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Elemental Content and Stoichiometry of SAR11 Chemoheterotrophic Marine Bacteria

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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.*

Keywords: elemental stoichiometry, SAR11, bacterioplankton

Author contributions: *SG and CC conceived of the study. AW, SG, CC, and YZ conducted all experiments and analyzed all samples; KV compiled data necessary to estimate the global census of SAR11. AW and SG drafted the initial manuscript and all authors contributed to revision and editing of the final manuscript.*

I. 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 $\times 10^{13}$ 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 ^{3}H-thymidine, but do incorporate ^{3}H-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 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.

II. Introduction

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 (Cermak et al. 2017; Tripp et al. 2008) 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. 2014b).

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 ^{3}H-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.

III. 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 µmol L^{-1}), glycine (5 µmol L^{-1}), methionine (5 µmol L^{-1}), $FeCl_3$ (1 µmol 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 RPM under a 12 h light: 12 h dark cycle. Light levels during the day were held at 140–180 µmol photons m^{-2} 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 *g* for 1.0 h at 20°C). Cell pellets were washed twice with growth medium (without added inorganic phosphorus, P_i) and re-suspended in one of the following conditions: i) P_i-replete (100 µmol L^{-1}); or ii) P_i-deplete growth medium (no P_i added). Each resuspension was monitored for growth and subsampled by centrifugation (48,298g for 1.0 h at 4°C) at t = 0, 2, 4, 6 and 8 days. 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 (Figure S1). 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. 3 sets of 6 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 ~10^8 – 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 while 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 (Figure 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 pre-combusted, acid-washed, DI rinsed glass test tubes for 4-5 hours 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, three milliliters of sample were acidified with 2N HCL (1.5%), and sparged for 1.5 minutes with CO_2-free gas. Three to five replicates (100 µl) of sample were injected into the combustion tube heated to 680° C that had CO_2 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 non-dispersive infrared detector. The resulting peak area was integrated with Shimadzu chromatographic software. Additional analytical details are described in the SOD.

Thymidine and leucine incorporation: Samples for SAR11 production were analyzed via ^{3}H-thymidine and ^{3}H-leucine incorporation following the methods of Simon and Azam (1989) with

slight modifications. In brief, triplicate samples and duplicate 5% TCA-killed controls of SAR11 cells in logarithmic growth phrase were incubated with 20 nmol L^{-1} ^{3}H-thymidine (specific activity 10.1 Ci $mmol^{-1}$; PerkinElmer, Boston, MA) or 20 nmol L^{-1} ^{3}H-leucine (specific activity 54.1 Ci $mmol^{-1}$; PerkinElmer, Boston, MA). Samples were incubated in the dark for 4 hours. 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 between ~5 and 60% as per Del Giorgio and Cole (1998). We have also estimated a singular value for SAR11 HTCC1062 bacterial growth efficiency (BGE) using data from Steindler et al. (2011), in which changes in O_2 concentration were measured in sealed bottles by non-invasive Optode sensor (PreSens). BGE is calculated as follows:

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

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 hours, O_2 dropped 180 µmol L^{-1} and cells increased to 3.01×10^8 cells ml^{-1}. Using a respiratory quotient of 0.91 CO_2 produced : O_2 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 SOD.

Results and Discussion

Global census of SAR11. Morris et al. (2002) estimated global SAR11 populations at 2.4×10^{28} 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 2.43×10^{28} cells, a number essentially identical to the original estimate. The details of this calculation can be found in the SOD.

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 Figure 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). Figure 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 HTC method is more sensitive and requires less volume (100 µL per analyses) than traditional CHN 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 4-8 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 4.5-4.6. 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 D_2O and H_2O (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 ten-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 (Grzymski 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 $cell^{-1}$); 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, CET 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 (TEM) and X-ray microanalysis (Gundersen et al. 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 ^{3}H-thymidine and ^{3}H-leucine by cultured strains of HTCC1061 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 (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 ^{3}H-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 ^{3}H-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 kg C mol^{-1}, and for HTCC7211 it was 0.74 kg C mol^{-1} ; values that are comparable to the conversion factor in common use for prokaryotic heterotrophic production, 1.5 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).

