

LETTER

Elemental content and stoichiometry of SAR11 chemoheterotrophic marine bacteriaAngelique E. White,^{1,2} Stephen J. Giovannoni ,^{3*} Yanlin Zhao,^{3a} Kevin Vergin,³ Craig A. Carlson⁴¹Department of Oceanography, University of Hawaii at Manoa, Honolulu, Hawaii; ²Daniel K. Inouye Center for Microbial Oceanography: Research and Education, Honolulu, Hawaii; ³Department of Microbiology, Oregon State University, Corvallis, Oregon; ⁴Marine Science Institute, University of California, Santa Barbara, California**Scientific Significance Statement**

SAR11 bacteria are the most abundant cells in the ocean and are members of the smallest class of plankton. Their elemental composition is important for assessing standing stocks of carbon and other elements and nutrient fluxes through marine food webs. However, estimates of elemental content are poorly constrained for this ubiquitous marine heterotroph. Here, we provide cellular carbon, nitrogen, and phosphorus quotas of SAR11 isolates and calculate global carbon standing stocks and preliminary estimates for the fraction of marine gross primary production that is oxidized by this abundant organism. This information raises anew the question of how small bacteria such as these compete successfully in the niche of organic carbon oxidation. Our results also provide values that may be useful for building geochemical models that evaluate the impacts of heterotroph foraging strategies on organic carbon cycling.

Abstract

We measured the carbon, nitrogen, and phosphorus content and production of cultured SAR11 cells in the genus *Pelagibacter*, from members of the 1a.1 and 1a.3 lineages, which are adapted to productive coastal waters and oligotrophic tropical/subtropical environments, respectively. The average growing SAR11 cell contained ~ 6.5 fg C, from which we calculated a global standing stock of 1.4×10^{13} g C. Calculations that consider uncertainties in cell turnover rates and growth efficiencies indicate this stock could oxidize 6% to 37% of gross ocean primary production. We also found that SAR11 do not incorporate ³H-thymidine but do incorporate ³H-leucine. We estimate conversion factors of 0.74–1.51 kg C mol⁻¹ leu, which are comparable to the low end of published leucine conversion factors for marine chemoheterotrophic bacterioplankton production. The molar

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ratio of elements C : N : P in growing cells was on average 25 : 6 : 1, significantly less than the mean (~ 50 : 10 : 1) for heterotrophic bacteria, indicating these strains are C and N poor relative to P.

We investigated the elemental stoichiometry and growth of SAR11 bacteria (*Pelagibacterales*), which are ubiquitous, free-living planktonic cells found at all depths and latitudes. SAR11 are estimated to number 2.4×10^{28} cells worldwide, about 25% of all plankton cells (Morris et al. 2002), with the greatest total and relative numbers in the most oligotrophic regions of the euphotic zone. Their main contributions to ocean biogeochemical cycles are the oxidation of labile forms of dissolved organic carbon (DOC) and the cycling of nitrogen (N) and phosphorus (P) through SAR11 biomass (Giovannoni 2017).

It is theorized that the extraordinary success of SAR11 is related to their simple cell architecture, small genome, and cell size (cell diameter ~ 0.4 μm), which in principle change membrane : cytoplasm and nucleic acid : biomass ratios and confer advantages both by increasing surface-to-volume ratios and decreasing cellular quotas for N and P (Giovannoni 2017). Streamlining theory, which was originally developed to understand the evolution of genome size, predicts selection for minimal cell size, and complexity will be strongest in the upper ocean where competition for N and P favors the reduction of cell quotas. The cellular C content of SAR11 cells has been estimated from measurements of cell mass (Tripp et al. 2008; Cermak et al. 2017) or cell volume (Romanova and Sazhin 2010), and one study reported cellular ratios of C : P of 36 for SAR11 strain HTCC1062 (Zimmerman et al. 2014).

SAR11 belong to the smallest size class of plankton and are the largest plankton group by census numbers. They also are one of the few significant bacterial plankton groups that have been cultured and can be manipulated in a controlled setting. Here, we report measurements of elemental stoichiometry for two strains of SAR11 and productivity estimates made by growing cells with ^3H -labeled thymidine and leucine. The data support the conclusion that SAR11 cells have very low quotas for C and N relative to P. We also demonstrate that SAR11 do not assimilate the pyrimidine thymidine, but accurate productivity estimates are obtained when growing cells are labeled with the amino acid leucine. We report cellular quotas that support previous claims of minimization in these plankton. These data will be useful for building geochemical models that consider the properties of the smallest classes of cells.

