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Radiocarbon in marine bacteria: Evidence for the ages of assimilated carbon

Abstract—It is generally accepted that marine bacteria utilize labile, recently produced components of bulk dissolved organic matter. This interpretation is based largely on indirect measurements using model compounds and plankton-derived organic matter. Here, we present an assessment of the relative proportions of modern and older dissolved organic carbon (DOC) utilized by marine bacteria. Bacterial nucleic acids were collected from both estuarine (Santa Rosa Sound, FL) and open-ocean (eastern North Pacific) sites, and the natural radiocarbon signatures of the nucleic acid carbon in both systems were determined. Bacterial nucleic acids from Santa Rosa Sound were significantly enriched in radiocarbon with respect to the bulk DOC and were similar to the radiocarbon signature of atmospheric CO_2 at the time of sampling, indicating that these bacteria exclusively assimilate a modern component of the estuarine bulk DOC. In contrast, bacterial nucleic acids from the oceanic site were enriched in ^{14}C relative to the bulk DOC but depleted in ^{14}C with respect to modern surface dissolved inorganic carbon (DIC) and suspended particulate organic carbon (POC_{susp}). This suggests that open-ocean bacteria assimilate both modern and older components of DOC. The distinct radiocarbon signatures of the nucleic acids at these two sites (i.e., $+120 \pm 17$‰ estuarine vs. -34 ± 24‰ oceanic) demonstrate that natural ^{14}C abundance measurements of bacterial biomarkers are a powerful tool for investigations of carbon cycling through microbial communities in different aquatic systems.

Marine DOC represents one of the largest exchangeable reservoirs of organic carbon ($\sim 0.6 \times 10^{18}$ g C) at the earth's surface (Druffel et al. 1992; Hedges 1992). Heterotrophic bacteria are the primary consumers of marine DOC and influence its persistence in seawater through preferential utilization of specific components of the bulk DOC pool. However, despite extensive research examining bacterial DOC utilization in different marine systems (e.g., Rakestraw 1947; Barber 1968; Coffin et al. 1990; Kirchman et al. 1991; Peterson et al. 1994; Carlson and Ducklow 1996; Cherrier et al. 1996), there remains uncertainty in our current understanding as to the relative ages of the specific bulk DOC components supporting bacterial growth. On the one hand,

short-term incubation studies (i.e., days to months) indicate that bacteria consume labile, recently produced DOC (Coffin et al. 1993; Carlson and Ducklow 1995, 1996; Cherrier et al. 1996; Coffin and Connolly 1997). On the other hand, as the concentration of oceanic bulk DOC is assumed to be at steady state (Norrman et al. 1995; Carlson and Ducklow 1996) and has an average conventional age of $\sim 4,000$–$6,000$ yr B.P. (Williams and Druffel 1987; Bauer et al. 1992; Druffel et al. 1992), it is generally thought that bacterial remineralization must also be one of the ultimate sinks for older, presumably more refractory bulk DOC constituents (Williams and Carlucci 1976). The latter, however, has yet to be directly demonstrated. The objective of this study was to examine and contrast the relative proportions of modern and older bulk DOC constituents assimilated by estuarine and oceanic bacteria using natural radiocarbon abundances of a specific biomarker, nucleic acids.

The carbon isotopic signatures of heterotrophic organisms, including bacteria, reflect the isotopic composition of the organic carbon sources they assimilate (Peterson and Fry 1987; Coffin et al. 1990). It is thus possible to determine what substrates are being consumed in situ by indigenous bacterial populations by measuring their isotopic compositions. Differential filtration cannot effectively isolate natural bacterial assemblages from inorganic and detrital particles of the same size (0.2–1.0-μm effective diameter) for whole-cell isotopic analysis (Coffin et al. 1989). An approach to separate bacteria from the detrital background is to isolate and analyze a biomarker that is specific to bacteria. Bacterial nucleic acids have been shown to have an isotopic composition similar to that of whole cells (Blair et al. 1985; Coffin et al. 1990; Kelly et al. 1998). The specificity of nucleic acids as a bacterial biomarker was confirmed by Coffin et al. (1990), who found that 95% of the 16S rRNA extracted from the 0.2- to 0.8-μm size fraction of estuarine water was bacterial. Comparison of the natural ^{14}C signatures of bacterial nucleic acids extracted from free-living bacteria with the ^{14}C signatures of bulk DOC constituents would additionally allow us to evaluate which age fractions of bulk DOC

are utilized in situ by heterotrophic marine bacterial assemblages.