IV. 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, HTCC7211 and HTCC1062, 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 sub-tropical 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 ^{3}H-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 there could be a systematic underestimate in bacterial production when using the thymidine method in oligotrophic region. The use of ^{3}H-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 to were used to establish a likely range for the contribution of SAR11 to the ocean carbon budget (see SOD). Our measurements indicate global SAR11 standing stocks of 1.6×10^{14} g C. Global ocean gross primary production (GPP) is estimated at ~140 – 170 $\times10^{15}$ 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 (~5-60%, 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 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 ~ 50% and growth rates of 0.1-0.5 d^{-1}, SAR11 C demand would be estimated to account for ≤ 37% of the mid-range of GPP (155 $\times10^{15}$ g C yr^{-1}). Assumptions of a fixed and slower growth rate of 0.05 d^{-1} and variable BGE (5 – 60%) yield C demands estimated to be between 6 and 37% of GPP (Figure 3). Examples of these calculations can be found in SOD.

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 DOM resources that

are not encompassed by NPP, which is typically estimated by measuring particulate matter production. The estimates of SAR11 carbon demand constrain the 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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VII. Tables

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 (molar)	C:N (molar)	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. (2014a)	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 Sazchin (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.

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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 - 140m)	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 (n=13), early-stationary phase	HTC	145	37	5	5	(Zimmerman et al. 2014a)
Cultured strains (n=4), exponential phase	X-ray	150	35	12		(Vrede et al. 2002)