Methods

Organism source

"*Candidatus Pelagibacter ubique*" str. HTCC1062 and *Pelagibacterales* sp. str. HTCC7211 were revived from 10% glycerol stocks and propagated in artificial medium for SAR11 (AMS1), amended with pyruvate ($100 \mu\text{mol L}^{-1}$), glycine ($5 \mu\text{mol L}^{-1}$),

methionine ($5 \mu\text{mol L}^{-1}$), FeCl_3 ($1 \mu\text{mol L}^{-1}$), and vitamins (Carini et al. 2013).

Cultivation details

All cultures were grown in acid-washed and autoclaved polycarbonate flasks. Cultures were incubated at 20°C with shaking at 60 revolutions per minute under a 12 h light : 12 h dark cycle. Light levels during the day were held at $140\text{--}180 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Cell densities were determined by staining with SYBR green I and counting cells with a Guava Technologies flow cytometer at 48–72 h intervals as described elsewhere (Tripp et al. 2008).

Cell harvesting for elemental analyses

Strain HTCC7211 and strain HTCC1062 cells were grown in artificial seawater medium (AMS1) and harvested in exponential growth phase (ca. 1.0×10^8 cells mL^{-1}) and stationary growth phase by centrifugation ($17,664 \times g$ for 1.0 h at 20°C). Cell pellets were washed twice with growth medium (without added inorganic phosphorus, P_i) and resuspended in one of the following conditions: (i) P_i replete ($100 \mu\text{mol L}^{-1}$) or (ii) P_i deplete growth medium (no P_i added). Each resuspension was monitored for growth and subsampled by centrifugation ($48,298 \times g$ for 1.0 h at 4°C) at $t = 0, 2, 4, 6,$ and 8 d. The supernatant was removed from centrifuged samples, and cell pellets were immediately frozen at -80°C until elemental analysis.

Calculation of elemental content per cell: Dilution series of cell suspensions

Elemental content of cells were derived from a dilution series prepared from exponential and stationary growth-phase cultures (Supporting Information Fig. S1; White et al. 2018). First, cultures were pelleted via centrifugation, and a subsample was collected for C : N analyses. Second, the remaining pellet isolated from each growth stage was separated into 18 fractions (e.g., three sets of six masses per growth phase) with a set for C analyses, a set for cell number, and a set for P analyses. For cell densities and C analyses, cell pellets were resuspended into AMS1 media with no added nutrients to achieve a dynamic range of cell densities spanning $\sim 10^8\text{--}10^{11}$ cells L^{-1} . Samples reserved for C analyses were stored frozen at -20°C in combusted glass vials with Teflon-coated septa caps, whereas cell density samples were counted as described above using a Guava Technologies flow cytometer. The set of cell pellets for P were analyzed without resuspension as described below. Elemental content per cell was calculated via linear regression of cell counts and elemental content in each fraction, where the slope of a model II least squares regression (using the MATLAB function `lsqfitgm.m`) is considered the elemental content per cell (Supporting Information Fig. S2).

C/N ratios

Cells were cultured, harvested, pelletized, and washed in AMS1 as described above. Following washing, a fraction of the cell pellet was removed from centrifuge tubes with a combusted spatula and deposited in combusted aluminum boats. C/N ratios were determined with an Exeter Analytics CE-440 elemental analyzer calibrated with acetanilide following manufacturer protocols.

Measurement of bacterial phosphorus

For P content, cell pellets were heated in precombusted, acid-washed, deionized water-rinsed glass test tubes for 4–5 h at 450°C in a muffle furnace. Samples were then allowed to cool and immersed in 10 mL of 0.15 mol L⁻¹ hydrochloric acid. P was analyzed in the extracted samples using molybdenum blue spectrophotometry as per the protocol of Hebel and Karl (2001). Accuracy was assessed from the analysis of a known dry weight of certified reference material (National Institute of Standards, NIST 1515, orchard leaves, certified 0.159% P by weight). The measured P content of NIST 1515 reference material averaged 0.152% (SE = 0.003%, *n* = 16).

