

Supporting Information

Hansman et al. 10.1073/pnas.0810871106

SI Text

Assessing Contamination from Dissolved Organic Carbon (DOC) Coisolation. We cannot use process blank results to constrain any contamination introduced from ambient DOC adsorption during sample isolation, because seawater volumes filtered during sample isolation were much larger than those used for lab cultures. In addition, DOC concentrations were much lower for mesopelagic samples (DOC composition is also different at depth) (1). Instead, we use the C/N of our samples to constrain the potential importance of DOC adsorption. This approach has been used previously for riverine systems (2). Data shown in Table 1 were used for the calculation, and in the case of the 0.2–0.5- μm sample from 670 m, where C/N was not measured, the highest sample C/N (5.32 for the 915 m >0.5- μm size fraction) was used. In this section, we use a C/N for pure DNA of 3 (based on the measured elemental composition of *E. coli* DNA, Sigma), and we assume that any deviation from this ideal value results from the adsorption and subsequent precipitation of ambient DOC with our DNA sample. We note that this is the extreme case because the deviation may also be caused by coprecipitation of other intracellular biochemicals, which does not represent contamination. In addition, we use relatively low C/N values for deep DOC (16; surface values are assumed to be 14; 3) and so the calculated contribution from DOC contamination is likely an overestimate. We further assume that the absorbed and co-precipitated DOC has C/N and $\Delta^{14}\text{C}$ signatures identical to the bulk DOC pool (we note here that our $^1\text{H-NMR}$ spectra are not consistent with a large contamination by bulk DOC, but we cannot rule out contamination by a small amount of aliphatic DOC—likely with C/N values greater than those used in the calculation). The potential fractional contribution from DOC adsorption is calculated first using measured (samples) and assumed (ambient, bulk DOC) C/N values, and then

potential shifts in our experimental $\Delta^{14}\text{C}$ -DNA values are calculated based on a 2 end-member mass balance using $\Delta^{14}\text{C}$ values for bulk DOC in the mesopelagic North Central Pacific (4) (Table S2). Errors for $\Delta^{14}\text{C}$ -DNA values corrected in this manner incorporate C/N measurement errors (ranging from 0.03 to 0.20), in addition to propagated AMS errors and blank corrections.

Process Blanks. In the text it was suggested that differences between $\Delta^{14}\text{C}$ -DNA and carbon sources observed in laboratory cultures may have resulted from coisolation of an exogenous carbon source, such as DOC. Because no consistent contamination from a modern or radiocarbon-dead component would satisfy the observed deviations, any contaminant would need to have an intermediate $\Delta^{14}\text{C}$ value. A mass balance calculation using the 2 process blanks from 2007 (Table 2) and their source carbon can be solved by a 24- μg C contaminant with a $\Delta^{14}\text{C}$ value of -235‰ . An absolute blank, rather than a fraction of the DNA yield, is favored for the 2007 experiments as seawater volumes used for the incubation, volumes of culture sample filtered (8 L), and process chemicals used were all identical for each experiment. This “contaminant” $\Delta^{14}\text{C}$ value is moderately enriched in ^{14}C relative to that of bulk DOC in surface waters of the eastern North Pacific Ocean, -302‰ in 2004 at an open ocean site (34°50'N, 123°00'W) (5), and it is still possible that adsorption from this pool onto our filters and coprecipitation with DNA during our extraction procedure leads to the observed deviation in sample $\Delta^{14}\text{C}$ from the source. However, source carbon and bacterial DNA from these process blanks had very similar $\delta^{13}\text{C}$ values, which can constrain adsorption of bulk DOC (with a $\delta^{13}\text{C}$ value of -22.5 in surface waters of the eastern North Pacific; 1) to 6–8%. Coffin et al. (6) showed that the $\delta^{13}\text{C}$ signature of bacterial DNA reflected the isotopic composition of source carbon to within 2.4‰.