VIII. Figures

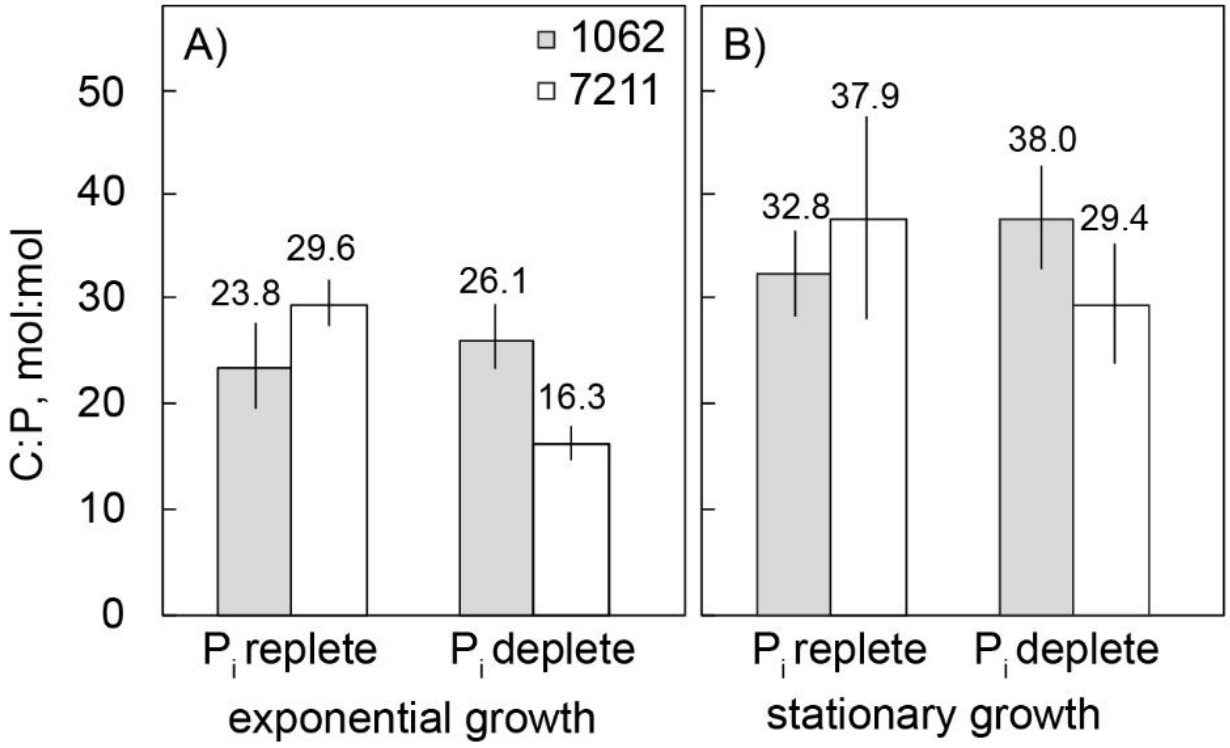

Figure 1. Measured C:P stoichiometry for strains HTCC1062 and HTCC7211 harvested during (A) exponential growth or (B) stationary growth phase from P_i deplete and P_i replete cultures. Error bars are calculated via error propagation of C cell^{-1} and P cell^{-1} measurements. Mean values are noted in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

Prepared for submission as a letter to Limnology & Oceanography Letters

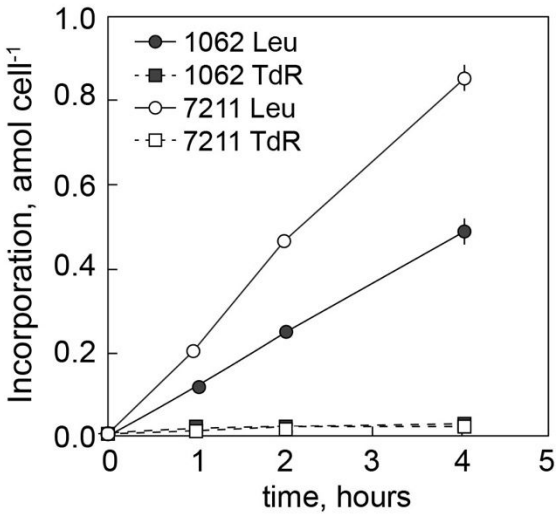

Figure 2. Incorporation of ^{3}H-leucine or ^{3}H-thymidine into HTCC1062 cells growing in culture. Bars represent the standard error for triplicate treatments.

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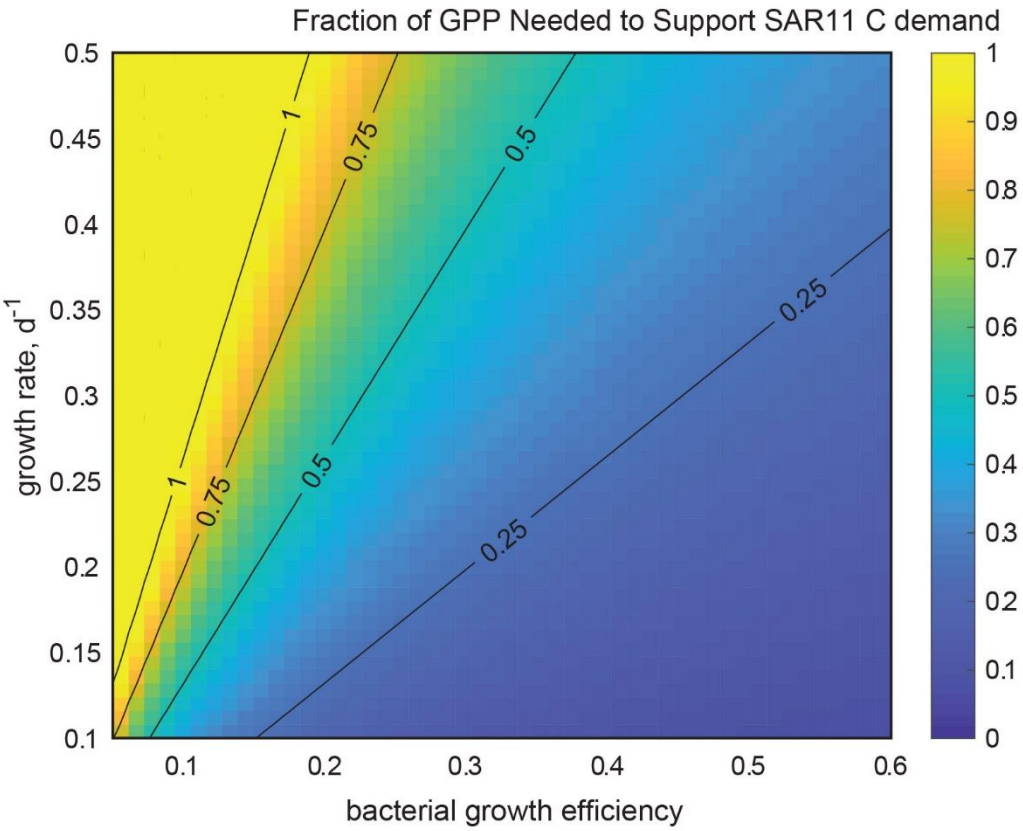

