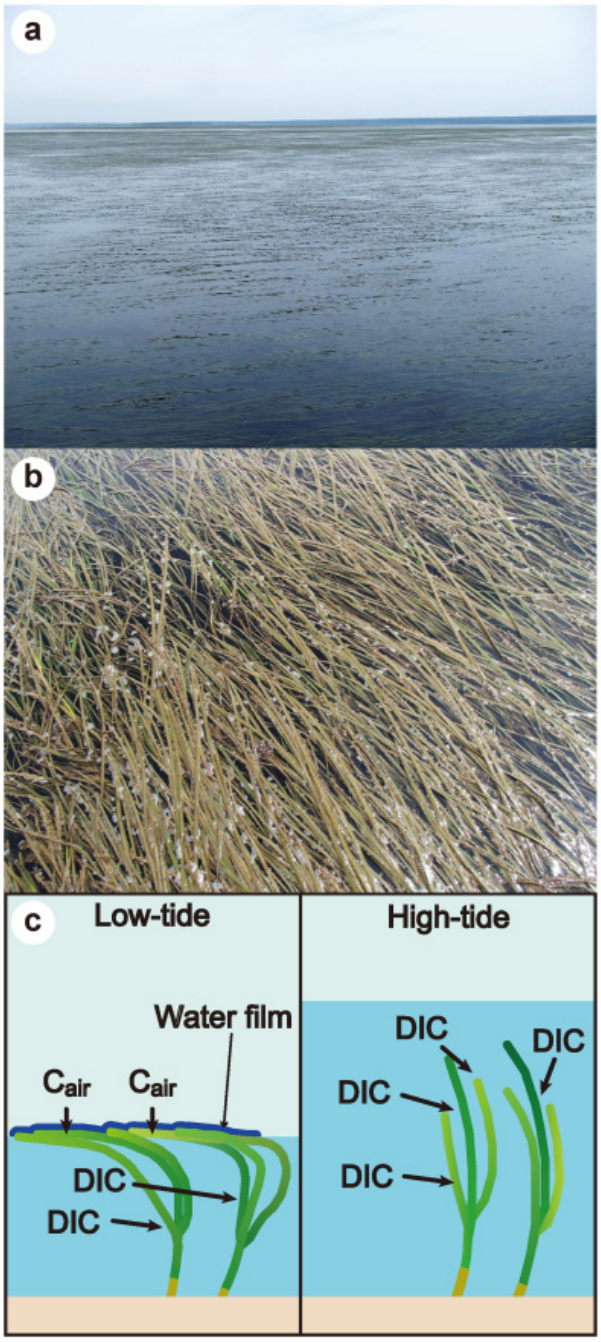

Figure 5. (a) Distant and (b) close-up views of the seagrass leaves exposed to the air during low tide in Furen Lagoon, Japan. (c) Conceptual diagram of the uptake of atmospheric CO_2 (C_{air}) across the surface-water film on the seagrass leaves during low tide (left), and the uptake of DIC during high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