1. Williams PM (1986) in *Plankton Dynamics of the Southern California Bight*, ed Eppley RW (Springer, Berlin), pp 53–83.
2. McCallister SL, Bauer JE, Cherrier JE, Ducklow HW (2004) Assessing sources and ages of organic matter supporting river and estuarine bacterial production: A multiple-isotope ($\Delta^{14}\text{C}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$) approach. *Limnol Oceanogr* 49:1687–1702.
3. Bronk DA (2002) in *Biogeochemistry of Marine Dissolved Organic Matter*, eds Hansell DA, Carlson CA (Academic, Amsterdam), pp 153–247.
4. Druffel ERM, Williams PM, Bauer JE, Ertel JR (1992) Cycling of dissolved and particulate organic matter in the open ocean. *J Geophys Res* 97:15639–15659.
5. Beaupre SR, Druffel ERM, Griffin S (2007) A low-blank photochemical extraction system for concentration and isotopic analysis of marine dissolved organic carbon. *Limnol Oceanogr Meth* 5:174–184.
6. Coffin RB, Velinsky DJ, Devereux R, Price WA, Cifuentes LA (1990) Stable carbon isotope analysis of nucleic acids to trace sources of dissolved substrates used by estuarine bacteria. *Appl Environ Microbiol* 56:2012–2020.