510
511
512 **Figure 3.** Contour of the fraction of GPP needed to support SAR11 C demand over a range of
513 assumed bacterial growth efficiencies and specific growth rates. The color axis is fixed from 0-1.
514

Supporting Online Documentation

Elemental Content and Stoichiometry of SAR11 Chemoheterotrophic Marine Bacteria

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I. Workflow and results of the serial dilution method

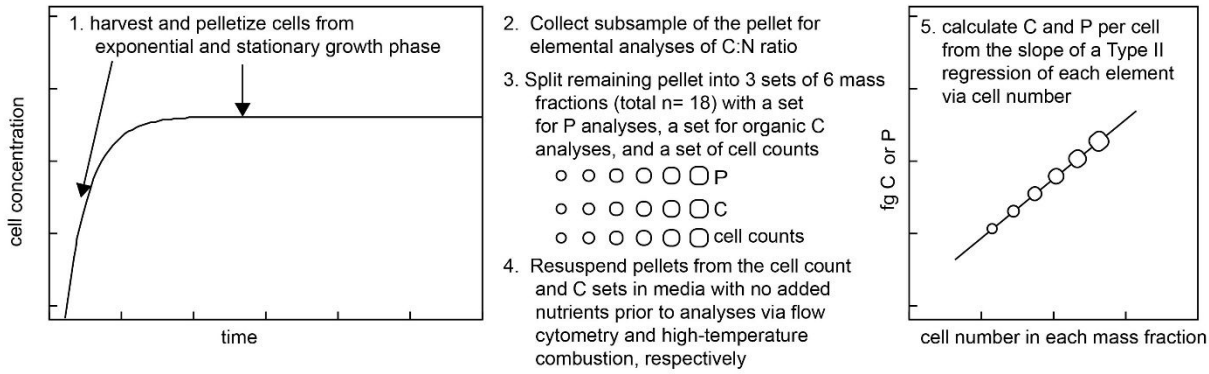

Figure S1. A schematic showing the five general steps followed to calculate elemental stoichiometry per cell in two strains of SAR11.

