LETTERS
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Significant silicon accumulation by marine picocyanobacteria

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The marine silicon cycle is thought to be intimately tied to the carbon cycle through its effect on the growth of diatoms. These unicellular algae form substantial blooms in cold, nutrient-rich waters. Their dense, siliceous cell walls promote the sinking of particulate matter, and all the carbon and nutrients contained therein[1]. As such, diatoms are thought to be the primary organisms responsible for the low levels of dissolved silicon observed in the surface ocean and the export of mineral silica to depth. Here, we use synchrotron X-ray fluorescence microscopy to determine the elemental composition of individual diatoms and cyanobacterial cells from the eastern equatorial Pacific and the Sargasso Sea. We show that cells of *Synechococcus*, a small unicellular marine cyanobacterium that dominates in nutrient-depleted waters[2], can exhibit cellular ratios of silicon to sulphur, and silicon to phosphorus, approaching those detected in diatoms in the same location. Silicon accumulation was also observed in cultured *Synechococcus* strains. We estimate that the water column inventory of silicon in *Synechococcus* can exceed that of diatoms in some cases. We suggest that picocyanobacteria may exert a previously unrecognized influence on the oceanic silicon cycle, especially in nutrient-poor waters.

A sizeable accumulation of Si by cells of the picocyanobacterium *Synechococcus* was indicated by single-cell analyses of Si, P and S in cells collected from two distinct regions of the ocean with relatively low silicic acid concentrations: the eastern equatorial Pacific (EEP) and the Sargasso Sea. Background-corrected X-ray fluorescence spectra of *Synechococcus* and diatom cells from the EEP analysed by synchrotron-based X-ray fluorescence microscopy (SXRF) exhibit a prominent peak at 1,732 eV that corresponds to the Si Kα fluorescence emission line (Fig. 1a,c). This peak was not apparent in the X-ray fluorescence spectra of non-siliceous protists collected from the same waters and analysed under the same beamline conditions (Fig. 1d). Spectra with sizeable Si emission peaks were also observed for *Synechococcus* cells collected from the Sargasso Sea as part of the FeAST II cruise[3] (Fig. 1b). These findings resemble some previous indications of significant Si accumulations by colonial cyanobacteria in a freshwater lake, Rostherne Mere[4,5], and by acid-resistant spores of *Bacillus cereus*[6].

Concentrations of Si associated with some *Synechococcus* cells were comparable to those of co-occurring diatoms, although significant spatial variability among and within stations was also observed. Cellular Si contents were normalized to cellular P and S (as a proxy for intracellular biomass) to allow comparisons with larger co-occurring protists. Mean ratios of Si/P and Si/S for *Synechococcus* cells from the EEP were 48% and 61%, respectively, of the averages for all diatoms examined from the same cruise (Table 1). This similarity was not due to low Si content of the EEP diatoms, as the silica content per cell volume of these cells was 50% larger than the average for cultured marine diatom species grown in Si-replete media[7]. Confusion with diatoms was highly unlikely as the *Synechococcus* cells imaged were always <1.5 μm in diameter, and no known diatom is <2 μm in diameter[8]. Si contents of *Synechococcus* cells from the Sargasso Sea were lower than those in the EEP and varied 50-fold among stations and depths (Table 1).

The reason for this variation is unclear at present. *Synechococcus* cells within a station typically varied in Si content by an order of magnitude, with Si/P and Si/S exhibiting less variability (Supplementary Table S1). A statistical model that treated station as a random effect estimated that variance in Si content among stations and depths in the Sargasso was threefold greater than that within stations and depths. Si contents were higher on average in deeper water where growth rates are slower, suggesting some effect of growth dilution. Although Si contents of cells were also lower than in the EEP where silicic acid concentrations were higher, there was no correlation to ambient dissolved silicic acid concentration (Supplementary Fig. S1) or to hydrography among stations within the Sargasso (Supplementary Fig. S2). Observed spatial variation could indicate facultative accumulation or loss of accumulated Si as induced by unknown environmental triggers, or sequential replacement of clones exhibiting variable levels of Si accumulation.