Measurement of bacterial carbon

High-temperature combustion was used to directly measure the total organic carbon content for each vial of a dilution series. Samples were analyzed on a modified Shimadzu TOC-V as described in Carlson et al. (2010). Briefly, 3 mL of sample was acidified with 2 mol L⁻¹ HCl (1.5%) and sparged for 1.5 min with CO₂-free gas. Three to five replicates (100 μL) of sample were injected into the combustion tube heated to 680°C that had CO₂-free gas flowing through the system at 168 mL min⁻¹. A magnesium perchlorate trap and copper mesh trap were used to ensure removal of water vapor and halides from the gas line prior to entering a nondispersive infrared detector. The resulting peak area was integrated with Shimadzu chromatographic software. Additional analytical details are described in the Supporting Information.

Thymidine and leucine incorporation

Samples for SAR11 production were analyzed via ³H-thymidine and ³H-leucine incorporation following the methods of Simon and Azam (1989) with slight modifications. In brief, triplicate samples and duplicate cultures of cells killed with 5% trichloroacetic acid of were incubated with 20 nmol L⁻¹ ³H-thymidine (specific activity 10.1 Ci mmol⁻¹; PerkinElmer) or 20 nmol L⁻¹ ³H-leucine (specific activity 54.1 Ci mmol⁻¹; PerkinElmer). Samples were incubated in the dark for 4 h. At each time point, subsamples were killed with TCA (5% final concentration), filtered onto 0.2-μm Nucleopore filters, and washed with ice-cold 5% TCA and 80% ethanol. Radioactivity was analyzed after addition of scintillation cocktail by a Beckman Coulter LS6500 Multipurpose Scintillation Counter.

Growth efficiency estimates

In evaluating SAR11 C demand, we consider a range of bacterial growth efficiency (BGE) between ~ 5% and 60% as per Del Giorgio and Cole (1998). We have also estimated a singular value for SAR11 HTCC1062 BGE using data from Steindler et al. (2011), in which changes in O₂ concentration were measured in sealed bottles by noninvasive Optode sensor (PreSens). BGE is calculated as follows:

$$\text{BGE} = \text{bacterial carbon production} \times (\text{bacterial carbon production} + \text{bacterial respiration})^{-1}$$

Oxygen consumption was assessed for SAR11 cells growing on a defined medium containing pyruvate (80 μmol L⁻¹), oxaloacetate (40 μmol L⁻¹), taurine (40 μmol L⁻¹), betaine (1 μmol L⁻¹), glycine (50 μmol L⁻¹), and methionine (50 μmol L⁻¹). In that experiment, between the zero time point and 92 h, O₂ dropped 180 μmol L⁻¹ and cells increased to 3.01 × 10⁸ cells mL⁻¹. Using a respiratory quotient of 0.91 CO₂ produced : O₂ consumed and our directly measured values of carbon per cell (6.5 fg C cell⁻¹), we estimated ~ 50% of consumed DOC was converted to biomass C under these conditions. This value suggests that BGE for SAR11 grown on an optimal defined medium is in the upper range of BGE cited by Del Giorgio and Cole (1998) for natural populations. Details of this calculation can be found in the Supporting Information.

Results and discussion

Global census of SAR11

Morris et al. (2002) estimated global SAR11 populations at 2.4 × 10²⁸ cells by extrapolating from fluorescent in situ hybridization (FISH) data obtained from a few sites. Since then many additional studies have published SAR11 cell counts obtained with FISH methods. We used all published data to re-evaluate global standing stocks of SAR11, arriving at 2.43 × 10²⁸ cells, a number essentially identical to the original estimate. The details of this calculation can be found in the Supporting Information.

Elemental composition of cultured isolates

To our knowledge, this is the first study to use regressions of dilution series to measure both cellular C and P in cultured marine plankton (Table 1). A schematic diagram explaining this approach can be found in Supporting Information Fig. S1. The essence of this approach is that cells can be collected and washed free of their growth medium by centrifugation and then diluted in a series, yielding a regression line when elemental composition measurements are plotted. The slope of the model II regression yields elemental composition per cell, while the y intercept is the value of the carrier (i.e., AMS1 media for C). Supporting Information Fig. S2 provides examples of regression plots obtained with this approach. After trying several methods, we found this approach to yield reliable regressions, without involving filtration methods, which are challenging to control. This dilution series approach avoids the loss of bacteria through glass fiber filters, and the

Table 1. Elemental stoichiometry of SAR11 during exponential growth and stationary growth under P deplete or P replete conditions and compared to literature-derived values of elemental content and stoichiometry.