Sample collection and analysis—Samples were collected from an estuarine site (Santa Rosa Sound, FL) and an oceanic site (34°50′N 123°00′W, Sta. M, eastern North Pacific) to compare the natural radiocarbon signatures of bacterial carbon and bulk DOC in both systems. A total of six bacterial samples for the nucleic acid extractions were collected and analyzed for ^{14}C abundances: two from the Santa Rosa Sound (estuarine) and four from Sta. M (oceanic). The estuarine bacterial samples were collected from the Gulf Breeze Environmental Research Laboratory (GBERL) pier in March 1995. The average salinity at the time of sampling was 18 ppt. The oceanic bacterial samples were collected from a depth of 4 m on two different cruises, one in June and one in September 1994. Bacteria were concentrated in 0.2-μm Gelman filter capsules following prefiltration through a sequential 120-, 10-, and 0.8-μm (0.6 μm for Sta. M samples) filter series. Approximately 100 liters of seawater was filtered for each of the estuarine samples, whereas 500–1,000 liters was filtered for each of the oceanic samples. Once the bacteria were concentrated, the 0.2-μm filter capsules were cleared of excess water, sealed with combusted aluminum foil, and frozen at $-20°C$ for subsequent processing in the laboratory. For each bacterial concentrate collected for nucleic acid extraction, an additional 0.2-μm Gelman filter capsule was placed in-line and downstream of the bacterial concentrate capsule to serve as a sample blank.

The detailed methodology used for the extraction of the bacterial nucleic acids is described in Cherrier (1997). Briefly, bacterial cells were lysed directly in the filter capsules by adding a solution of 20 mM Tris, 2 mM ethylenediaminetetraacetic acid, and 2% sodium dodecyl sulfate (SDS) and then boiling the sealed capsule for 15 min in a 100°C water bath. Following the precipitation and removal of the SDS from the lysate, the bacterial nucleic acids were isolated and purified using dialysis and a series of ethyl alcohol, phenol, and isoamyl alcohol/chloroform precipitations. The purity of the extracted nucleic acids was estimated by spectrophotometric ultraviolet (UV) A_{260}/A_{280} ratios (Sambrook et al. 1989). The average UV absorbance ratio for these bacterial nucleic acids was 1.9 $\pm$ 0.1 ($n = 5$); because pure nucleic acid would have a ratio of 2, this ratio indicates that the nucleic acids were relatively pure and free of protein.

Once extracted, the nucleic acids were combusted to CO_2, converted to graphite targets (Vogel et al. 1987), and analyzed for ^{14}C activities at the Center for Accelerator Mass spectrometry (CAMS) at Lawrence Livermore National Laboratory. A small portion ($\sim$10%) of each CO_2 sample was analyzed for its $\delta^{13}C$ composition (with $\pm$0.01‰ analytical precision) on a Finnigan MAT Delta S Isotope mass spectrometer at Florida State University.

Sample blanks consisted of 0.2-μm filter capsules that were placed downstream of sample capsules as described above. These blanks were treated, extracted, and measured for natural ^{14}C abundance in the same manner as described for the bacterial concentrates. The amount of carbon collected in each individual sample blank was below the detection limit for accelerator mass spectrometry (i.e., <4 μg C).

