Assessment of the influence of benzalkonium chloride addition on radiocarbon analysis of dissolved inorganic carbon

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Abstract

This study aimed to assess whether benzalkonium chloride (BAC) addition to water samples could be used as a disinfectant in the radiocarbon (14C) analysis of dissolved inorganic carbon (DIC). We investigated the effectiveness of BAC addition in inhibiting 14C changes in DIC due to microbial activity during water preservation. The DIC and 14C concentration of natural waters (groundwater, seawater, river water, and lake water) without BAC increased by 1.4–3.3 mg L–1 and 1.8–4.2 percent modern carbon (pMC) for 2 weeks, and 2.2–7.9 mg L–1 and 3.0–11.9 pMC for 4 weeks, respectively, while none of the BAC-treated water samples, excluding seawater, exhibited changes in DIC or 14C concentration. Although slight increases in DIC and 14C concentrations in seawater with BAC treatment were observed, 0.4 mg L–1 and 0.4 pMC for 2 weeks, and 1.6 mg L–1 and 1.5 pMC for 4 weeks, respectively, these changes were 20–40% lower than that in seawater without BAC treatment. Furthermore, carbon contamination into the sample water due to BAC decomposition and BAC-induced microorganism decomposition was negligible in 14C analysis, albeit using BAC solution without atmospheric CO2 contamination could be a significant issue. Our results demonstrate that BAC is an effective inhibitor of biological DIC changes for 14C analysis of freshwater samples, although slightly less effective in seawater.

The radiocarbon (14C) concentration of dissolved inorganic carbon (DIC) in water has been used in several studies to clarify water residence time, ocean circulation, anthropogenic carbon behavior, and magmatic or geogenic carbon supply (McNichol et al. 1994; Rose and Davisson 1996; Sikes et al. 2000; Matsumoto 2007; Takahashi et al. 2013; Han and Plummer 2016; Cartwright et al. 2020). Since natural water usually contains microorganisms, the DIC concentration, 14C concentration, and δ13C can change due to biological activities during sample preservation. Therefore, a disinfection process is needed to inhibit such DIC changes. The standard method for marine samples in international programs, such as WOCE, GLODAP, and GEOTRACES (Key et al. 2002; Anderson 2020; Olsen et al. 2020), is disinfection by mercuric chloride (HgCl2) solution (McNichol et al. 1994; McNichol et al. 2010; Abrams 2013). However, the release of mercury into the environment is heavily restricted owing to its toxicity to the ecosystem. Regarding the measurement of the 13C and δ13C of DIC, as well as alkalinity in nonmarine water samples, many methods have been suggested to inhibit biological activities during preservation. Refrigeration has been one of the most effective methods used for a long time. (Ascough et al. 2010), but it cannot be used for water sampling in study areas where controlling temperature is impossible (Wilson et al. 2020). Moreover, DIC concentrations, δ13C, and 14C concentrations in natural waters sometimes vary despite refrigeration (Takahashi et al. 2019b). Filtration is also effective (Doctor et al. 2008; Takahashi et al. 2019a; Wilson et al. 2020; Mos et al. 2021) as it has the advantage that water samples can be analyzed without contamination derived from chemical reagents. However, water samples containing a lot of suspended materials can clog the filter media, preventing their filtration. Another effective method is acid-mediated CO2 gas extraction in small vials performed in the field (Taipale and Sonninen 2009; Olack et al. 2018), but it is unrealistic for 14C preparation as 14C analysis necessitates the processing of large volumes. Regarding toxic substances, Taipale and Sonninen

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(2009) reported that copper hydroxide complexes, formed from copper added to lake water, influence DIC speciation by decreasing pH and altering $\delta^{13}$C by selective precipitation of $^{12}$C in the copper complexes. Mos et al. (2021) found that mercury reduces the total alkalinity of lake water by forming complexes with dissolved organic matter in the water. They mentioned that the reduction in total alkalinity by HgCl$_2$ was not apparent in seawater, where mercury tends to form complexes with Cl, suggesting that the use of HgCl$_2$ should be avoided when analyzing freshwater and groundwater samples to prevent analytical uncertainties. Miyakawa and Okumura (2018) also reported that adding HgCl$_2$ to drilling core groundwater can potentially vary the $\delta^{13}$C of CH$_4$ due to the isotopic fractionation associated with the reaction of mercury and methane which generates HCl and leads to CO$_2$ outgassing. Therefore, metal additives should be avoided when analyzing freshwater samples. Benzalkonium chloride (BAC) inhibits microbial activity by lysing cells, inactivating enzymes, and denaturing proteins. It has been used as a common disinfectant in food, medical, and other industrial applications. Previous studies (Knoepfle et al. 2009; Takahashi et al. 2019a; Wilson et al. 2020) have shown that BAC works well for DIC analysis of freshwater and seems effective (Table 1). However, the influence on $^{14}$C analysis by BAC addition to water sample has not been assessed. Thus, the present study aims to determine whether BAC influences $^{14}$C analysis. Considering that this analysis is considerably more sensitive to carbon contamination than $\delta^{13}$C analysis, the influence of BAC addition on the carbon blank must be investigated as follows: (1) Assessment of whether BAC addition is effective in inhibiting $^{14}$C changes in freshwater samples. Benzalkonium chloride (BAC) inhibits microbial activity by lysing cells, inactivating enzymes, and denaturing proteins. It has been used as a common disinfectant in food, medical, and other industrial applications. Previous studies (Knoepfle et al. 2009; Takahashi et al. 2019a; Wilson et al. 2020) have shown that BAC works well for DIC analysis of freshwater and seems effective (Table 1). However, the influence on $^{14}$C analysis by BAC addition to water sample has not been assessed. Thus, the present study aims to determine whether BAC influences $^{14}$C analysis. Considering that this analysis is considerably more sensitive to carbon contamination than $\delta^{13}$C analysis, the influence of BAC addition on the carbon blank must be investigated as follows: (1) Assessment of whether BAC addition is effective in inhibiting $^{14}$C changes in