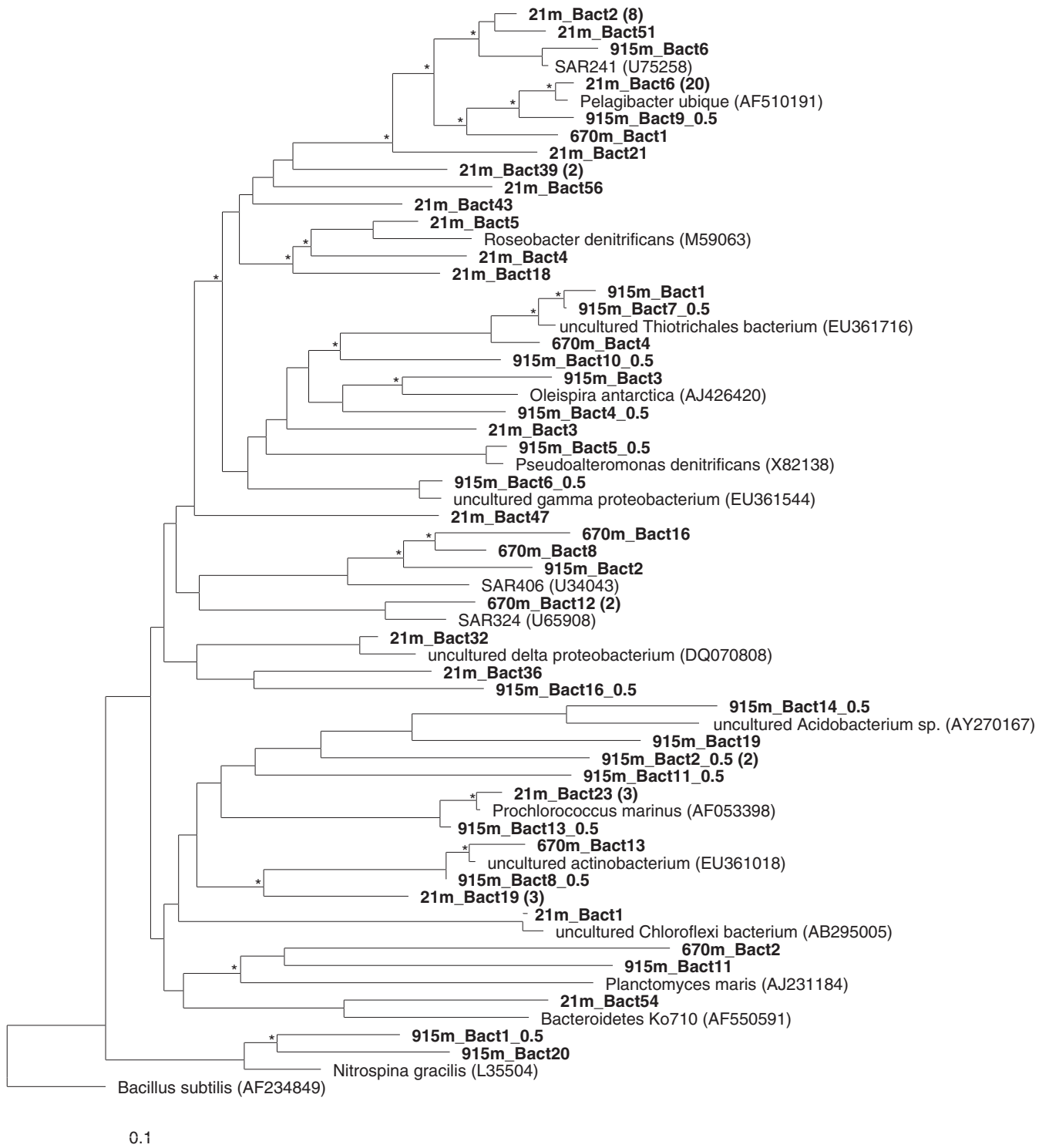


Fig. S1. Neighbor-joining phylogenetic tree of bacterial (A) and archaeal (B) 16S rRNA genes from extracted DNA samples. Sequences with $>97\%$ identity for each particular depth and size fraction were grouped into operational taxonomic units (OTU). The number of sequences in each OTU is indicated in parentheses next to a representative clone from the group. Sequences are from randomly picked clones. Asterisks indicate bootstrap values $\geq 60\%$ (based on 1,000 replicates). (Scale bars represent 0.1 substitutions per site.) Difficulties amplifying the approximate 1,400-bp bacterial 16S rRNA fragment from the $>0.5\text{-}\mu\text{m}$ sample from 670 m lead us to believe that this sample is partially degraded (confirmed by examination on an agarose gel), therefore sequences from that sample are absent from the bacterial phylogenetic data.