Conditions	Strain	fg P cell ⁻¹	fg C cell ⁻¹	C : P (mol)	C : N (mol)	C : N : P
This study: cells harvested during exponential growth, quotas estimated via dilution series						
P replete	HTCC1062	0.70±0.02	6.6±1.1	24.3±0.7	4.5±0.1	24:5:1
P limited	HTCC1062	0.41±0.03	4.3±0.4	26.8±0.4	4.6±0.3	27:6:1
P replete	HTCC7211	0.51±0.02	6.4±1.6	32.7±2.1	4.5	33:7:1
P limited	HTCC7211	0.51±0.03	3.2±0.3	16.4±0.2	4.6	16:4:1
This study: cells harvested during stationary growth, quotas estimated via dilution series						
P replete	HTCC1062	0.31±0.01	4.0±0.4	33.8±0.5	4.5±0.1	34:8:1
P limited	HTCC1062	0.40±0.02	6.1±0.7	38.7±0.7	4.5±0.1	38:9:1
P replete	HTCC7211	0.50±0.03	8.0±2.0	41.4±2.6	4.5	41:9:1
P limited	HTCC7211	0.43±0.02	5.2±1.1	31.4±1.4	4.5	31:7:1
Prior reports: Cells harvested onto nominal 0.3 μm pore size GF-75 filter at early stationary phase						
Zimmerman et al. (2014)	HTCC1062	2.9	32.2	36	NA	NA
Prior reports: Carbon content estimated from cell volume or cell mass						
Tripp et al. (2008)	HTCC1062		5.8*			
Cermak et al. (2017)	HTCC1062		6.0*			
Cermak et al. (2017)	HTCC7211		8.0*			
Prior reports: Volume measured and C content estimated here as per Romanova and Sazhin (2010) assuming fg cell ⁻¹ = 133.75 × [μm ³] ^{0.428}						
Steindler et al. (2011)	HTCC1062		31.9			
Rappé et al. (2002)	HTCC1062		22.2			
Malmstrom et al. (2005)	In situ		34.1			
Zhao et al. (2017)	HTCC1062		30.1			

*Calculated assuming 50% C by mass and cell density of 1 g cm⁻³, Cermak et al. (2017) measured dry mass for HTCC1062 and HTCC7211 to be 11.9 ± 0.7 and 16.0 ± 0.8 fg cell⁻¹. Error reported in this table reflects the standard error of the slope generated by the model II regression.

high-temperature catalytic oxidation method is more sensitive and requires less volume (100 μL per analyses) than traditional CHN (carbon-hydrogen-nitrogen) analysis.

Prior estimates of SAR11 cell volumes, cell masses, and elemental quotas that apply different methods have been reported (Table 1). Under nutrient replete conditions, we found similar carbon contents of ~ 6.5 fg C cell⁻¹ (Table 1) for both strains assayed, with C content decreasing significantly under P limitation to 3.2 and 4.3 fg C cell⁻¹ for HTCC7211 and HTCC1062, respectively. Carbon quotas ranged from 4 to 8 fg C cell⁻¹ when cells were harvested during stationary growth phase. Across strains and nutrient status, the molar ratio of C : N was tightly conserved, ranging from 4.5 to 4.6. The molar ratio of C : P was more variable (16–39), with increases in C : P values observed for both strains in stationary phase as compared to exponential phase (Table 1).

The C quotas we report, ~ 6.5 fg C cell⁻¹, are very close to estimates made by Cermak et al. (2017), who used Archimedes principle and the difference in mass between cells in D₂O and H₂O (Table 1) to estimate dry biomass at 12–16 fg cell⁻¹. They then applied the assumption of 50% carbon by weight in biomass to arrive at C quotas. These values for *Pelagibacter* cell carbon quotas are approximately 10-fold less than that of the

highly abundant photosynthetic prokaryote *Prochlorococcus* (45–60 fg C cell⁻¹; Bertilsson et al. 2003) and are considerably reduced compared to published estimates for marine heterotrophic bacteria in general (Table 2). Our findings are consistent with reports that indicate SAR11 belong in the smallest class of plankton cells (Rappé et al. 2002).

We measured C : N ratios in the narrow range of 4.5–4.6 : 1, close to published values for marine bacteria (5 : 1; Table 1). Signatures of evolution to economize N content have been reported from marine bacterial proteomes (Grzyski and Dussaq 2012), including SAR11, while other studies have indicated that the low G + C content of genomic DNA in some plankton, including SAR11, is more likely to be a consequence of C limitation (Hellweger et al. 2018). Regardless, our findings indicate a relatively small fraction of C and N biomass in these cells.