### Table 1. Summary of previous studies on BAC assessment (excluding the studies using BAC as disinfectant only).

| Measured component                                      | Treatment compared with BAC | Results                                                                 | References                |
|----------------------------------------------------------|-----------------------------|------------------------------------------------------------------------|---------------------------|
| $\delta^{13}$C of DIC in pond and spring waters          | Filter                      | Good effectiveness: 0.2-$\mu$m filter, H$_3$PO$_4$, BAC, and HgCl$_2$ (no statistically significant difference) for ~ 2 months of preservation | Wilson et al. (2020)       |
|                                                           | HgCl$_2$                    | Significantly worse: Low concentration of CuSO$_4$, acidification with HCl and ZnCl$_2$ |                           |
|                                                           | CuSO$_4$                    | Filtering is recommended because samples without additive could be used in other analyses |                           |
|                                                           | ZnCl$_2$                    |                                                                         |                           |
|                                                           | HCl                         |                                                                         |                           |
|                                                           | H$_3$PO$_4$                 |                                                                         |                           |
| $\delta^{13}$C of DIC in groundwater, seawater, river, pond, and hot spring waters | HgCl$_2$                    | Both of BAC and NaCl showed good effectiveness in the freshwater samples for ~ 1–2 months of preservation, but the effect diminished for seawater and brackish water. BAC can be used for 10–15 days of preservation for saline waters. | Takahashi et al. (2019a) |
| $\delta^{13}$C of DIC in lake water                       | HgCl$_2$                    | Samples with BAC and HgCl$_2$ (N-6) showed identical $\delta^{13}$C values within the standard deviation of duplicate water samples. | Knoepfle et al. (2009) |
| $\delta^{13}$C of CO$_2$ and CH$_4$ in gases adsorbed on sediment drilling cores or cuttings (preserved with in situ water) | HgCl$_2$                    | BAC solution must be larger than 0.3%* in preserved sample jar to measure $\delta^{13}$C of CH$_4$ (probably also CO$_2$). | Miyakawa and Okumura (2018) |
| O$_2$/Ar and chlorophyll a (Chl a) in seawater           | HgCl$_2$                    | BAC was as effective as HgCl$_2$ at preserving O$_2$ concentrations in low Chl a seawater samples for up to 7 days, but only for 3 days for seawater samples containing Chl a concentrations of $\leq$ 1 mg m$^{-3}$. | Gloël et al. (2015) |
| Carboxylic acids in drinking water                       | HgCl$_2$                    | Minimum amount of BAC in water samples must be 30 mg L$^{-1}$ to eliminate biodegradation for 30 days of preservation. | Kuo (1998) |

*The high concentration required for their sediment-containing samples was likely caused by the higher adsorption affinity of BAC for solids than for water.
DIC due to microbial activity during sample preservation. (2) Verification of the effect of BAC addition on $^{14}$C analysis depending on the chemical composition of the water. (3) Investigation of the level of carbon contamination caused by BAC addition and its influence on $^{14}$C measurement of DIC.

**Materials and procedures**

**Natural water samples**

To confirm the inhibitory effect of BAC addition on microbial activities when determining $^{14}$C concentrations of DIC, verification with natural water samples containing microorganisms is necessary. In our study, three seawater (SW01–SW03), two groundwater (GW04 and GW05), two lake water (LW06 and LW07), and one river water (RW08) samples were used (nos. 1–8 in Table 2). SW01 was taken at a depth of 10 m at a site far from the coast in the Sea of Japan. SW02 and SW03 were collected from the sea surface of the Pacific Coast at Rinku Beach (Tokoname City), Aichi Prefecture, and Kashima port, Ibaraki Prefecture, respectively, in central Japan. GW04 and GW05 were taken from shallow wells at Tsukuba City, Ibaraki Prefecture, in eastern Japan. The two wells were located within 5 m of each other, with depths of 60 and 30 m, respectively. LW06, LW07, and RW08 were collected from Lake Kasumigaura, Lake Kitaura, and the Yata River, respectively, in Ibaraki Prefecture. No disinfectant treatment or filtering was carried out during sample collection for any of the natural water samples unless otherwise stated.

To stimulate microbial activity and allow detection of even the slightest DIC changes that may have occurred via biological influences, beet sugar powder was added to SW03, GW04, GW05, LW06, LW07, and RW08. The amounts dissolved were 0, 0.02, 0.1, 0.2, 1, and 2 g L$^{-1}$ of sample water. Samples in which 2 g of beet sugar was added (nos. 3–8, Table 2) were used for studying the suppression of microbial activity by BAC addition, and samples in which six different amounts (0–2 g) of beet sugar were added (nos. 9–10 in Table 2) were used for the comparison of DIC changes due to beet sugar addition. Since DIC derived from beet sugar likely has a high $^{14}$C concentration, water samples with a high $^{14}$C concentration may not exhibit $^{14}$C changes caused by the decomposition of beet sugar to DIC associated with microbial activity. Therefore, to decrease the $^{14}$C concentration in the water sample with sugar addition, NaHCO$_3$ solution (1 mol L$^{-1}$ solution, Kanto Table 2). Natural and artificial water samples for assessments of the effects of BAC addition on DIC changes, the comparison of DIC change by sugar addition, and $^{14}$C changes.

| No | Sample | Water type       | Additives | Preservation |
|----|--------|------------------|-----------|--------------|
|    |        |                  | Initial   | BAC-free     | BAC-added   | Period | Bottle |
| 1  | SW01   | Seawater         | —         | —            | B           | 32 days | PAN    |
| 2  | SW02   | Seawater         | —         | —            | B           | 67 days | PAN    |
| 3  | SW03   | Seawater         | S, N      | S, N         | S, N, B     | 14 and 29 days | PAN  |
| 4  | GW04   | Groundwater      | S, N      | S, N         | S, N, B     | 15 and 29 days | PAN  |
| 5  | GW05   | Groundwater      | S, N      | S, N         | S, N, B     | 15 and 32 days | PAN  |
| 6  | LW06   | Lake water       | S, N      | S, N         | S, N, B     | 15 and 29 days | PAN  |
| 7  | LW07   | Lake water       | S, N      | S, N         | S, N, B     | 15 and 30 days | PAN  |
| 8  | RW08   | River water      | S, N      | S, N         | S, N, B     | 14 and 28 days | PAN  |
| 9  | With sugar |            | S* N      | S* N        | Not prepared | 7 days | Glass |
| 10 | Without sugar |        | N         | N           | Not prepared | 7 days | Glass |
| 11 | Without sugar |        | —         | Not prepared | Not prepared | —   | —   |
| 12 | W09    | Shallow groundwater† | —  | —          | B           | 30 days | PAN  |
| 13 | W10    | Hot spring water (SO$_4$ type)† | —  | —          | B           | 31 days | PAN  |
| 14 | W11    | Hot spring water (Cl type)† | —  | —          | B           | 31 days | PAN  |
| 15 | W12    | Groundwater (volcanic region)† | —  | —          | B           | 35 days | PAN  |
| 16 | W13    | Seawater†        | —         | —          | B           | 35 days | PAN  |
| 17 | W14    | Deep groundwater† | —         | —          | B           | 36 days | PAN  |

Additives: beet sugar (S), NaHCO$_3$ solution (N), and BAC (B). “—” indicates that samples without additives. PAN: acrylonitrile butadiene methyl acrylate bottle (one bottle each for BAC-free and BAC-added waters). Glass: glass vials sealed with butyl rubber septa and aluminum caps (three vials for BAC-added water).