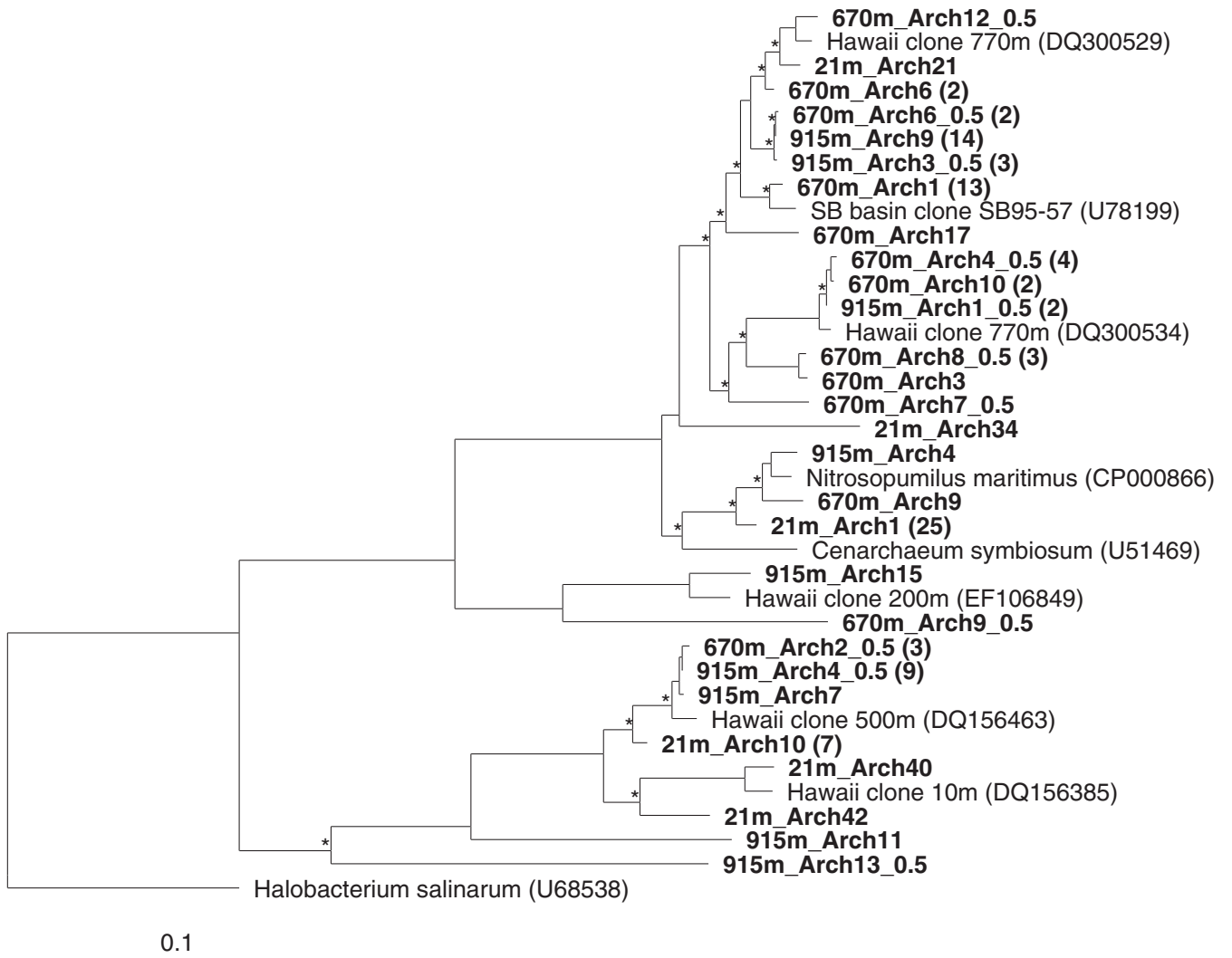


Fig. S1 continued.

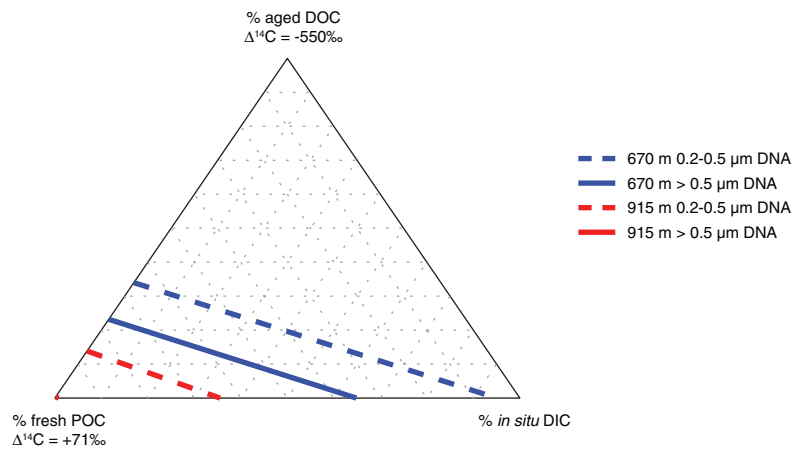


Fig. S2. Ternary plot of possible % contributions of fresh particulate organic carbon (POC) ($\Delta^{14}\text{C} = +71\text{‰}$), aged DOC ($\Delta^{14}\text{C} = -550\text{‰}$), and in situ dissolved inorganic carbon (DIC) ($\Delta^{14}\text{C} = -151\text{‰}$ at 670 m and -171‰ at 915 m) to microbial $\Delta^{14}\text{C}$ -DNA. Calculations were performed using corrected values from Table 1, with uncertainties up to $\pm 8\%$ based on radiocarbon errors. Note that results from 2 end-member mass balance calculations, for example, when considering only DIC and POC input to free-living microorganisms, are the intercepts of each axis. (Gridlines indicate 10% increments.)

Table S1. General phylogenetic classification and accession numbers of nearest BLAST hits of bacterial and archaeal 16S rRNA clone libraries from extracted DNA samples