We therefore combined sample blanks (6 $\pm$ 2 μg C, $n = 3$). The combined blank $\Delta^{14}C$ value was -73 $\pm$ 47‰ and represents 2–4% of the amount of nucleic acid carbon extracted from each sample. All nucleic acid samples were blank corrected. This correction did not significantly alter any of the final nucleic acid $\Delta^{14}C$ values over their precorrected values. If the nucleic acid samples had been contaminated by trace amounts of solvents used during the extraction process, the $\Delta^{14}C$ value of the blank would have been substantially depleted in ^{14}C relative to the samples.

DOC use in the estuary—$\Delta^{14}C$ is defined as the deviation in parts per thousand from the ^{14}C activity of nineteenth-century wood (Stuiver and Polach 1977). The $\Delta^{14}C$ values of the estuarine bacterial nucleic acids were $+108$ $\pm$ 8‰ and $+132$ $\pm$ 8‰ (average $\Delta^{14}C$ = $+120$ $\pm$ 12‰; Table 1; Fig. 1a). These values were significantly greater (i.e., enriched in ^{14}C) than bulk estuarine DOC ($+22$ $\pm$ 7‰, $n = 2$; Table 1; Fig. 1a) and were equal to the radiocarbon signature of atmospheric CO_2 in March 1995 (i.e., $\Delta^{14}C$ = $+113$ $\pm$ 5‰, $n = 3$; Table 1; Fig. 1a) at the time of collection (Levin and Kromer 1997). They were also similar to the two $\Delta^{14}C$ values for DIC of 97 $\pm$ 6‰ and 90 $\pm$ 6‰, (average $\Delta^{14}C$ = $+94$ $\pm$ 5‰; Table 1; Fig. 1a) measured at this study site 1 yr later when atmospheric $\Delta^{14}CO_2$ values would have declined further. The similarities between the $\Delta^{14}C$ values of the estuarine bacterial nucleic acids, DIC, and atmospheric CO_2 indicate that all of the assimilated DOC in this estuarine system was modern or approximately <10 yr old. This finding agrees with the currently accepted assumption that recently produced, labile DOC supports heterotrophic bacterial production in marine systems (Coffin et al. 1993; Carlson and Ducklow 1995, 1996; Cherrier et al. 1996).

The $\delta^{13}C$ values of the two estuarine bacterial nucleic acid samples (-21.0 and -20.8‰) were similar to values observed by Coffin et al. (1990) at the same study site in January 1989 (i.e., average = -21.1‰, $n = 2$). Although a number of potential sources from seagrass to benthic algae contribute to organic matter in the estuary, two primary sources are estuarine phytoplankton and terrestrial vegetation (Peterson et al. 1985). The range of $\delta^{13}C$ values for carbon derived from a mixture of coastal phytoplankton and terrestrial organic matter is typically -21 to -29‰ (Peterson et al. 1985, 1994; Chanton and Lewis in press). The $\delta^{13}C$ value of the estuarine bulk DOC at the time of collection was -25.7‰ (Table 1), indicating that bulk DOC at this study site was likely comprised of a mixture of both marine and terrestrial carbon (Coffin and Cifuenties unpubl. data). The $\delta^{13}C$ values of the bacterial nucleic acids, however, were significantly enriched relative to the bulk DOC, suggesting that ^{14}C-enriched (i.e., modern) marine not terrestrial organic matter was the dominant carbon source supporting bacterial growth and production at this study site (Coffin et al. 1990).

DOC use in the open ocean—In contrast to the estuarine samples, the $\Delta^{14}C$ values of the oceanic bacterial nucleic acids ranged from -13 $\pm$ 10‰ to -61 $\pm$ 12‰ (average $\Delta^{14}C$ = -34 $\pm$ 24‰, $n = 4$; Table 1; Fig. 1b). The nucleic acid $\Delta^{14}C$ values were all greater than -70‰, which indi-

Table 1.   Isotopic measurements of bacterial nucleic acids and the potential carbon sources available for bacterial utilization at an estuarine site (Santa Rosa Sound) and an oceanic site (Sta. M).