*Different amounts of beet sugar (0.02, 0.1, 0.2, 1, and 2 g L$^{-1}$ of sample water).
†Assumed type of natural water reported by Takahashi et al. (2021).
Chemical Co. Inc.), which is verified to have a low \(^{14}\)C concentration to be \(~0.7\) percent modern carbon (pMC) (Takahashi et al. 2021), was added to the water sample to approximately double the DIC concentration (nos. 3–10 in Table 2). The artificial depletion of \(^{14}\)C in the water sample by adding NaHCO\(_3\) solution allowed us to highlight \(^{14}\)C changes that would occur if beet sugar had a high \(^{14}\)C concentration was used in microbial metabolism.

To investigate DIC change during preservation, three kinds of water samples, initial, BAC-free, and BAC-added, were measured for nos. 1–8 and 12–17 samples in Table 2. The initial water was a sample before preservation and mixed with the same additives as preserved waters except for BAC. At the beginning of preservation, all samples were placed in the preservation bottle at the same time as the initial water treatment, and CO\(_2\) extraction (nos. 1–8 and 12–17 in Table 2) and Gas Chromatograph-Isotope Ratio Mass Spectrometer (GC-IRMS) measurement (nos. 9–11 in Table 2), were performed as described below. The BAC-free and BAC-added waters were the samples preserved for a certain period, differing only in BAC addition.

**Artificial water samples**

To evaluate the influence of BAC addition alone, it is necessary to conduct an assessment by eliminating biological influence that could vary the \(^{14}\)C concentration of DIC during the experiment. Therefore, artificial water samples without microorganisms (W09–W14) prepared by Takahashi et al. (2019c), for interlaboratory comparisons of the \(^{14}\)C concentrations of DIC by adding chemical reagents to ultra-purified water, were used (nos. 12–17 in Table 2). They were designed to have similar chemical contents as natural waters, shallow and deep groundwaters, hot spring waters (SO\(_4\) type and Cl type), groundwater (volcanic region), and seawater. Their \(^{14}\)C concentrations were reported to range from 0.61 to 93.54 pMC (Takahashi et al. 2021). Three bottles each of W09–W14 were randomly chosen from the unused stocks and used for initial, BAC-free, and BAC-added waters.

**Water preservation**

For natural water samples, BAC-free and BAC-added waters were preserved in acrylonitrile butadiene methyl acrylate (PAN) bottles (full fill volume is 313 mL) for 32 days (SW01), 67 days (SW02), 30–36 days (W09–W14), and for two periods of 14–15 and 28–32 days (SW03, GW04, GW05, LW06, LW07, and RW08) at room temperature (nos. 1–8 and 12–17 in Table 2). Since the PAN bottle has a high-performance gas barrier (Takahashi et al. 2019b), it is suitable for water preservation with protection from CO\(_2\) contamination. All sample waters, except SW01, were treated up to \(~15\) days after sampling; SW01 had been stored at room temperature (10–25\(^{\circ}\)C) for 8 yr until it was used in the present study. SW02 was directly aliquoted into each bottle at the water sampling site, while the water from other sampling sites was stirred separately in a large beaker to ensure homogeneity before being aliquoted into the bottles. W09–W14, which had been preserved in PAN bottles, were used directly for BAC-free and BAC-added waters from respective unused stock bottles without homogenization.

As reported by Takahashi et al. (2019a), 1 mL of 1% BAC solution per 100 mL of water sample was added unless otherwise indicated. The 1% BAC solution was prepared from 10% BAC stock solution (FUJIFILM Wako Pure Chemical Co.) by dilution with ultra-purified water (Milli-Q Direct 8 or Milli-Q Integral 3, Merck Millipore Co.). BAC was added to natural water samples prior to filling the preserved bottles. For artificial waters, the bottle was temporarily opened and closed for the addition of BAC before preservation.

To compare DIC changes between water samples with and without sugar addition, natural waters with six different amounts of beet sugar were preserved for 7 days in three glass vials (13 mL) sealed with butyl rubber septa and aluminum caps. These were not prepared with BAC-added water, but only initial and BAC-free waters (nos. 9 and 10 in Table 2). This experiment was carried out \(~90\) days after collecting the water.

**Procedures**

To extract CO\(_2\) from water samples (nos. 1–8 and 12–17 in Table 2), the ReCEIT procedure (Takahashi et al. 2021) was used, which can treat a variety of water samples with a wide range of DIC concentrations (0.4–100 mmol L\(^{-1}\), in the case of 1.2 mg of carbon) and produces high CO\(_2\) yields (\(~98\%)\). The ReCEIT is a simple method for extracting CO\(_2\) from DIC in water samples without a carrier gas, which consists of repeated cycles of CO\(_2\) extraction from water into the headspace of the reaction container, expansion of the extracted gas into the vacuum line, and cryogenic trapping of CO\(_2\). The CO\(_2\) gas after cryogenic purification was quantified by pressure measurement in a known volume of vacuum line and subsequently reduced to graphite for accelerator mass spectrometry (AMS) measurements (Kitagawa et al. 1993). Approximate DIC concentration was obtained from amounts of treated water and extracted CO\(_2\) (nos. 3–8 and 12–17 in Table 2), and its error was assumed to be 2.9%, based on the standard deviation of CO\(_2\) extraction yield of the ReCEIT procedure (Takahashi et al. 2021). As appropriate, Sulfix treatment (8–20 mesh, Kishida Chemical Co., Ltd) was conducted to remove sulfide gases before graphitization. The \(^{13}\)C concentrations of artificial and natural waters were measured using AMS (model 4130-AMS, HVEE) at the Institute for Space-Earth Environmental Research, Nagoya University, Japan (Nakamura et al. 2000). Isotopic fractionation was corrected using the \(^{13}\)C/\(^{12}\)C ratio measured by the AMS system. The \(^{14}\)C concentration is expressed as pMC (Stuiver and Polach 1977). The standard deviations (1σ) in \(^{14}\)C concentration of extracted CO\(_2\) were 0.02–0.04 pMC for waters below 1 pMC, and below 0.8% of \(^{14}\)C concentration for waters above 10 pMC.
(Takahashi et al. 2021). The average δ13C discrepancy between the extracted CO2 by the ReCEIT procedure and direct analyses by the GC-IRMS was 0.02 ± 0.06‰ (Takahashi et al. 2021).

The radiocarbon concentration and δ13C of BAC and beet sugar were measured using the AMS system mentioned above and an IRMS with a dual inlet system (Delta-V Advantage, Thermo Fisher Scientific, Inc.) at the Geological Survey of Japan, in order to evaluate DIC contamination decomposed from the additives. For combustion to CO2, the BAC was sealed in capillary glass tubes and the beet sugar was sealed in vacuum quartz tubes with CuO; both were heated stepwise up to 900°C (Minami et al. 2013) and 850°C for 3 h, respectively. DIC concentrations and δ13C values of water samples with and without sugar addition (nos. 9 and 10 in Table 2) and water samples without any additives (no. 11 in Table 2) were measured using the IRMS with gas chromatography (GasBench II, Thermo Fisher Scientific Inc.). The measurement errors were approximately ±2% for DIC concentration and ±0.04‰ for δ13C (Takahashi et al. 2019a). The error of DIC concentration was calculated as the standard deviation of the measured value of three different vials of the preserved water samples, or propagation error from ±2% of the individual measurement of DIC concentration, and the larger value from these was used. It was also used the error of individual measurement for the initial water since it was measured only once.