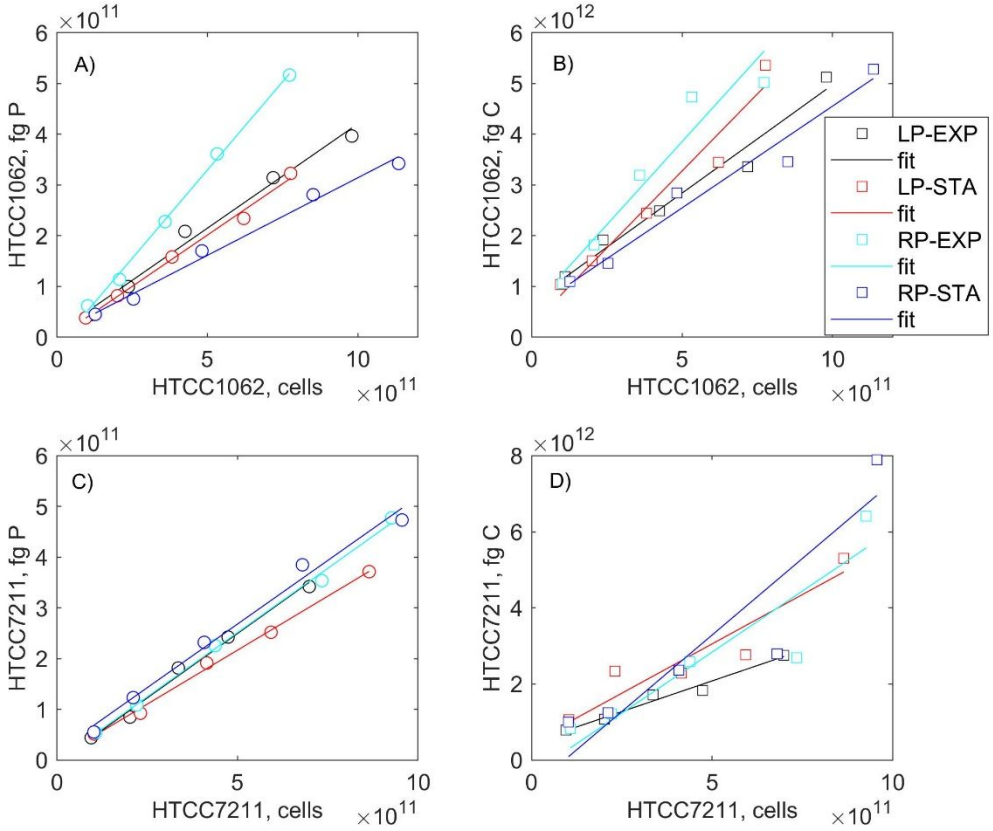

Figure S2. Results of dilution series of cells isolated during exponential and stationary phase for strain HTCC1062 (A-B) and HTCC7211(C-D) with panel A and C showing regressions of cell number and P content in isolated pellets and B and D showing regressions of cell number and organic C content in isolated pellets. In all panels, symbols are actual measurements and lines are the result of a Type II regression.

II. SAR11 global census

Several studies used fluorescence in situ hybridization (FISH) or quantitative PCR (qPCR) to estimate SAR11 cell abundance in seawater samples. Since these techniques are labor intensive, sampling tends to be in more easily accessible locations and conducted over short time spans. These studies include surveys of the Baltic Sea (Herlemann et al. 2014), Mediterranean Coast (Alonso-Sáez et al. 2007), Atlantic Ocean transect (Schattenhofer et al. 2009), Southern Ocean (Straza et al. 2010; Thiele et al. 2012), Hawaii Ocean Time-Series site (HOT)(Eiler et al. 2009), and the Bermuda Atlantic Time-Series Study site (BATS)(Morris et al. 2002). Unfortunately, these study sites are not well-representative of the vast ocean volume (Eakins and Sharman 2010). Seas such as the Baltic, Mediterranean, and South China represent about 1% of the total volume. Coastal regions represent about 7.4% of the ocean volume (Costello et al. 2010) and the top 100 m of the surface layer where most of the photosynthesis is occurring represents about 0.1% of the total volume (Costello et al. 2015). The remaining 92.6% of the ocean is represented by deeper samples. A three-year time series of depth profiles from BATS (Carlson et al. 2009; Morris et al. 2002) is the most thorough sampling of surface and mesopelagic horizons so the