Sample (CAMS#/UCDI#)	Volume seawater filtered (liter)	Carbon obtained from combustion of nucleic acids (μg C)	Δ^{14}C* (‰)	δ^{13}C† (‰)
Santa Rosa Sound				
Bacterial nucleic acids				
I, 6 Mar 95	75	148§	+132 ± 8	−21.0 ± 0.1
II, 6 Mar 95	75	148§	+108 ± 8	−20.8 ± 0.1
Average	n/a‡	n/a	+20 ± 17 (n = 2)	−20.9 ± 0.2
Potential carbon sources				
Atmospheric CO_2‖	n/a	n/a	+113 ± 5 (n = 3)	−6.5
Bulk DIC (42196/2655,42197/2656)	n/a	n/a	+97 ± 6, 90 ± 6	−0.5, −0.6
Bulk DOC	n/a	n/a	+22 ± 7	−25.7 ± 0.1
Sta. M				
Bacterial nucleic acids				
P-20, 19–22 Jun 94	900	130	−13 ± 10	−22.3 ± 0.1
P-22 Ib, 20–21 Sept 1994	530	110	−61 ± 12	−21.1 ± 0.1
P-22 IIa, 22–23 Sept 94	460	50	−49 ± 10	−21.2 ± 0.1
P-22 IIb, 23–25 Sept 94	460	60	−14 ± 12	−20.7 ± 0.1
Average	n/a	n/a	−34 ± 24 (n = 4)	−21.3 ± 0.7
Potential carbon sources				
Surface DIC¶	n/a	n/a	+70 ± 7 (n = 11)	−2.1 ± 0.1
Surface POC_{susp}#	n/a	n/a	+66 ± 11 (n = 4)	−22.5 ± 0.1
Surface DOC_{bulk}**	n/a	n/a	−258 ± 20 (n = 4)	−21.6 ± 0.1
Deep DOC_{bulk}**	n/a	n/a	−549 ± 14 (n = 17)	−21.2 ± 0.1

* For nonreplicated samples, Δ^{14}C errors are reported as ±1σ resulting from a combination of counting statistics and sample δ^{13}C and blank Δ^{14}C corrections. For replicated samples (n), Δ^{14}C errors are reported as ±1 SD of the replicate mean. The amount of carbon in the individual sample blanks was below the detection limit for measurement by AMS (i.e., <4 μg C). Individual blanks were therefore combined to yield 6 ± 2 μg C (n = 3). The combined blank Δ^{14}C value was −73 ± 47‰, representing ~2–4% of the amount of nucleic acid carbon extracted from each sample.

† δ^{13}C = ([R_{sample}/$R_{standard}$] −1) × 1,000, where R = ^{13}C/^{12}C, and the standard is the Pee Dee Belemnite.

‡ Not applicable.

§ Carbon content estimated from spectrophotomentric measurement of nucleic acid concentration.

‖ Average Δ^{14}C values for atmospheric CO_2 during March 1995 (Levin and Kromer 1997).

¶ Average Δ^{14}C-DIC values for Sta. M surface waters measured between February 1992 and July 1995 (Massiello et al. 1998).

Average suspended Δ^{14}C-POC values for Sta. M surface waters measured between February 1992 and July 1993 (Druffel et al. 1996).

** Average surface and deep Δ^{14}C DOC values, respectively, for Sta. M measured between February 1992 and July 1993 (Bauer et al. 1998).

cates the presence of postbomb ^{14}C (Berger et al. 1966) produced from atmospheric testing of nuclear weapons in the late 1950s and early 1960s. However, these bacterial Δ^{14}C values were significantly lower than the respective surface (25 m) water POC_{susp} and DIC Δ^{14}C values of 66 ± 11‰ (n = 4, Druffel et al. 1996) and 70 ± 7‰ (n = 11, Massiello et al. 1998) measured at Sta. M between 1992 and 1995. The δ^{13}C signatures of the oceanic bacterial nucleic acids ranged from −20.7 to −22.3‰ (Table 1). These δ^{13}C values are very similar to the average value observed for surface bulk DOC (−21.5 ± 0.9‰) (Bauer et al. 1998) at the same study site, confirming that both were derived from an autochthonous marine source (Williams and Gordon 1970).