**Evaluation of boost effect by sugar addition on DIC change**

In this study, the boost effect (BE) is defined as an index of how many times the DIC change in the sugar-added sample (boosted) is greater than the DIC concentration change in the no-sugar sample (no-boosted) during the preservation. It was quantified by the ratio of the DIC concentration changes between the boosted with sugar and no-boosted without sugar during an identical preservation period as follows:

\[
BE = \frac{\text{DIC}_{\text{boost}} \text{DIC}_{\text{boost-ini}}}{\text{DIC}_{\text{no-boost}} \text{DIC}_{\text{no-boost-ini}}},
\]

(1)

where DIC_{boost}, DIC_{boost-ini}, DIC_{no-boost}, and DIC_{no-boost-ini} are measured DIC concentrations of boosted waters after and before preservation, and those of no-boosted waters after and before preservation, respectively. The denominator and numerator of Eq. 1 are, respectively, expressed as follows:

\[
\begin{align*}
\text{DIC}_{\text{boost}} \text{DIC}_{\text{boost-ini}} & = \text{DIC}_{\text{sugar}}, \\
\text{DIC}_{\text{no-boost}} \text{DIC}_{\text{no-boost-ini}} & = \text{DIC}_{\text{org}},
\end{align*}
\]

(2) (3)

where subscripts “sugar” and “org” are sugar dissolved to water sample and organic matter in initial water, respectively. DIC_{sugar} and DIC_{org} indicate the DIC concentration increased by boosting with sugar addition, and the original DIC concentration change without boosting with sugar during the preservation, respectively.

When the value of DIC_{org} was negative, for example, DIC_{no-boost-ini} was measured to be 21.5 ± 0.4 mg L⁻¹, and DIC_{no-boost} was 20.5 ± 0.4 mg L⁻¹, it was considered that DIC concentration during the preservation was not changed. However, DIC_{org} must be greater than zero to calculate BE. Thus, we assumed the value DIC_{org} as the error range propagated from measurement errors of DIC_{no-boost} and DIC_{no-boost-ini}. So, in the case of the example above, it was considered that DIC_{org} was not −1.0 ± 0.6 mg L⁻¹ but 0.0 ± 0.6 mg L⁻¹, and assumed as 0.6 mg L⁻¹. Moreover, when the error range of DIC_{org} reached negative values, such as 0.2 ± 0.4 mg L⁻¹, it was considered the same way, and the DIC_{org} was assumed to be 0.4 mg L⁻¹.

The value of DIC_{sugar} can be obtained using the isotopic mass balance calculation, expressed as follows:

\[
\text{DIC}_{\text{boost-ini}} \delta^{13}C_{\text{boost-ini}} \text{DIC}_{\text{sugar}} \delta^{13}C_{\text{sugar}} = \left( \text{DIC}_{\text{boost-ini}} + \text{DIC}_{\text{sugar}} \right) \delta^{13}C_{\text{boost}},
\]

(4)

where δ13C_{boost}, δ13C_{boost-ini}, and δ13C_{sugar} are the δ13C values of boosted waters after and before preservation, and that of beet sugar, respectively. The approach using the δ13C can clearly indicate that the DIC that increased during preservation is derived from beet sugar.

**Evaluation of DIC changes without BE**

To estimate the DIC change without BE by sugar addition during preservation, 14C mass balance can be used as follows:

\[
\text{DIC}_{\text{ini}} ^{14}C_{\text{ini}} + \text{DIC}_{\text{org}} ^{14}C_{\text{org}} + \text{DIC}_{\text{sugar}} ^{14}C_{\text{sugar}} = \left( \text{DIC}_{\text{ini}} + \text{DIC}_{\text{org}} + \text{DIC}_{\text{sugar}} \right) ^{14}C_{\text{after}},
\]

(5)

where subscripts “ini” and “after” are initial water, and water after preservation, respectively. The target for estimation in Eq. 5 is DIC_{org}. The (DIC_{ini} + DIC_{org} + DIC_{sugar}) on the right-hand side of Eq. 5 could be taken as the DIC_{after}, which was obtained by CO2 quantification at CO2 extraction, but CO2 extraction has uncertainty, and it cannot be verified; therefore, DIC_{after} was not adopted. DIC_{sugar} can be expressed in terms of DIC_{org} and BE values using Eqs. 1–3, and by substituting this value into Eq. 5, DIC_{org} can be obtained even if DIC_{sugar} is unknown. DIC_{ini} was assumed by DIC concentration obtained from CO2 quantification at CO2 extraction. Since this value would define the approximate DIC concentration, the error was assumed to be zero. For 14C_{ini} and 14C_{sugar}, the values measured in this study were used. 14C_{org} was assumed to be 100 pMC. Since natural waters used in this study were collected in terrestrial and coastal regions, carbon in them was expected as recently formed, that is, ~100 pMC.

Using the value of DIC_{org} estimated by Eq. 5, 14C change during the preservation, which eliminated the sugar added BE (14C_{ini-org}), can be calculated as follows:
\[ 14C_{ini+org} = \frac{DIC_{ini} \cdot 14C_{ini} + DIC_{org} \cdot 14C_{org}}{DIC_{ini} + DIC_{org}} \] (6)

where subscript “ini + org” is the mixed component of “ini” and “org.” All values of parameters in Eqs. 5 and 6 were evaluated with errors of propagation calculation from the values of analytical and assumed errors.

**Assessment**

**14C changes of natural water samples preserved with and without BAC**

The 14C concentrations of initial water, BAC-free water, and BAC-added water were measured to be 97.2 ± 0.3 pMC, 97.6 ± 0.3 pMC, and 97.2 ± 0.3 pMC in SW01, and 102.5 ± 0.3 pMC, 102.6 ± 0.3 pMC, and 102.2 ± 0.3 pMC in SW02, respectively, and they were identical within respective samples (Fig. 1). It can be interpreted as suggesting that the addition of BAC suppresses DIC changes due to microbial activity, or as suggesting that the addition of BAC is not necessary. For SW01, since the organic material that is the source of DIC depleted owing to the long time since the sampling (8 yr), the 14C concentration would not have changed without the addition of BAC. This suggests that no change in 14C concentration can be detected when BAC is added to seawater. For SW02, organic materials can be degraded to DIC by microbial activity, but those carbons might be the same carbons with high 14C concentration as in the DIC, since SW02 was taken at the coast where terrestrial carbon was supplied. Hence, we considered that the 14C concentration of SW02 would not change with microbial activity.