mean values from this study were used mainly to extrapolate to the total ocean. The BATS system has a strong seasonal cycle, the main feature of which is the annual deep mixing event in late winter or early spring where water cooling and storm activity mixes the upper 200-300 m of the water column. SAR11 cell numbers are at their lowest during the three-month period immediately preceding the deep mixing period and at their highest during the three-month period corresponding to the month of deepest mixing and the two succeeding months. This situation at BATS may represent the extremes of SAR11 abundance at other oceanic sites, although the timing may be different. At BATS, the period immediately prior to deep mixing is likely the most oligotrophic and the period during and immediately following deep mixing is the least oligotrophic during the year. The study of an Atlantic transect (Schattenhofer et al. 2009) temporally overlaps with the BATS time series study (Carlson et al. 2009) and confirms the values obtained at BATS for that time period. Additionally, it suggests that SAR11 abundance may fluctuate such that abundance is greater in one hemisphere (north or south) while simultaneously less abundant in the opposite hemisphere, with intermediate values in the equatorial region. Using this assumption, highest estimated abundance was applied to Northern Hemisphere regions and lowest estimated abundance was used for Southern Hemisphere regions. Without more extensive sampling, it is unclear if another method for apportioning abundance would be more accurate. The least sampled regions are the Arctic Ocean and Southern Ocean. There are a few summer surface samples that suggest that abundance is in the same range as for more temperate regions (Straza et al. 2010; Thiele et al. 2012). One study (Garneau et al. 2008) documents a 75% reduction in total cell counts during the Arctic winter but it is not clear if this reduction affects all cells equally so no reduction was applied to our calculation, potentially resulting in a small overestimation. In conclusion, the deep ocean is the main driver for calculating the total abundance of SAR11. With the sparse sampling reported in the literature, we are extrapolating using relatively few samples so our estimate may be very inaccurate. More widespread sampling of deep waters over a time span of several years would produce a more accurate estimate of total SAR11 abundance. However, it is interesting that this study is quite similar to two previous estimates using other methods (Morris et al. 2002; Schattenhofer et al. 2009).

Supplementary Table 1. Calculations of the concentration of SAR11 cells in various ocean basins. Total volume for each basic was derived from (Eakins and Sharman 2010) with the relative volume in the coastal ocean, surface ocean (<100 m) and deep ocean calculated via assuming 7.4% of volume as coastal ocean (Costello et al. 2010), 10% of volume in the upper 100m in the open ocean (Costello et al. 2015) and the remaining volume as deep ocean. The fraction of the SAR11 in the coastal, surface, and deep ocean are derived from the studies described above. We assume a population of 5×10^8 cells L^{-1} in the surface and coastal ocean and 5×10^7 in the deep ocean and the total population is then calculated as the sum of the fraction of SAR11 in each habitat multiplied by the volume of that specific habitat.

Body of Water	Coastal Volume, L	Surface Volume, L	Deep Volume, L	SAR11 coastal	SAR11 <100m	SAR11 deep	Total
Arctic Ocean	1.39×10^{18}	1.74×10^{16}	1.73×10^{19}	0.17	0.49	0.159	2.60×10^{26}
Baltic Sea**	2.09×10^{16}			1.0			9.35×10^{23}
Mediterranean	3.25×10^{17}	4.07×10^{15}	4.06×10^{18}	0.27	0.378	0.206	8.65×10^{25}
North Atlantic	1.08×10^{19}	1.35×10^{17}	1.35×10^{20}	0.17	0.378	0.206	2.34×10^{27}
South Atlantic	1.18×10^{19}	1.48×10^{17}	1.48×10^{20}	0.38	0.348	0.112	3.10×10^{27}
Indian Ocean	1.95×10^{19}	2.44×10^{17}	2.44×10^{20}	0.38	0.348	0.112	5.12×10^{27}
North Pacific	2.45×10^{19}	3.07×10^{17}	3.06×10^{20}	0.17	0.378	0.206	5.29×10^{27}
South Pacific	2.43×10^{19}	3.05×10^{17}	3.04×10^{20}	0.38	0.348	0.112	6.38×10^{27}
South China Sea	7.31×10^{17}	9.15×10^{15}	9.14×10^{18}	0.18	0.348	0.112	1.19×10^{26}
Southern Ocean	5.31×10^{18}	6.65×10^{16}	6.64×10^{19}	0.38	0.49	0.159	1.55×10^{27}
Total							**2.43×10^{28}**

**The Baltic Sea is composed of a freshwater to brackish to marine habitat that is reported here as coastal simply to minimize the number of categories shown.