Although the Sta. M bacterial nucleic acids were significantly enriched in ^{14}C with respect to surface bulk DOC, they were also depleted in ^{14}C relative to surface DIC and POC, indicating that bacteria in surface water at Sta. M assimilated both modern and older DOC. The Δ^{14}C values of DOC in open-ocean surface waters have previously been found to range from −150 to −258‰ (Williams and Druffel

1987; Bauer et al. 1992, 1998; Druffel et al. 1992). These values represent the mixing of recent postbomb inputs (greater than −70‰) of DOC from surface waters with older DOC (−393 to −525‰) derived from deeper waters (Williams and Druffel 1987). Using mass-balance calculations of DOC concentration and Δ^{14}C values, Williams and Druffel demonstrated that bulk DOC (with average Δ^{14}C = −146‰) in the mixed layer of the central North Pacific was consistent with its being comprised of two major components. One component (~44% of the total) was assumed to consist of older and presumably refractory DOC having the same concentration and Δ^{14}C signature as in deeper waters (Δ^{14}C = −525‰, below 1,000 m). The second component (~56% of the total) was assumed to consist of modern postbomb DOC (Δ^{14}C = 150‰, Williams and Druffel 1987; Druffel et al. 1992) derived from recent primary production and its subsequent trophic utilization. As Williams and Druffel pointed out, this two-component model is a simplification of an undoubtedly more complex age structure in the bulk DOC pool; however, it is useful as an initial starting point for
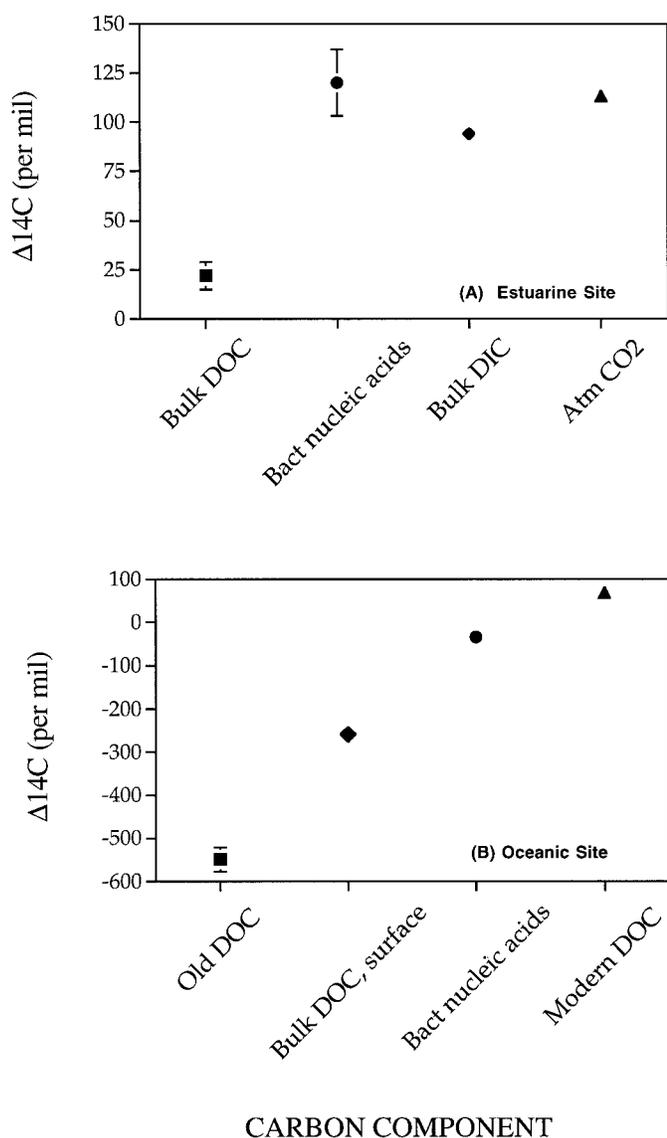
CARBON COMPONENT