The DIC and 14C concentrations of initial water were 54.2 ± 1.6 mg L\(^{-1}\) and 46.5 ± 0.2 pMC in SW03, 44.9 ± 1.3 mg L\(^{-1}\) and 9.7 ± 0.1 pMC in GW04, 40.7 ± 1.2 mg L\(^{-1}\) and 40.7 ± 0.2 pMC in GW05, 34.5 ± 1.0 mg L\(^{-1}\) and 46.8 ± 0.2 pMC in LW06, 39.0 ± 1.1 mg L\(^{-1}\) and 48.7 ± 0.2 pMC in LW07, and 30.1 ± 0.9 mg L\(^{-1}\) and 40.7 ± 0.2 pMC in RW08, respectively. Since DIC and 14C of each sample were added and diluted by NaHCO\(_3\), respectively, the original concentrations will be approximate half and twice of these values, respectively. For BAC-free water, the 14C concentrations exhibited large, nearly proportional increases over the preservation periods (Fig. 2). The incremental 14C and DIC concentrations were 5.6–19.3 pMC and 3.9–10.0 mg L\(^{-1}\) after ~15 days, and 10.0–42.0 pMC and 5.0–27.1 mg L\(^{-1}\) after ~30 days, respectively. DIC increase might be explained by the supply of beet sugar-derived DIC with a 14C concentration of 103.3 pMC, so GW04 with the lowest 14C concentration in initial water showed the greatest change. Regarding the BAC-added water, the 14C concentration was relatively constant during the preservation period, except for SW03. The 14C concentrations between the initial water and BAC-added water were identical for GW05 at both ~15 days and ~30 days of preservation, but samples showed changes slightly larger than the error ranges, 0.3 pMC and 0.3 pMC for GW04, 0.4 pMC and 0.6 pMC for LW06, 0.6 pMC and 0.3 pMC for LW07, and 0.8 pMC and 0.7 pMC for RW08, respectively, than those in other natural waters. Since DIC changes are augmented by sugar addition, the actual 14C changes must be smaller than the measured values.

**BE by sugar addition on DIC change**

The DIC concentration and \(\delta^{13}C\) changes in natural waters during the 7-days preservation period differed between water samples with added beet sugar and those with no added sugar (Fig. 3). Sugar-added samples showed greater changes in DIC concentration and \(\delta^{13}C\), 2.7–6.4 mg L\(^{-1}\) (Table 3) and −3.9‰ to −1.8‰, respectively. These changes did not show a significant difference regardless of the amount of sugar added. Regarding sugar-free samples, DIC concentration and \(\delta^{13}C\) changes were small, −1.4 to +1.6 mg L\(^{-1}\) (Table 3) and −0.0‰ to +0.2‰, respectively. DIC concentrations of sugarfree LW06, LW07, and RW08 decreased during the preservation, but this was likely due to analytical problems rather than consumption of DIC during preservation. While it is difficult to determine the appropriate amount of sugar, as it is related to the preservation period, we can conclude that the experimental conditions provided sufficient carbon. The 14C concentration and \(\delta^{13}C\) of beet sugar were measured to be 103.3 ± 0.7 pMC and −26.2‰, respectively.

Estimated results of the BEs on DIC changes resulting from the increase of microbial activity by the addition of sugar are shown in Table 3. Although the BE estimated from the isotopic mass balance tends to be larger, there was no significant difference between the results of the two calculation methods. The averaged value of BEs obtained from two estimations for the 7-days preservation period following the addition of sugar...
indicated 3.0 ± 1.4 in SW03, 5.3 ± 1.8 in GW04, 6.3 ± 1.9 in GW05, 5.9 ± 1.6 in LW06, 6.3 ± 1.9 in LW07, and 7.3 ± 1.8 in RW08, respectively.

\section*{14C change of artificial waters by BAC addition}

All the artificial water results (W09–W14) showed identical 14C concentrations within the error ranges of AMS
Table 3. Changes in DIC concentration during 7-days preservation of natural water samples and BE on DIC change induced by sugar addition. The results with superscript 1 were obtained from DIC concentration measurements and Eq. 1. The results with superscript 2 were derived from the isotopic mass balance and calculated using Eqs. 1 and 4.