III. Example calculation of bacterial growth efficiency (BGE) and the amount of global production oxidized by SAR11

BGE = BP/ (BP + BR) * 100% where BP is bacterial carbon production and BR is bacterial respiration

Conversion of cell counts from Steindler et al. (2011) to carbon were estimated to determine the SAR11 biomass production over a 92 hr incubation:

$$BP = (3.0 \times 10^{11} \text{ cells } L^{-1}) \times (6.5 \times 10^{-15} \text{ g C cell}^{-1}) \times (1 \text{ mol C}/ 12.01 \text{ g}) = 1.6 \times 10^{-4} \text{ mol C } L^{-1}$$

Over the same incubation period BR was estimated from the oxygen consumption measured in Steindler et al. (2011) and converted to CO_2 respired using a commonly assumed respiratory quotient (RQ; CO_2 produced:O_2 consumed) of 1 for carbohydrates and a more conservative RQ of marine organic matter of 0.72 (Anderson 1995).

The consumption of 1.8×10^{-4} mol O_2 L^{-1} over the 92 hr incubation is equivalent to BR of 1.8×10^{-4} mol CO_2 L^{-1} and 1.3×10^{-4} mol CO_2 L^{-1} using an RQ of 1 and 0.72, respectively.

As such the estimates of BGE is equivalent to 47 – 55% depending on RQ used. These values are high but well within the range assumed (~5%-60%) for natural populations (Del Giorgio and Cole 1998).

The total carbon reservoir of the global SAR11 population (assuming average weight of 1062 and 7211) is then:

$$(6.5 \times 10^{-15} \text{ g C cell}^{-1}) \times (2.43 \times 10^{28} \text{ cells}) = 1.58 \times 10^{14} \text{ g C}$$

Using this global SAR11 C content, we can then estimate the fraction of gross primary production (GPP) required to support SAR11 C demand. Below we show an example calculation using a specific growth rate of 0.1 d^{-1} and BGE of 50%:

Global SAR11 carbon production can be calculated assuming a specific growth rate of 0.1 d^{-1}:

$$(1.58 \times 10^{14} \text{ g C}) \times (365 \text{ d yr}^{-1} \times 0.1 \text{ d}^{-1}) = 5.77 \times 10^{15} \text{ g C yr}^{-1}$$

From these values, the fraction of the GPP (155×10^{15} g C yr^{-1} as per the mean of Marra 2002 and Westberry et al., 2008) needed to support the growth and standing stock of SAR11 can then be calculated via assumption of a growth efficiency of 50%:

$$(5.77 \times 10^{15} \text{ g C yr}^{-1}) / (0.5 \times 155 \times 10^{15} \text{ g C yr}^{-1}) \times 100 = 7\% \text{ of GPP oxidized by SAR11}$$

This calculation is an example and is of course sensitive to the estimate of growth efficiency and specific growth rate, both of which are challenging to assess for natural populations and to determine over an annual cycle. For this reason, we have calculated the solution for a range of BGE and growth rates (Figure 3, main text) using fixed values for GPP (155×10^{15} g C yr^{-1}) and global C content of SAR11 (1.58×10^{14} g C).

IV. Evidence that the SAR11 genome lacks thymidine salvage genes

Supplementary Table 2 - Distribution of two key thiamine salvage genes among SAR11 genomes from isolates. The strains from Hawaii and the Sargasso Sea belong to the Ia.1 ecotype, whereas those from the Oregon Coast belong to the Ia.1 ecotype. BlastP was performed with reference sequences for thymidine phosphorylase (PZA13145.1) and thymidine kinase (WP_062426622.1).

Strains	Thymidine	Thymidine kinase	Origin
HIMB058	*	0	Hawaii
HIMB083	0	0	Hawaii
HIMB114	0	0	Hawaii
HIMB122	0	0	Hawaii
HIMB1321	0	0	Hawaii
HIMB140	0	0	Hawaii
HIMB4	0	0	Hawaii
HIMB5	0	0	Hawaii
HIMB59	0	0	Hawaii
HTCC1002	0	0	Oregon Coast
HTCC1013	0	0	Oregon Coast
HTCC1016	0	0	Oregon Coast
HTCC1040	0	0	Oregon Coast
HTCC1062	0	0	Oregon Coast
HTCC7211	0	0	Sargasso Sea
HTCC7214	0	0	Sargasso Sea
HTCC7217	0	0	Sargasso Sea
HTCC8051	0	0	Oregon Coast
HTCC9022	0	0	Oregon Coast
HTCC9565	0	0	Oregon Coast
IMCC9063	0	0	Arctic Ocean