Fig. 1. (a) Average $\Delta^{14}C$ values of bacterial nucleic acids (circle) and potential DOC sources available for bacterial assimilation in the surface waters of Santa Rosa Sound. Sources of DOC include bulk estuarine (square), which is comprised of an unknown combination of old (not shown), and modern (represented by bulk DIC and atmospheric CO_2, diamond and triangle, respectively) DOC fractions in this system. (b) Average $\Delta^{14}C$ values of bacterial nucleic acids (circle) and potential DOC sources available for bacterial assimilation in the surface waters at Sta. M. Sources of DOC include surface bulk DOC (diamond), which is comprised of an old DOC fraction (square), and a modern DOC fraction (triangle). We assume that the $\Delta^{14}C$ of the modern fraction is approximately equal to surface DIC and POC $\Delta^{14}C$ values. Error bars represent the standard deviation of the averaged $\Delta^{14}C$ values.

trying to understand how bacterial processes ultimately affect bulk DOC.

Using Williams and Druffel's (1987) approach, we hypothesized that two distinct DOC components with different ages were available for bacterial utilization in surface waters at Sta. M: a modern ^{14}C-enriched component and an older

DOC ^{14}C-depleted component (*see Fig. 1b*). The average concentration and $\Delta^{14}C$ of bulk DOC at 25-m depth at Sta. M between February 1992 and July 1993 was $72 \pm 2\mu M$ C and $-258 \pm 20‰$ ($n = 4$), respectively (Bauer et al. 1998). The average concentration and $\Delta^{14}C$ value of the deep-water bulk DOC between ~1,500- and 4,100-m depth during this same period was $39 \pm 1 \mu M$ C and $-549 \pm 14‰$ ($n = 17$), respectively (Bauer et al. 1998). We assumed that these latter values represented the older component in surface-ocean bulk DOC and that the balance of surface DOC was of recent origin (Williams and Druffel 1987). Using the concentration differences between surface bulk DOC and the older DOC fraction, we estimated that the average concentration of the modern DOC fraction in Sta. M surface waters was approximately $33\mu M$ (72 minus $39\mu M$). Because planktonic carbon is believed to be the primary source of modern DOC to oceanic surface waters (Williams and Druffel 1987), we further assumed that the $\Delta^{14}C$ signature of the modern DOC fraction was approximately 68‰, equal to the average $\Delta^{14}C$ values of POC_{susp} ($66 \pm 11‰$, Druffel et al. 1996) and DIC ($70 \pm 7‰$, Massiello et al. 1998) in Sta. M surface waters.

On the basis of this distinction between surface- and deep-ocean DOC, the bulk DOC ($\Delta^{14}C = -258‰$) components potentially available to free-living heterotrophic bacteria in the surface waters at Sta. M therefore includes a modern DOC component (46% of the total at $\Delta^{14}C = 68‰$) and an older DOC component (54% of the total at $\Delta^{14}C = -549‰$) (Fig. 1b). If bacteria nonselectively assimilate DOC constituents of all ^{14}C ages in surface seawater, then the $\Delta^{14}C$ values of the bacterial nucleic acids should be similar to the average $\Delta^{14}C$ value ($-258‰$) observed for bulk DOC. Instead, bacterial nucleic acids were significantly enriched in ^{14}C with respect to bulk DOC, indicating that bacteria preferentially utilized the modern constituent of the surface bulk DOC. However, it is significant to note that bacteria did not assimilate modern DOC exclusively. This is reflected in the observation that the mean $\Delta^{14}C$ value of the bacterial nucleic acids ($-34‰$) was significantly ($P = 0.002$, t-test) lower than the $\Delta^{14}C$ value (68‰) of modern recently produced surface-ocean (25 m) organic matter (Druffel et al. 1996; Massiello et al. 1998).