Three strains of *Synechococcus* isolated from different regions of the ocean and exhibiting distinct ecologies were studied in culture using techniques designed to avoid production of mineral Si (Table 1). As with the field samples, cellular Si concentrations varied widely among strains and conditions (Table 1). For strain WH5701 grown in f/2-amended Sargasso sea water with EEP levels of $[\mathrm{Si(OH)_4}]$, Si contents were within twofold of those observed in the EEP. In contrast, strain WH7803 grown at a similar $[\mathrm{Si(OH)_4}]$ in Aquil artificial sea water[9] exhibited substantially lower cellular Si concentrations, approaching the lowest measurements for cells collected from the Sargasso Sea. For another strain (CC9311) there is some evidence that growth dilution can affect Si content; cells

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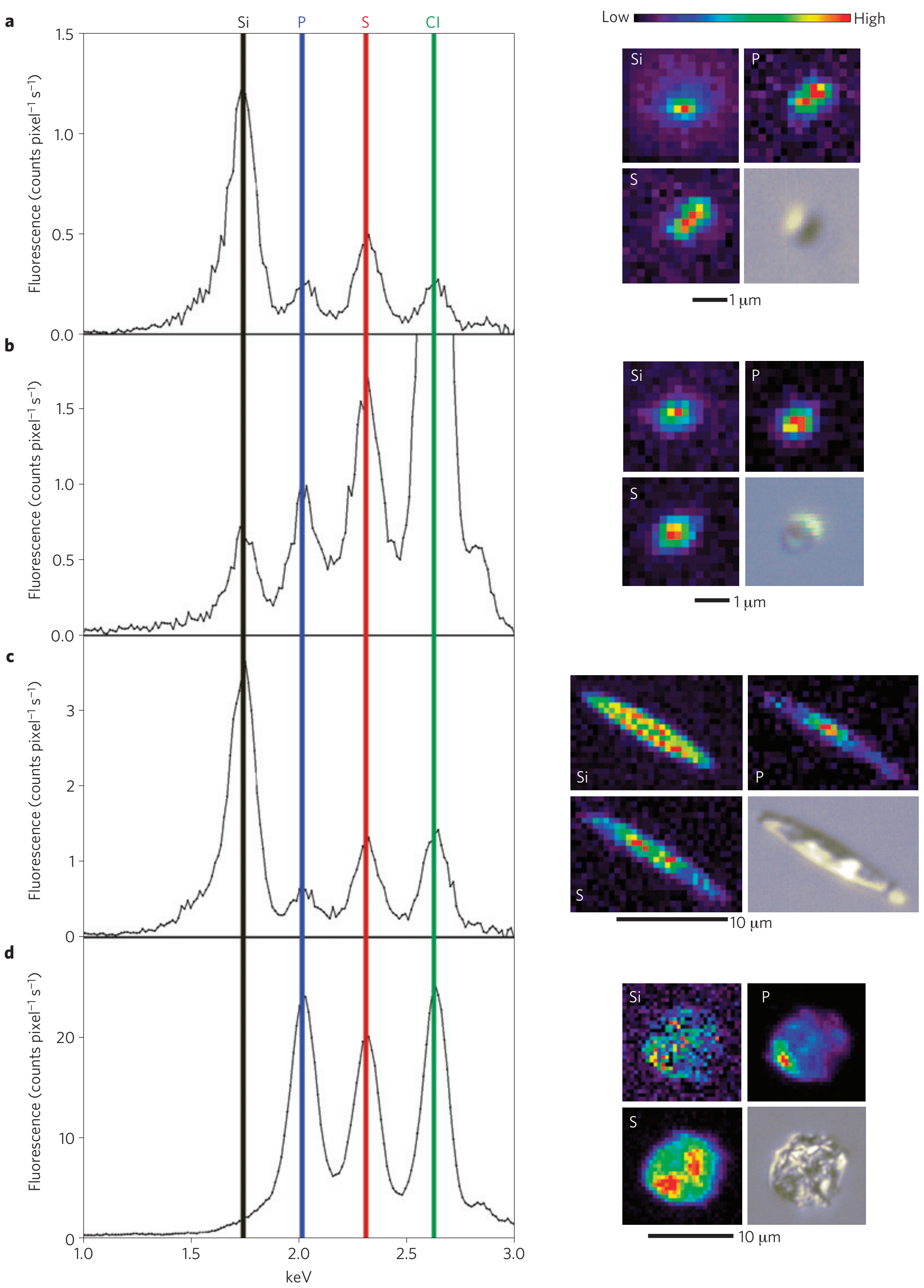