| Sample | Amount of beet sugar added (g) | DIC change $^1$ (mg L$^{-1}$) | BE$^1$ (added/not added) | DIC change $^2$ (mg L$^{-1}$) | BE$^2$ (added/not added) |
|--------|--------------------------------|--------------------------------|--------------------------|--------------------------------|--------------------------|
| SW03   | 0                              | 1.6 ± 1.3                      | —                        | —                              | —                        |
| SW03   | 0.02                           | 5.2 ± 1.4                      | 3.2 ± 4.4                | 5.0 ± 0.6                      | 3.1 ± 4.2                |
| SW03   | 0.1                            | 5.2 ± 1.4                      | 3.3 ± 4.4                | 5.0 ± 0.3                      | 3.1 ± 4.1                |
| SW03   | 0.2                            | 5.4 ± 1.4                      | 3.4 ± 4.6                | 5.1 ± 0.4                      | 3.2 ± 4.2                |
| SW03   | 1                              | 3.9 ± 1.3                      | 2.4 ± 3.3                | 5.0 ± 0.3                      | 3.1 ± 4.2                |
| SW03   | 2                              | 4.6 ± 1.3                      | 2.9 ± 3.9                | 4.7 ± 0.3                      | 2.9 ± 3.9                |
| Average of | BE = 3.0 ± 1.4 | Average of | BE$^1$ = 3.0 ± 1.9 | Average of | BE$^2$ = 3.1 ± 1.8 |
| GW04   | 0                              | 1.1 ± 1.1*                     | —                        | —                              | —                        |
| GW04   | 0.02                           | 4.9 ± 1.1                      | 4.7 ± 5.0                | 5.1 ± 0.4                      | 4.8 ± 5.1                |
| GW04   | 0.1                            | 5.6 ± 1.1                      | 5.3 ± 5.6                | 5.9 ± 0.5                      | 5.6 ± 5.9                |
| GW04   | 0.2                            | 4.8 ± 1.1                      | 4.5 ± 4.9                | 5.8 ± 0.6                      | 5.5 ± 5.8                |
| GW04   | 1                              | 5.4 ± 1.0                      | 5.1 ± 5.5                | 7.5 ± 0.9                      | 7.1 ± 7.5                |
| GW04   | 2                              | 4.8 ± 1.0                      | 4.6 ± 4.9                | 6.4 ± 0.6                      | 6.1 ± 6.5                |
| Average of | BE = 5.3 ± 1.8 | Average of | BE$^1$ = 4.8 ± 2.3 | Average of | BE$^2$ = 5.8 ± 2.8 |
| GW05   | 0                              | 0.9 ± 0.9*                     | —                        | —                              | —                        |
| GW05   | 0.02                           | 4.5 ± 1.0                      | 4.8 ± 4.6                | 6.6 ± 0.3                      | 7.0 ± 6.6                |
| GW05   | 0.1                            | 4.8 ± 1.0                      | 5.1 ± 4.9                | 7.0 ± 0.2                      | 7.5 ± 7.0                |
| GW05   | 0.2                            | 6.4 ± 1.1                      | 6.8 ± 6.5                | 7.7 ± 1.1                      | 8.2 ± 7.8                |
| GW05   | 1                              | 4.6 ± 0.9                      | 4.9 ± 4.7                | 6.6 ± 0.3                      | 7.1 ± 6.6                |
| GW05   | 2                              | 3.7 ± 0.9                      | 3.9 ± 3.8                | 7.0 ± 0.5                      | 7.4 ± 7.0                |
| Average of | BE = 6.3 ± 1.9 | Average of | BE$^1$ = 5.1 ± 2.3 | Average of | BE$^2$ = 7.4 ± 3.1 |
| LW06   | 0                              | 0.8 ± 0.8*                     | —                        | —                              | —                        |
| LW06   | 0.02                           | 3.3 ± 1.0                      | 4.1 ± 3.5                | 5.6 ± 0.8                      | 7.0 ± 5.7                |
| LW06   | 0.1                            | 3.3 ± 0.9                      | 4.0 ± 3.4                | 5.9 ± 0.2                      | 7.3 ± 5.9                |
| LW06   | 0.2                            | 3.2 ± 0.8                      | 4.0 ± 3.4                | 5.6 ± 0.2                      | 6.9 ± 5.6                |
| LW06   | 1                              | 4.0 ± 1.1                      | 4.9 ± 4.2                | 8.1 ± 1.7                      | 10.0 ± 8.3               |
| LW06   | 2                              | 2.7 ± 0.9                      | 3.4 ± 3.0                | 6.0 ± 0.5                      | 7.5 ± 6.1                |
| Average of | BE = 5.9 ± 1.6 | Average of | BE$^1$ = 4.1 ± 1.6 | Average of | BE$^2$ = 7.7 ± 2.9 |
| LW07   | 0                              | 0.9 ± 0.9*                     | —                        | —                              | —                        |
| LW07   | 0.02                           | 4.8 ± 1.0                      | 5.1 ± 5.0                | 5.7 ± 0.4                      | 6.2 ± 5.8                |
| LW07   | 0.1                            | 4.9 ± 1.0                      | 5.3 ± 5.0                | 6.7 ± 0.7                      | 7.2 ± 6.7                |
| LW07   | 0.2                            | 5.3 ± 1.0                      | 5.7 ± 5.4                | 6.4 ± 0.3                      | 6.9 ± 6.4                |
| LW07   | 1                              | 5.0 ± 0.9                      | 5.4 ± 5.1                | 6.2 ± 0.6                      | 6.6 ± 6.2                |
| LW07   | 2                              | 5.6 ± 0.9                      | 6.1 ± 5.7                | 7.5 ± 0.2                      | 8.1 ± 6.1                |
| Average of | BE = 6.3 ± 1.9 | Average of | BE$^1$ = 5.5 ± 2.3 | Average of | BE$^2$ = 7.0 ± 2.9 |
| RW08   | 0                              | 0.7 ± 0.7*                     | —                        | —                              | —                        |
| RW08   | 0.02                           | 3.9 ± 0.8                      | 5.3 ± 4.0                | 6.4 ± 0.2                      | 8.8 ± 6.4                |
| RW08   | 0.1                            | 4.1 ± 0.9                      | 5.6 ± 4.5                | 6.3 ± 0.6                      | 8.7 ± 6.4                |
| RW08   | 0.2                            | 3.7 ± 0.8                      | 5.1 ± 3.9                | 3.9 ± 0.1                      | 8.5 ± 6.3                |
| RW08   | 1                              | 4.5 ± 0.8                      | 6.1 ± 4.6                | 3.9 ± 0.1                      | 9.4 ± 6.9                |
| RW08   | 2                              | 4.1 ± 0.7                      | 5.6 ± 4.2                | 3.9 ± 0.1                      | 10.4 ± 7.7               |
| Average of | BE = 7.3 ± 1.8 | Average of | BE$^1$ = 5.5 ± 1.9 | Average of | BE$^2$ = 9.1 ± 3.0 |

The error values with the DIC change and the averaged BE of respective samples are propagated from the measurement errors of DIC concentration before and after preservation and from errors of Average of BE$^1$ and Average of BE$^2$, respectively. The error values with Average of BE$^1$ and Average of BE$^2$ are standard deviations (1σ) of the values of the BE within each sample.

*The assumed value from the propagation error.

measurement among initial water, BAC-free water, and BAC-added water samples (Fig. 4). No $^{14}$C changes in the BAC-free water after preservation indicate that the samples were stored well. The $^{14}$C concentration and δ$^{13}$C for the carbon of BAC itself, not the carbon dissolved in the BAC solution, were measured to be 60.7 ± 0.14 pMC ($n = 3$) and −29.7‰ ($n = 2$), respectively.
Discussion

Inhibitory effects of BAC on microbial activity in preserved water

DIC and $^{14}$C concentrations which eliminated the BE by sugar addition $(\text{DIC}_{\text{org}}$ and $^{14}$C$_{\text{ini} + \text{org}}$) are listed in Table 4. These values indicate the biogenic DIC changes during preservation; if it is zero or no change from the initial value, it shows that DIC production due to microbial activity has been suppressed. The $\text{DIC}_{\text{org}}$ and $^{14}$C$_{\text{ini} + \text{org}}$ of BAC-added water, except for SW03, did not change during preservation. The slight changes in $\text{DIC}_{\text{org}}$ and $^{14}$C$_{\text{ini} + \text{org}}$ in SW03 were observed, 0.4 mg L$^{-1}$ and 0.4 pMC for 2 weeks, and 1.6 mg L$^{-1}$ and 1.5 pMC for 4 weeks, respectively. Although these changes are much smaller than BAC-free water showing increases in $\text{DIC}_{\text{org}}$ and $^{14}$C$_{\text{ini} + \text{org}}$, 2.0 mg L$^{-1}$ and 1.8 pMC for ~2 weeks, and 3.9 mg L$^{-1}$ and 3.4 pMC, respectively, they are not negligible. For other BAC-free waters, $\text{DIC}_{\text{org}}$ and $^{14}$C$_{\text{ini} + \text{org}}$ increased during the preservation period, 1.3–3.3 mg L$^{-1}$ and 1.9–4.2 pMC for ~2 weeks, and 2.2–7.9 mg L$^{-1}$ and 3.0–11.9 pMC for 4 weeks, respectively. DIC change in SW03 is not larger than those of other waters in BAC-free water, so the suppression effect of BAC is not likely related to the magnitude of the DIC.