Sample	Phylogenetic classification	No. of clones	Accession no. of nearest BLAST hits	% identity	
21 m 0.2–0.5 μm	alpha proteobacteria (41/49)	20	EU805169	99	
		8	EU8054456	100	
		3	EU805169	100	
		2	EF573034	99	
		1	EU861201	99	
		1	EU805167	99	
		1	EU346850	95	
		1	EU804260	99	
		1	EF572240	99	
		1	EF572229	99	
		delta proteobacteria (3/49)	1	DQ070808	97
			1	DQ395309	98
			1	EU249716	93
			3	EU804473	99
			1	AB295005	99
		cyanobacteria (3/49)	1	EU010168	98
			1	EU010168	98
670 m 0.2–0.5 μm	delta proteobacteria (2/8)	2	DQ396254	97	
		1	AB193918	98	
	SAR406 (2/8)	1	DQ300753	99	
		1	AB193895	98	
	alpha proteobacteria (1/8)	1	AF469226	99	
	gamma proteobacteria (1/8)	1	AY381291	87	
	planctomycetes (1/8)	1	EU361010	99	
	actinobacteria (1/8)	1	EU361010	99	
	915 m 0.2–0.5 μm	gamma proteobacteria (2/7)	1	DQ513059	99
			1	DQ906763	91
alpha proteobacteria (1/7)		1	U75258	97	
delta proteobacteria (1/7)		1	DQ396048	99	
planctomycetes (1/7)		1	AY381291	87	
915 m > 0.5 μm	SAR406 (1/7)	1	EU092071	95	
		1	EU361544	99	
	gamma proteobacteria (5/14)	1	AF469226	99	
		1	DQ396109	96	
		1	AY907800	99	
		1	AF434117	98	
		alpha proteobacteria (2/14)	1	AF469348	99
			1	EF574992	87
		verrucomicrobia (2/14)	2	EU686604	97
		bacteroidetes (1/14)	1	AY279054	99
		actinobacteria (1/14)	1	DQ396300	99
		acidobacteria (1/14)	1	EU491382	95
		delta proteobacteria (1/14)	1	EF646130	99
		cyanobacteria (1/14)	1	EF574918	99
21 m 0.2–0.5 μm	group I crenarchaeota (27/36)	25	EU283425	99	
		1	AY627460	98	
		1	DQ300510	100	
		7	DQ299286	99	
		1	DQ156396	100	
	group II euryarchaeota (9/36)	1	EF106797	100	
		13	EU486950	99	
		1	EU686615	99	
		2	U46680	99	
		1	EF414502	99	
670 m 0.2–0.5 μm	group I crenarchaeota (20/20)	2	EF645850	99	
		1	AB193963	96	
		3	AB193995	99	
		4	EF645850	99	
		2	EU791558	99	
	group II euryarchaeota (3/15)	1	AF121995	99	
		3	EU686615	99	
		1	EU696620	88	
		1	EU686642	99	
		1	EU686642	99	
670 m > 0.5 μm	group I crenarchaeota (12/15)	4	DQ641746	99	
		1	AB193977	99	
	group II euryarchaeota (3/15)	14	EU791558	99	
		1	DQ270603	98	
		1	AB193995	99	
		2	EF645850	99	
		3	EU791558	99	
		9	AB193995	100	
		1	AB177280	98	
		1	AB177280	98	
915 m 0.2–0.5 μm	group I crenarchaeota (16/18)	1	EU283425	99	
		1	AB193977	99	
		14	EU791558	99	
		1	DQ270603	98	
		1	AB193995	99	
915 m > 0.5 μm	group I crenarchaeota (5/15)	2	EF645850	99	
		3	EU791558	99	
		9	AB193995	100	
		1	AB177280	98	
		1	AB177280	98	

Table S2. Salinity and temperature data for sample collection depths and inputs for $\Delta^{14}\text{C}$ -DNA corrections based on bulk DOC adsorption

Sample	Salinity	Temp, °C	Sample C/N	Bulk DOC C/N, $\Delta^{14}\text{C}$	Measured $\Delta^{14}\text{C}$, ‰	% DOC adsorption	Corrected $\Delta^{14}\text{C}$, ‰
21 m 0.2–0.5 μm	34.7	24.5–27.5	4.05	14, –191‰	+36	10%	+60
670 m 0.2–0.5 μm	34.4	6.5	n.a.	16, –405‰	–187	–	–140*
670 m > 0.5 μm			4.31	16, –405‰	–106	10%	–73
915 m 0.2–0.5 μm	34.5	5.8	5.06	16, –470‰	–87	16%	–15
915 m > 0.5 μm			5.32	16, –470‰	–27	18%	+69

Data sample collection depths provided by Natural Energy Laboratory of Hawaii Authority (<http://www.nelha.org>). C/N, see ref. 3; $\Delta^{14}\text{C}$, see ref. 4. n.a., not available (amount of N in sample was too low for accurate measurement).

* $\Delta^{14}\text{C}$ -DNA corrections for this sample were calculated using the highest C/N ratio (5.32 for 915 m > 0.5 μm size fraction) as an upper bound.