Figure 1 | Single-cell analyses of S, P and Si in organisms collected from the field. X-ray fluorescence spectra (left) and corresponding two-dimensional maps of elemental concentration and visual images (right). **a**, *Synechococcus* cell from the EEP. **b**, *Synechococcus* cell from the Sargasso Sea. **c**, Diatom from the EEP. **d**, Photosynthetic dinoflagellate from the EEP. Vertical lines represent energies of Kα emission lines for Si, P, S and Cl.

grown at [Si(OH$_4$)] ~100 μM in Aquil grew 78% slower than when grown in similar concentrations of silicic acid in f/2-amended sea water, but contained 38% more Si per cell. Finally, the culture experiments imply that Si is not a required nutrient. When CC9311 and WH7803 cells were acclimated to Aquil medium without Si added, cellular Si content was reduced to levels undetectable with either bulk digestion or SXRF techniques while growth rates were unaffected (Table 1).

The mechanism of Si accumulation by *Synechococcus* is unknown, in part because the SXRF method cannot resolve the form and precise location of the Si associated with the cell. Any credible mechanism must be able to explain a substantial concentration gradient between the cell and its environment, and a substantial concentration of Si associated with the cell. Assuming a maximum equivalent spherical diameter for *Synechococcus* of 1.5 μm, we calculate a conservative intracellular concentration in EEP cells of

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Table 1 | Si contents of field-collected and cultured cells.

Cruise	Cell type	Station/clone	*N*	Hydrography/culture conditions	Depth	Method	[Si(OH)$_4$]	Si	Si/P	Si/S
EB04	*Synechococcus*	26	14	Equatorial upwelling	S	SXRF	3.0	413 ± 69	10.1 ± 1.8	8.8 ± 1.4
	Diatoms	All	62	Equatorial upwelling	S	SXRF	1.4-4.4	59,500 ± 8,300	21.0 ± 1.6	14.5 ± 0.9
	HFlag	All	85	Equatorial upwelling	S	SXRF	1.4-4.4	1,400 ± 360	1.0 ± 0.2	0.9 ± 0.2
	AFlag	All	146	Equatorial upwelling	S	SXRF	1.4-4.4	600 ± 270	0.3 ± < 0.1	0.2 ± < 0.1
FeAST-2	*Synechococcus*	K1	7	Cyclonic eddy	S	SXRF	0.8	120 ± 15	3.8 ± 0.6	1.1 ± 0.2
	Synechococcus	K2	19	Mode-water eddy	DCM	SXRF	1.3	100 ± 17	1.7 ± 0.1	1.4 ± 0.1
	Synechococcus	K3	10	Cyclonic eddy	S	SXRF	0.8	25 ± 9	0.9 ± 0.2	0.2 ± < 0.1
	Synechococcus	K3	22	Cyclonic eddy	DCM	SXRF	0.8	46 ± 8	2.2 ± 0.3	1.4 ± 0.1
	Synechococcus	K4	10	Cyclonic eddy	S	SXRF	0.9	7 ± 3	0.2 ± < 0.1	0.1 ± < 0.1
	Synechococcus	K4	13	Cyclonic eddy	DCM	SXRF	1.1	5 ± 2	0.8 ± 0.3	0.1 ± < 0.1
	Synechococcus	K5	10	Anticyclonic eddy	S	SXRF	1.1	26 ± 9	1.2 ± 0.4	0.3 ± < 0.1
	Synechococcus	K5	27	Anticyclonic eddy	DCM	SXRF	0.8	113 ± 22	5.7 ± 1.4	1.9 ± 0.6
Culture	*Synechococcus*	WH5701	3	f/2 − Si		BSi	∼5	194 ± 21		
	Synechococcus	WH7803	3	f/2 − Si		BSi	0.7-8.0	16-32		
	Synechococcus	WH7803	16	Aquil − Si		SXRF	0.4	n.d		
	Synechococcus	CC9311	3	f/2 + Si		BSi	100	40 ± 2		
	Synechococcus	CC9311	3	Aquil+Si		BSi	100	58 ± 3		
	Synechococcus	CC9311	3	Aquil-Si		BSi	0.4	n.d.		