Relative cellular quotas of P were much more variable than C : N. We found higher P content per cell for HTCC1062 relative to HTCC7211 during exponential growth (Table 1) with HTCC1062 also having greater flexibility of P quotas between P replete (0.70 fg cell⁻¹) and P deplete conditions (0.41 fg cell⁻¹). P quotas for HTCC7211 did not differ significantly as a function of P supply during exponential growth (~ 0.5 fg cell⁻¹);

Table 2. Elemental analyses for mixed assemblages of open-ocean bacterioplankton and mixed communities of cultured organisms.

Location	Method	fg C cell ⁻¹	fg N cell ⁻¹	fg P cell ⁻¹	C : N	Ref.
Sargasso Sea (20–140 m)	TEM X-ray	4.0–8.9	0.8–1.7	0.1–0.3	5.3–9.1	Gundersen et al. (2002)
Equatorial Pacific	HTC	5.9	1.2		5.7	
Subpolar, S. Pacific, 65°S	HTC	23.5	3.9		7	Fukuda et al. (1998)
Temperate, S. Pacific, 48°S	HTC	6.5	1.2		6.3	Fukuda et al. (1998)
Subtropical S. Pacific, 15°S	HTC	12.5	1.8		8.1	Fukuda et al. (1998)
Subtropical N. Pacific 15°N	HTC	12.8	1.8		8.3	Fukuda et al. (1998)
Subtropical N. Pacific, 31°N	HTC	13.3	2.9		5.4	Fukuda et al. (1998)
Subtropical N. Pacific	Inverse modeling	6.24				Christian and Karl (1994)
Cultured strains (<i>n</i> = 13), early-stationary phase	HTC	145	37	5	5	Zimmerman et al. (2014)
Cultured strains (<i>n</i> = 4), exponential phase	X-ray	150	35	12		Vrede et al. (2002)

however, P quotas were reduced under P limitation when cells were harvested during stationary phase (Table 1; *t*-test, *p* < 0.01). The ratio of C : P increased for both strains during stationary growth phase, regardless of P supply, as cellular P quotas were reduced relative to C. This indicates that the low C : P and N : P ratios observed are not due to P-rich cells, but rather C- and N-poor cells relative to other heterotrophic bacteria. Specifically, the mean C : N : P of heterotrophic bacteria has been estimated to be ~ 50 : 10 : 1 on a molar basis (Fagerbakke et al. 1996) compared to the 24 : 5 : 1 for HTCC1062 and 33 : 7 : 1 for HTCC7211 for nutrient replete, exponentially growing cells. Supporting this conclusion, cryo-electron tomography have indicated that the nucleoid of SAR11 cells occupies nearly half of the cytoplasmic volume (Zhao et al. 2017). Given the genome size of SAR11 (1.3 Mb), the P quota required for DNA would be 0.13 fg cell⁻¹ or ~ 20% of the cellular P quota

we measured. This implies potential for sizeable allocation of P to non-nucleic acid compounds such as phospholipids (e.g., Carini et al. 2015).

There are a few studies that have evaluated the elemental content of mixed assemblage of open-ocean bacterioplankton (Table 2). Using the high-temperature combustion method, similar to that described in this study, Fukuda et al. (1998) found the C and N content of mixed population of open-ocean bacterioplankton to be greater (i.e., 12.4 ± 6.3 fg C cell⁻¹ and 2.1 ± 1.1 fg N cell⁻¹), than we report here for SAR11. The C content we report is consistent with Christian and Karl (1994), who reasoned, based on inverse modeling approach, that oceanic bacterioplankton cell content must be less than 10 fg C cell⁻¹. The C and N content per cell that we report here is similar to estimates of Sargasso Sea bacterioplankton made by transmission electron microscopy and X-ray microanalysis (Gundersen et al.