If modern ($\Delta^{14}C = 68‰$) and older ($\Delta^{14}C = -549‰$) DOC are assumed to be the only two components of surface bulk DOC available for bacterial utilization at Sta. M, we can estimate the relative assimilation of each component necessary to achieve the average observed bacterial nucleic acid $\Delta^{14}C$ value of $-34‰$ as

$$(\Delta^{14}C \text{ bacterial nucleic acids}) = (x)(\Delta^{14}C \text{ DOC}_{modern}) + (y)(\Delta^{14}C \text{ DOC}_{old})$$

where x is the fractional amount of modern DOC in nucleic acids, y is the fractional amount of old DOC in nucleic acids, $x + y = 1$, and $\Delta^{14}C$ DOC_{modern} and $\Delta^{14}C$ DOC_{old} are 68 and $-549‰$, respectively. Solving for x and y, we find that the estimated relative amount of the modern DOC fraction assimilated by bacteria is 83% and that that of the old DOC fraction is 17%.

Using 16S rRNA hybridization, Coffin et al. (1990) confirmed that 95% of the nucleic acid extracted from 0.2- to

1.0-μm size fractions of estuarine water was bacterial. It is thus possible that some small portion of the nucleic acids extracted from each sample was from other microorganisms in this size range. It is likely that these would be small microflagellates that graze on bacteria and consequently, would not have shifted the radiocarbon content of the bacteria. If other organisms, such as cyanobacteria, were concentrated in this size range and if their nucleic acids were extracted by our procedure, this would result in a more modern isotope ratio, which means our results are conservative and underestimate heterotrophic assimilation of older carbon. Because of the wide range of potential sources (i.e., prokaryotic and eukaryotic) for nonliving particle-bound nucleic acids, it is difficult to speculate how any contributions from a submicron (0.2–0.8 μm) particulate fraction could affect the results reported here. While these cannot be completely ruled out (Paul et al. 1985), such contributions are likely to be small compared to the live bacterial component as a result of the relatively high rates of turnover and utilization of nonliving nucleic acids in aquatic environments (Paul et al. 1985, 1988). Due to this rapid turnover, we would not expect ^{14}C-depleted particle-bound nucleic acids to comprise a significant fraction of the total extracted nucleic acids (leading to lower observed Δ^{14}C values). However, this possibility cannot be excluded entirely without further study.

Although the assimilation of modern DOC exceeds that of old DOC by a factor of five for the Sta. M samples, our results also show that, in contrast to the estuarine bacteria where all of the DOC assimilated was modern, surface-ocean bacteria also utilize a significant amount of the older and presumably refractory component of bulk DOC. Recent studies have argued that the principal organic substrates supporting bacterial production in open-ocean systems are from a relatively small, labile, and recently produced fraction of the bulk DOC (6–7% of bulk DOC or ~25% of the modern fraction), which is turned over on the order of days to weeks (Kirchman et al. 1991; Carlson and Ducklow 1996; Cherrier et al. 1996). The present study confirms that recently produced DOC is the dominant component supporting heterotrophic bacterial biomass production, at least in surface waters. However, if bacterial remineralization was a sink for the recently produced fraction of bulk DOC only, then we might expect that the apparent residence time for DOC in the oceans would be much longer than the observed range of 4,000–6,000 yr (Williams and Druffel 1987; Druffel et al. 1992).

Utilization of older bulk DOC component—Cherrier et al. (1996) proposed that the bacterial populations in Sta. M surface waters were energy limited and that growth appeared to be constrained by the quality of the DOC substrates available for utilization. The older and presumably refractory DOC fraction must therefore be made available to bacteria either by: (1) cometabolism with labile DOC, and/or (2) processes such as photooxidation. Cometabolism of complex refractory substrates together with labile material has been observed in a wide range of aquatic ecosystems (Connolly and Coffin 1995). It is thus possible that during transient episodes of elevated concentrations of labile DOC (i.e., following bloom events), when abundant energy is available, bacteria concurrently utilize older bulk DOC constituents as well as labile DOC.