Table 4. DIC and $^{14}$C concentrations of the initial natural water samples and DIC concentrations derived from microbial activity ($\text{DIC}_{\text{org}}$) and $^{14}$C concentration ($^{14}$C$_{\text{ini} + \text{org}}$), which eliminated the sugar-added BE. Both DIC and $^{14}$C concentrations did not show their original values because of NaHCO$_3$ addition to water samples.

| Sample | Initial water | BAC-added water | BAC-free water |
|--------|---------------|-----------------|----------------|
|        |               | 2 weeks | 4 weeks | 2 weeks | 4 weeks |
|        | DIC (mg L$^{-1}$) |        |        |        |        |
| SW03   | 54.2          | 0.4 ± 0.2 | 1.6 ± 0.7 | 2.0 ± 0.9 | 3.9 ± 1.7 |
| GW04   | 44.9          | 0.0 ± 0.0 | 0.0 ± 0.0 | 2.2 ± 0.8 | 7.0 ± 2.4 |
| GW05   | 40.7          | 0.0 ± 0.0 | 0.1 ± 0.0 | 1.3 ± 0.4 | 2.2 ± 0.7 |
| LW06   | 34.5          | 0.0 ± 0.0 | 0.0 ± 0.0 | 1.4 ± 0.4 | 4.1 ± 1.2 |
| LW07   | 39.0          | 0.1 ± 0.0 | 0.0 ± 0.0 | 3.3 ± 1.0 | 7.9 ± 2.4 |
| RW08   | 30.1          | 0.1 ± 0.0 | 0.0 ± 0.0 | 1.7 ± 0.4 | 4.1 ± 1.0 |

|        | $^{14}$C (pMC) | $^{14}$C$_{\text{ini} + \text{org}}$ (pMC) |
|--------|---------------|----------------|
| SW03   | 46.5 ± 0.2    | 48.3 ± 0.8     |
| GW04   | 9.7 ± 0.1     | 13.9 ± 1.4     |
| GW05   | 40.7 ± 0.2    | 42.5 ± 0.6     |
| LW06   | 46.8 ± 0.2    | 48.8 ± 0.6     |
| LW07   | 48.1 ± 0.2    | 52.0 ± 1.1     |
| RW08   | 40.7 ± 0.2    | 43.8 ± 0.8     |

Fig. 4. $^{14}$C concentrations of artificial water samples (W09–W14) before and after preservation for 30–36 d ($\bigcirc$, initial water; $\bigtriangleup$, BAC-free water; $\bullet$, BAC-added water). DIC concentrations were obtained from Takahashi et al. (2019c).
change. These results suggest that adding BAC is an effective treatment to obtain DIC data as it inhibits microbial activities, although it is slightly less effective in seawater.

Gloël et al. (2015) and Takahashi et al. (2019a) also reported that BAC is less effective in seawater, and noted that BAC could inhibit microbial activities for short preservation periods, a few days for O2/Ar analysis, and ~ 15 days for the δ13C analysis of DIC. It is unclear why BAC is not fully effective in seawater, but the presence of spores, that have specialized resistance mechanisms and structures that promote survival, might be one of the reasons (Gloël et al. 2015). They presumed that viable bacteria were killed immediately by BAC exposure, but spores germinated within a few days after BAC exposure. However, although the spores survive BAC exposure, the germinated microorganisms should be killed by BAC. This suggests two possibilities: the effects of DIC produced when the spores germinate and the inability of BAC to maintain its effects for long periods. The latter possibility seems unlikely since the experiments by Gloël et al. (2015) showed no effect when the amount of BAC added to seawater was varied. It remains to be verified whether the bactericidal ability of BAC is temporary and not long-lasting. BAC is a very stable substance, so it is expected that the ability of BAC is maintained for a longer period than the ~ 30 days or so tested in the present study. Of course, if the samples are completely sealed after the microorganisms are killed, there should be no problem with even longer preservation, but it will need to be verified in the future. Takahashi et al. (2019a) have reported that BAC is effective for preservation within 10–15 days to measure δ13C. Meanwhile, the results of the present study show slight DIC changes even during short-term preservation, though the seawater samples in both studies were treated similarly with BAC. This is probably caused due to the difference between biogenic DIC and seawater DIC values which is larger for 14C than for δ13C. We surmise that BAC can be used to inhibit DIC changes due to microbial activity as a toxic substance alternative to HgCl2 for both 14C and δ13C carbon isotopic analyses of DIC in freshwater. BAC cannot inhibit DIC changes in seawater, but it can reduce DIC changes significantly.

14C change by BAC addition

The influence of blank carbon in the BAC solution on 14C analysis in water samples is discussed here. The DIC concentration of a 1% BAC solution was reported to be 0.5 mg L−1, and increased by 0.26 mg L−1 with each repeated use of the solution from a single bottle (Takahashi et al. 2019a). They reported that the δ13C value of a BAC solution approached that of atmospheric CO2 with the increasing number of times the container was opened. This occurs because BAC solutions are slightly alkaline (pH ≈ 8 for 10% solution, data from Safety Data Sheet) and readily absorb CO2 from the air. The 14C concentration of the BAC itself was 60.7 pMC, but that of the contaminating carbon from the BAC solution was considered to be approximately equal to current atmospheric CO2.

Using the reported DIC concentration of BAC solution, 14C changes in water samples owing to carbon contamination derived from BAC absorbed atmospheric CO2 were estimated (Fig. 5). We can determine whether the 14C change is detectable by comparison with the expected error in 14C analysis by AMS. The 14C change due to the addition of BAC solution was larger with lower DIC and 14C concentrations in sample water. The 14C change had a negative linear relationship with the initial 14C concentration in sample water and positive relationships with the inverse DIC concentrations in sample water. The 14C change was a more influential parameter than 14C concentration, and therefore, DIC concentration must be monitored closely when using BAC. The measurements from the artificial water showed identical 14C concentrations between the initial water and BAC-added water samples. Even with artificial water having a low 14C concentration of < 1 pMC, the influence of BAC addition was not detected (Fig. 4). However, even a small change in DIC concentration can have a large impact on samples with low DIC concentrations, and thus caution is warranted.

As mentioned above, blank carbon in BAC solution increases with the number of times the bottle of BAC solution is opened. The increase of DIC blank in BAC solution had a
positive linear relationship with the number of repeated uses of BAC solution (Fig. 5). For each $^{14}$C and DIC concentration in the sample water, we can determine the approximate number of times that we can use the BAC solution from a single bottle so that the $^{14}$C concentration does not vary beyond the analytical error.