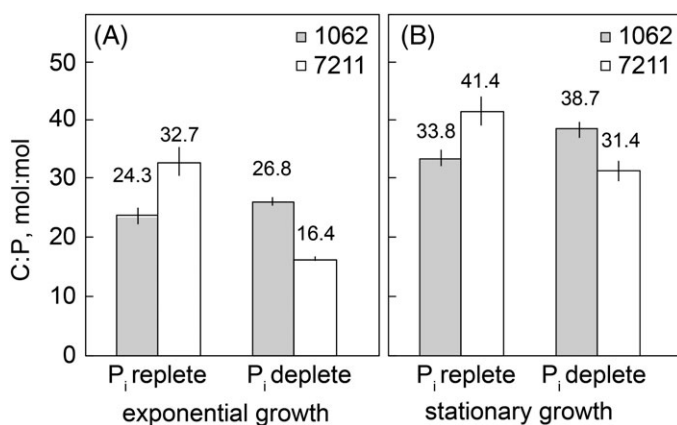


Fig. 1. Measured C : P stoichiometry for strains HTCC1062 and HTCC7211 harvested during (A) exponential growth or (B) stationary growth phase from P_i deplete and P_i replete cultures. Error bars are calculated via error propagation of C cell⁻¹ and P cell⁻¹ measurements. Mean values are noted in text.

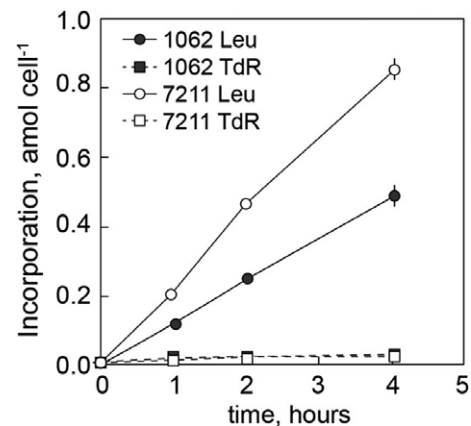


Fig. 2. Incorporation of ³H-leucine or ³H-thymidine into HTCC1062 cells growing in culture. Bars represent the standard error for triplicate treatments.

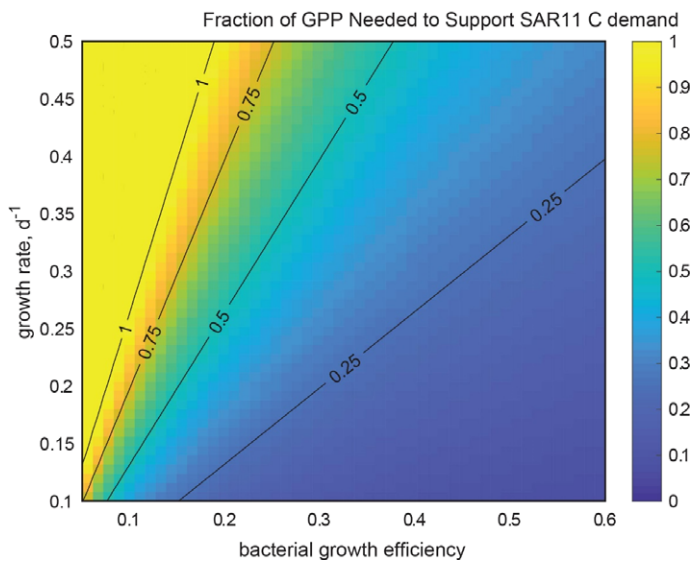


Fig. 3. Contour of the fraction of GPP needed to support SAR11 C demand over a range of assumed bacterial growth efficiencies and specific growth rates. The color axis is fixed from 0 to 1.

2002); however, SAR11 isolates appear to be enriched in P compared to the mixed Sargasso Sea bacterioplankton assemblage.

Bacterial production

We measured the uptake of ^3H -thymidine and ^3H -leucine by cultured strains of HTCC1062 and HTCC7211. Neither HTCC1062 nor HTCC7211 assimilated thymidine, consistent with genome analyses which show that most SAR11 cells lack thymidine phosphorylase and thymidine kinase, two key enzymes in salvage pathways for pyrimidine deoxynucleosides (Supporting Information Table S2). We speculate that the absence of these genes is another example of the evolutionary trend to genome reduction in SAR11 that sacrifices some seemingly valuable functions to yield a cell architecture that utilizes scarce resources efficiently.

In contrast, both strains incorporated ^3H -leucine a proxy for bacterial biomass production (Kirchman et al. 1986). Because direct measurements of growth rates and biomass were available, we were able to compare the estimated productivity from the uptake of ^3H -leucine to the actual increase in biomass allowing us to empirically derive factor necessary to convert leucine incorporation to C production. For HTCC1062, the empirically derived leucine conversion factor was $1.51 \text{ kg C mol}^{-1}$, and for HTCC7211, it was $0.74 \text{ kg C mol}^{-1}$ —values that are comparable to the conversion factor in common use for prokaryotic heterotrophic production, $1.5 \text{ kg C mol leu}^{-1}$ (Simon and Azam 1989) and to those reported for a variety of marine environments (Alonso-Sáez et al. 2007; Calvo-Díaz and Morán 2009).