* low similarity to anthranilate phosphoribosyltransferase CDS -1 330 332

IV. *Thymidine and leucine uptake*

1062 carbon content: 6.6×10^{-15} g C cell^{-1}
1062 growth rate: 0.0275 h^{-1}
Estimated biomass increase in 1 hour: $N_2/N_1 = e^{0.0275} = 1.028$
Leu incorporation rate: 0.122 amol cell^{-1} h^{-1}
Empirical conversion factor: 1.51 kg C (mol leu)$^{-1}$

7211 carbon content: 6.4×10^{-15} g C cell^{-1}
7211 growth rate: 0.025 h^{-1}
Estimated biomass increase in 1 hour: $N_2/N_1 = e^{0.025} = 1.025$
Leu incorporation rate: 0.217 amol cell^{-1} h^{-1}
Empirical conversion factor: 0.74 kg C (mol leu)$^{-1}$

132 ***V. Additional details regarding measurement of bacterial carbon***
133
134 After extensive conditioning of the combustion tube with repeated injections of low carbon water
135 (LCW) and seawater the system response was standardized daily with a four-point calibration curve
136 of glucose solution in Nanopure water. All samples were systematically referenced against low
137 carbon water, reference sea waters (every 6 – 8 analyses, (Carlson et al. 2010). The standard
138 deviation of the seawater references analyzed throughout a run generally had a coefficient of
139 variation (C.V.) ranging between 1-2% over the 3-7 independent analyses. Analytical precision of
140 samples was < 2% C.V. As was done for P, organic carbon content per cell was determined by Model
141 II least square regression of TOC concentration vs cell abundance where the slope represents mean
142 cell organic C content.
143

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Table 1. Description of the fields needed to describe the creation of your dataset.

Title of dataset	Data from: Elemental Content and Stoichiometry of SAR11 Chemoheterotrophic Marine Bacteria
URL of dataset	https://doi.org/10.5061/dryad.1749362
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 $\times 10^{13}$ g C. Conservative estimates of turnover rates and growth efficiency indicate this stock could oxidize up to ~40% of gross ocean primary production. We also found that SAR11 do not incorporate ^{3}H-thymidine, but do incorporate ^{3}H-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 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.
Keywords	elemental stoichiometry, SAR11, bacterioplankton
Dataset lead author	Angelicque E. White
Position of data author	Associate Professor
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Email address of data author	aewhite@hawaii.edu
Primary contact person for dataset	aewhite@hawaii.edu
Position of primary contact person	aewhite@hawaii.edu
Address of primary contact person	Daniel K. Inouye Center for Microbial Oceanography: Research and Education, Honolulu, Hawaii, USA
Email address of primary contact person	aewhite@hawaii.edu
Organization associated with the data	Oregon State University
Usage Rights	publicly available and free to use
Geographic region	NA
Geographic coverage	NA
Temporal coverage - Begin date	NA
Temporal coverage - End date	NA
General study design	*Laboratory experiments*
Methods description	*Study design is described in detail in the associated manuscript*
Laboratory, field, or other analytical methods	*Describe the lab, field, or other processing methods for each variable included in the data table. This section may, and should, be long. You should insert additional rows in this table to complete this section.*
Quality control	
Additional information	Header information and units are described in the data file

Table 2. Description of the variables (i.e., columns) in the "data" sheet of the spreadsheet presented at DOI: https://doi.org/10.5061/dryad.1749362 under review with L&O letters

Column name	Definition	Units
Elemental Analysis Experiment ID	refers to successive experiments in which strains of SAR11 (1062 or 7211) were grown and elemental stoichiometry was characterized	NA
Treatment ID	refers to growth conditions (LP = limited phosphorus, RP = replete phosphorus) and growth stage at harvest (EXP = exponential, ST= Stationary)	NA
N	N = successive identifier for experiment/treatment	NA
cells	Number of SAR11 cells in each pellet fraction	Number of cells
fgP	fg P = fg P in each pellet fraction	fg P
fgC	fg C = fg C in each pellet fraction	fg C