Bacterial utilization of photochemically degraded components of old refractory DOC is a second potential explanation for the slight ^{14}C depletion in bacterial nucleic acids, relative to modern organic matter, observed at Sta. M. Mopper et al. (1991) estimated that for each ocean-mixing cycle (~1,000 yr), 12–48% of oceanic humic material was degraded to biologically labile and volatile organic compounds by photooxidation. The effective light penetration depth for production of these compounds in the open ocean was estimated to be ~5 m (Mopper et al. 1991), close to our sampling depth of 4 m at Sta. M. Bacterial uptake of fatty acid and nitrogen-rich compounds produced from photooxidation of humic material has been observed in both freshwater and marine systems (Kieber et al. 1989; Lindell et al. 1995; Wetzel et al. 1995; Bushaw et al. 1996). Thus, given the energy constraints of the bacterial populations in the eastern North Pacific, we speculate that older, more refractory DOC may be made available for bacterial utilization and assimilation via cometabolism and/or photooxidative processes.

If we assume a bacterial DOC utilization rate of 0.4 μM d^{-1} (i.e., as observed in Sargasso Sea surface waters during nonbloom conditions; Hansell et al. 1995) and that ~17% of what is utilized is older DOC as estimated in the present study, then we calculate a utilization rate of 0.068 μM d^{-1} for the old component of bulk DOC. This utilization rate is nearly equivalent to the estimate of Hansell et al. (0.07 μM d^{-1}) for the production rate of labile DOC from old refractory DOC via photooxidation in the Sargasso Sea. If we further assume that, during nonbloom conditions, older DOC is made available to the bacteria only by photooxidation (i.e., in the upper 5 m, Mopper et al. 1991), we can estimate a global ocean residence time for the older DOC component by integrating the DOC utilization rate (both assimilated and respired) of 0.4 μM d^{-1} to a depth of 5 m (i.e., effective light penetration depth for photooxidation, Mopper et al. 1991) over the surface area of the ocean (3.61 × 10^{14} m^2) and multiplying by 0.17 (the fraction of old DOC in the bacterial nucleic acids, assuming that there is no ^{14}C fractionation between respired and assimilated DOC). This gives an estimated rate of 5.4 × 10^{14} g C yr^{-1} for the global annual bacterial utilization of the old component of bulk DOC. If we then divide the oceanic DOC reservoir (0.6 × 10^{18} g C, Hedges 1992) by this globally integrated rate, we obtain a residence time estimate of ~1,100 yr for the older DOC component. This is within the estimated range given by Mopper et al. (~500–2,100 yr) for the oceanic residence time of biologically refractory, photochemically reactive DOC and is also within a factor of five of the ^{14}C age of the older component of surface bulk DOC (Williams and Druffel 1987; Druffel et al. 1992).

In conclusion, using natural ^{14}C and ^{13}C measurements, we have demonstrated that bacterial biomass in a coastal estuarine system (Santa Rosa Sound, FL) is supported exclusively by modern (less than or equal to ~10 yr) DOC originating from autochthonously produced organic matter. Utilization of this recently produced DOC may thus attenuate the flux of young labile DOC from productive coastal waters to the open ocean. Because sampling was conducted

only in the spring in this study, additional measurements are needed to investigate seasonal variation in modern carbon fluxes through estuarine systems. We have also shown that, while bacterial biomass in eastern North Pacific surface waters is supported predominantly by modern forms of DOC, there is also measurable and concomitant assimilation of older material. The estimated global annual bacterial utilization rate of the old bulk DOC component (5×10^{14} g C yr^{-1}) is sufficiently large to suggest that bacterial utilization is an important sink for both modern and older DOC. Additional measurements are needed to define natural variability in bacterial radiocarbon and to address the difference between our calculated residence time of 1,100 yr and the estimated 6,000-yr age of the old DOC reservoir (Williams and Druffel 1987; Druffel et al. 1992). Measurements of natural ^{14}C abundances in deep-ocean bacterial nucleic acids may also help to resolve these discrepancies. Finally, the activity of surface-ocean bacteria may be more important than previously expected for the utilization of deep-ocean DOC and hence, the turnover of the total ocean DOC pool.

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