Conclusions

These experiments were done with two strains that represent the most abundant lineage of SAR11, *Pelagibacter* 1a,

found throughout the global surface ocean. The two strains we investigated, HTCC1062 and HTCC7211, represent the 1a.1 and 1a.3 ecotypes of *Pelagibacter*, which have different biogeographical distributions with latitude: the 1a.1 ecotype is found in cool temperate and polar waters (Brown et al. 2012), whereas the 1a.3 ecotype is abundant in warm equatorial and subtropical waters. In some temperate regions, these two ecotypes oscillate seasonally (Eren et al. 2013). We report elemental stoichiometry of these strains to be relatively C and N poor relative to P; the mean molar C : N : P stoichiometry of growing cells was 25 : 6 : 1. The reduction in P during P limitation exhibited by HTCC1062 and not HTCC7211 suggests variable P-allocation strategies among strains.

The ^3H -thymidine tracer method is a widely used for assessing heterotrophic bacterial production in aquatic systems (Fuhrman and Azam 1982). However, the absence of thymidine labeling with SAR11 suggests that there is potentially a bias in estimates of rates of heterotrophic microbial production made with this method. Because SAR11 cells become proportionately more abundant with increasingly oligotrophic conditions and can reach as much as 40% of planktonic cell communities, our findings suggest that there could be a systematic underestimate in bacterial production when using the thymidine method in oligotrophic region. The use of ^3H -leucine as a tracer of bacterioplankton biomass production is a more appropriate assay.

The carbon quotas we measured and the global census of SAR11 cells were used to establish a likely range for the contribution of SAR11 to the ocean carbon budget (see Supporting Information). Our measurements indicate global SAR11 standing stocks of $1.6 \times 10^{14} \text{ g C}$. Global ocean gross primary production (GPP) is estimated at $\sim 140\text{--}170 \times 10^{15} \text{ g C yr}^{-1}$ (Marra 2002; Westberry et al. 2008). Uncertainties in the estimation of SAR11 contributions to global ocean carbon oxidation mainly reside in uncertainties about specific growth rates and BGE. We estimated BGE from the oxygen uptake measurements of Steindler et al. (2011), and our C quotas to be 50% for cells growing on defined carbon compounds. This measurement is at the high end of the range reported for natural populations ($\sim 5\text{--}60\%$; Del Giorgio and Cole 1998). In cultures of SAR11, specific growth rates of 0.5 d^{-1} are common, and for bacterioplankton communities, typical bacterial turnover rates are $< 0.2 \text{ d}^{-1}$ (Kirchman 2016). Figure 3 shows SAR11 contributions to GPP over a range of values for BGE and growth rate. Using our BGE estimate of $\sim 50\%$ and growth rates of $0.1\text{--}0.5 \text{ d}^{-1}$, SAR11 C demand would be estimated to account for $\leq 37\%$ of the mid-range of GPP ($155 \times 10^{15} \text{ g C yr}^{-1}$). Assumptions of a fixed and slower growth rate of 0.05 d^{-1} and variable BGE ($5\text{--}60\%$) yield C demands estimated to be between 6% and 37% of GPP (Fig. 3). Examples of these calculations can be found in Supporting Information.

Despite uncertainties, these assessments establish the scale of SAR11 involvement in the carbon cycle, raising the question, what adaptations enable them to gather such a large

share of organic matter resources? Investigations of SAR11 metabolism have shown them to be specialists in the oxidation of low-molecular-weight, labile carbon compounds, including volatile organic compounds that are released by healthy, growing cells and via processes that involve cell death (Halsey et al. 2017). Thus, at least in part, SAR11 is targeting dissolved organic matter (DOM) resources that are not encompassed by Net Primary Productivity (NPP), which is typically estimated by measuring particulate matter production. The estimates of SAR11 carbon demand constrain scale their activity, but at least part of their success is likely due to their ability to exploit resources that would be part of GPP in most calculations. SAR11 cells are unusual, and better understanding their strategic success may help us understand features of cell biology that contribute to trophic interactions at large scales.

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