Cellular Si contents (amol cell^{-1}), Si/P (mol mol^{-1}) and Si/S (mol mol^{-1}) of *Synechococcus* and three protistan functional groups (diatoms, heterotrophic flagellates—HFlag, autotrophic flagellates—AFlag) measured during cruises to the equatorial Pacific Ocean (EB04), Sargasso Sea (FeAST-2) and in cultured strains. *Synechococcus* measurements are means ± standard error. *N* is the number of cells analysed in the field or number of replicate cultures used. For [Si(OH)$_4$], ranges of station means in the EEP are presented for each protistan functional group. Cellular Si for all field samples was measured with SXRF whereas all but one of the culture measurements (low-Si Aquil for WH7803) were made using a biogenic silica assay (BSi) that employs colorimetric determination of silico-molybdate after exposure of cells to hot 0.2 N NaOH. Silicic acid concentrations in the field or growth media are in units of moles per litre. Depth refers to the depth horizon sampled: S, surface mixed layer; DCM, deep chlorophyll maximum. n.d., not above detection limits.

0.45 mol l^{-1}. This minimum concentration is 200,000-fold higher than typical of dissolved Si concentrations in the equatorial Pacific and the Sargasso Sea. Moreover, the cellular concentration is 200 times the solubility for Si(OH)$_4$ in sea water[10].

A number of mechanisms can result in accumulation of Si external to the cell wall in cyanobacteria, but all are unlikely to occur under open ocean conditions. *Synechococcus* can act as a nucleating site for the formation of minerals[11] that could conceivably contain or adsorb Si under certain conditions[12]. Such mechanisms may have been responsible for the observations of Si accumulation by colonial freshwater cyanobacterium, *Microcystis*, in Rostherne Mere[4,5], which is characterized by substantially higher silicic acid concentrations (∼20 µmol l^{-1}), high and variable pH (8.5–9.5) and very high chlorophyll concentrations (>20 µg l^{-1}; ref. 4). Given lower silicic acid, pH and productivity of the open ocean sites studied here mineral formation is very unlikely to explain our observations[12]. In hot springs and stream biofilms where [Si(OH)$_4$] exceeds its solubility threshold, extensive polymerization of silica seems to be initiated and guided by initial associations of Si with surface polysaccharides comprising the cell sheath[13]. However, the presence of a substantial layer of amorphous silica that is on the cell surface and unprotected by an organic coating would be highly unlikely in the open ocean, where subsaturating conditions result in rapid dissolution of exposed amorphous silica from diatom frustules[14]. Although it is possible that Si could be bound directly or indirectly with uncharacterized organic ligands associated with the glycocalyx, no organism has yet been found that accumulates Si primarily in this form. Indeed, monomeric Si does not associate with surface ligands of the filamentous cyanobacterium *Calothrix* under subsaturated conditions even though this organism can serve as a nucleus for precipitation under saturated conditions in hot springs[15].

Known mechanisms that entail accumulation of Si internal to the cell are similarly problematic. The concentration of Si associated with *Synechococcus* cells is certainly sufficient to promote amorphous silica formation, much as occurs in diatoms and other eukaryotes. However, it is not clear how prokaryotes, without membrane-bound vacuoles that allow for manipulation of pH and accumulation of Si(OH)$_4$, could manage silica deposition intracellularly without severe consequences for their cellular biochemistry. *Bacillus cereus* sometimes deposits silica as part of the spore coat during endosporulation[6], but this process occurs within a space isolated from the cytoplasm by two invaginated layers of the cell membrane. Analogously, it is possible that silica deposition could also occur in the periplasmic space between the outer cell wall and the cell membrane, where it could serve various protective functions. Alternatively, a steep concentration gradient of dissolved Si across the cell boundary could be maintained if Si(OH)$_4$ was complexed with organic ligands or if Si was covalently bound as organo-silicon. Soluble Si concentrations within diatoms reach levels observed here for *Synechococcus*[16], presumably owing to prevention of intracellular amorphous silica formation by such ligands. However, the existence and identity of such silico-organic complexes have not been verified, much less shown to contribute greatly to Si content in any other organism.

Whatever the form or location of the cellular Si, or the mechanism of uptake, we estimate that the contribution of *Synechococcus* to particle-associated Si pools in the upper ocean can be substantial. The ratio of Si in *Synechococcus* to that in diatom cells was estimated for the EEP from cell counts and SXRF measurements. The average water column inventory of Si in *Synechococcus* during 2004 was 40% of that in diatoms whereas the inventory in 2005 was nearly twice that of diatoms (Fig. 2). We estimate that living diatoms and *Synechococcus* accounted for only 2–31% and 2–11%, respectively, of the total biogenic silica measured in the EEP. This low contribution is not surprising given that the preponderance of the total biogenic silica pool in the EEP is detrital and not associated with living cells[17]. Similar direct comparisons of Si in diatoms and *Synechococcus* are not