For $^{14}$C analysis, 100–1000 mL of water is used depending on the DIC concentration, and 1–10 mL of 1% BAC solution is used each time. If the sample volume is small, a large amount of BAC solution remained after 5, 10, or 20 uses; however, it is necessary to monitor the number of times that BAC solution is removed from a single bottle and to use a new bottle of BAC solution according to the $^{14}$C and DIC concentrations of the sample water. The lower the DIC concentration, the larger the amount of water sample needed for carbon extraction, and thus the larger the amount of blank-derived carbon contamination. The lower the $^{14}$C concentration in the water sample, the more pronounced the influence. When the DIC or $^{14}$C concentration of the sample water is low, the number of times the BAC solution is exposed to the atmosphere should be carefully monitored. We, therefore, conclude that the addition of BAC to water samples is appropriate so long as the user is aware of the effects on the water with low $^{14}$C and DIC concentrations and is also mindful to minimize the number of times that the BAC is sampled from a single bottle. Of course, it would be better if we could develop a method of using BAC solution that is not affected by atmospheric contamination, but at present, controlling the frequency of use is an easy and realistic measure.

**Decomposition of BAC and microorganisms**

BAC is a highly stable substance; however, we examined its influence in an unlikely event, which is decomposed into DIC. When BAC decomposes to DIC during preservation, the $^{14}$C concentration of DIC approaches the BAC value of 60.7 pMC, but the $^{14}$C concentrations were identical among all of the initial water, BAC-free water, and BAC-added water of artificial water samples (Fig. 4). Therefore, the resistance of BAC to decomposition is considered equally high in water of diverse chemical compositions. BAC is a quaternary ammonium compound with a chemical composition of $\text{C}_{2+\text{H}_2\text{N}^+\text{(CH}_3}_2\text{R-Cl}^-$ (where R ranges from C$_{9}$H$_{17}$ to C$_{18}$H$_{37}$). The main components are $R = C_{12}$H$_{25}$ and $R = C_{14}$H$_{29}$. Hence, the carbon content of BAC is calculated as 7.2 mg L$^{-1}$. The typical amount of BAC used is 0.07 mg L$^{-1}$ in sample water. When BAC decomposes to DIC in water samples during the preservation, carbon isotopes are shifted to the BAC values of 60.7 pMC and $-29.7\%$o. However, BAC is not easily degraded, as indicated by the fact that it has been detected at problematic levels in wastewater globally (Brycki et al. 2014). Reduction of BAC through biodegradation occurs, eventually producing CO$_2$, H$_2$O, NH$_3$, and Cl$^-$. However, it is unlikely that the decomposition of BAC itself will proceed rapidly, as it will be mineralized through many intermediate products. Even if BAC were completely decomposed, the DIC concentration would increase only negligibly; moreover, it is unlikely that all BAC would be decomposed or that all would be converted to DIC. Taken together, we can conclude that the direct contribution of BAC to $^{14}$C analysis is small and need not be considered.

When BAC is added to natural water, the microorganisms may be decomposed by the BAC and become a carbon supply source. Berninger et al. (1991) quantified the concentrations of bacteria, heterotrophic nanoplankton, and other microorganisms in 108 lakes, ponds, rivers, and bogs worldwide and found that they ranged from $3 \times 10^5$ to $4 \times 10^9$ heterotrophic nanoplankton and $3 \times 10^5$ to $1 \times 10^9$ picoplankton mL$^{-1}$. They estimated the cell carbon of heterotrophic nanoplankton and picoplankton to be $1.06 \times 10^{-12}$ g per $4.3 \times 10^4$ cells and $0.03 \times 10^{-12}$ g per $1.2 \times 10^7$ cells, respectively, in a productive pond (Priest Pot, Northern England). Using the worldwide abundances and cell carbon contents of heterotrophic nanoplankton and picoplankton, we assumed the carbon content of microorganisms in fresh water to be approximately $2.5 \times 10^{-8}$ to $2.5 \times 10^{-6}$ mg L$^{-1}$, which is also considered to be undetectable. Moreover, even if the microorganisms decompose, they would become dissolved organic carbon, not DIC. Therefore, we conclude that the decomposition of microorganisms by BAC addition does not affect the $^{14}$C analysis of DIC.

**Comments and recommendations**

The present study assessed several potential sources of carbon contamination of the water samples induced by BAC addition, including the contributions from BAC and microorganism decomposition, and blank carbon in the BAC solution. BAC addition to the water sample to inhibit DIC changes during sample preservation due to microbial activities showed effectiveness for freshwater samples such as groundwater, lake water, and river water. For samples with DIC concentration changes of $1.3 - 3.3$ mg L$^{-1}$ in 2 weeks and $2.2 - 7.9$ mg L$^{-1}$ in 4 weeks in freshwater without BAC, adding BAC can suppress the DIC changes. It is slightly less effective in seawater; slight changes in DIC and $^{14}$C concentration in seawater samples were observed, 0.4 mg L$^{-1}$ and 0.4 pMC for 2 weeks, and 1.6 mg L$^{-1}$ and 1.5 pMC for 4 weeks, respectively. However, it can reduce DIC changes significantly, even in seawater, by 20–40%. Artificial treatments, namely the addition of NaHCO$_3$ and beet sugar, were carried out in some samples to control the initial $^{14}$C concentration and stimulate microbial activity, respectively. Sugar addition indicated that DIC changes due to microbial activities likely increased 3.0–7.3 times. It was considered that the slight changes in DIC in seawater samples were detected by employing this treatment.

The decomposition of BAC itself has little influence on DIC, and the influence of carbon from the decomposition of microorganisms by BAC is even smaller, and these need not
be considered. When 1 mL of 1% BAC solution was added to
100 mL of sample water, the influence of blank carbon con-
tamination from the BAC solution on $^{14}$C concentration
was not detected in any artificial or natural water samples. How-
ever, the $^{14}$C change owing to BAC addition to natural water
having extremely low concentrations of $^{14}$C and DIC was esti-
mated to be larger than the error range of AMS measurements.
In contrast, we estimated the $^{14}$C change with BAC addition
when the same bottle of BAC solution was used repeatedly, as
the amount of blank carbon in the BAC solution increased. A
small change in the DIC concentration from BAC can have a
large impact on samples with low DIC concentrations. It is,
therefore, necessary to place more emphasis on monitoring
low DIC concentrations rather than low $^{14}$C concentrations.
Although the present study focused on BAC treatment,
BAC alone is not sufficient to prevent DIC changes due to
microbial activities in seawater. Filtering is also recommended
to prevent biological effects on carbon species in water sam-
pies (Wilson et al. 2020; Mos et al. 2021). Filtering is effective
for other analyses of water samples, owing to no contamin-
ination by chemical reagents, but filtration alone may also not be
sufficient to prevent biological changes (Takahashi et al. 2019a).
Therefore, treatment with a combination of BAC and
filtration can inhibit microbial influence on DIC changes
because the spores, which are of relatively larger ($\sim 1 \mu m$) size,
would get filtered off. The combination of BAC and filtration
will be the focus of future studies.

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Conflict of Interest
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