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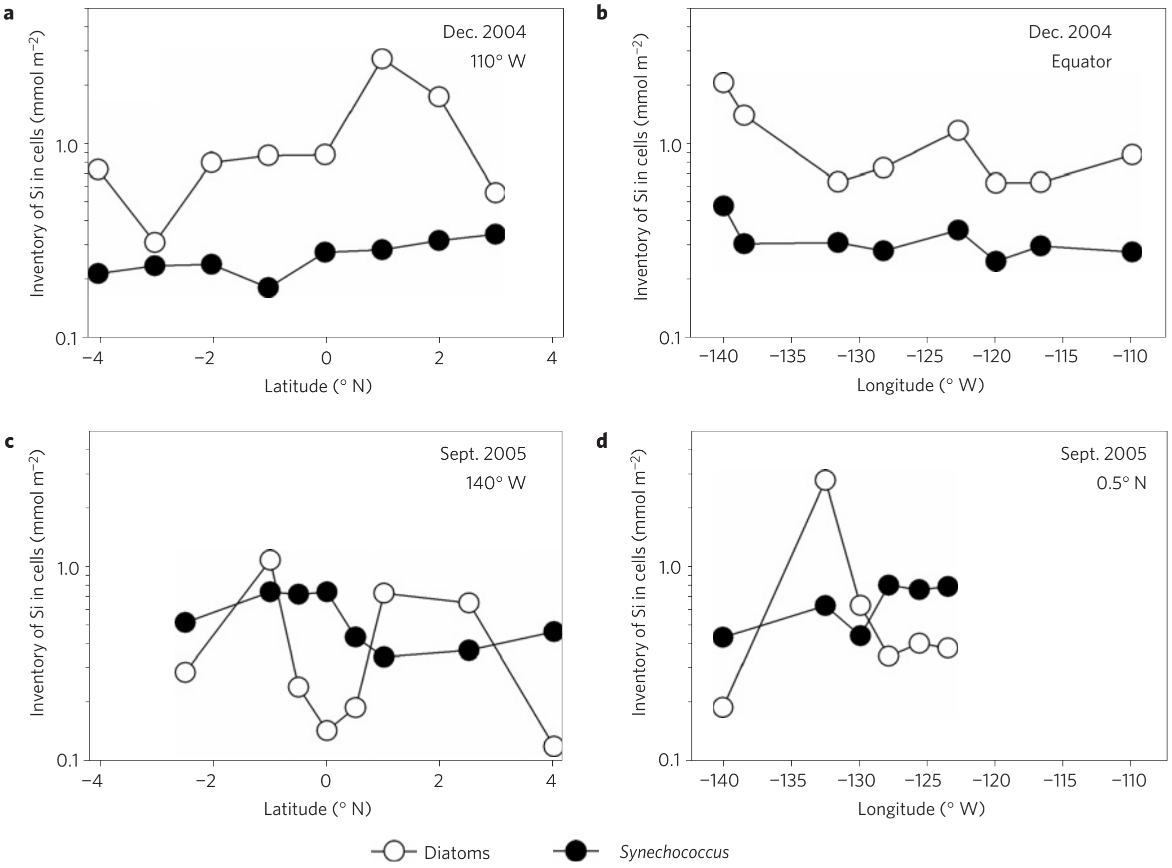

Figure 2 | Calculated inventories of Si within the euphotic zone of the EEP. Euphotic zone Si inventories for the meridional transect in December 2004 (**a**), the zonal transect along the Equator in December 2004 (**b**), the meridional transect in September 2005 (**c**) and the zonal transect along 0.5° N in September 2005 (**d**). Diatoms, open circles; *Synechococcus*, filled circles.

Table 2 | Contribution of different size fractions to particulate Si concentration ($\mathrm{nmol\ Si\ l^{-1}}$) in the Sargasso Sea.

	Total				0.4–3 μm fraction				Percentage in 0.4–3 μm fraction			
	Surface		DCM		Surface		DCM		Surface		DCM	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
Biogenic silica	22.0	3.6	22.6	6.3	4.3	2.1	3.5	1.1	20	9	16	3
Lithogenic silica	21.3	9.7	17.3	14.1	12.7	6.8	6.5	3.2	55	5	76	24
Corrected biogenic silica	18.8	3.4	20.0	4.9	2.4	1.2	2.6	0.6	14	7	14	2

Data collected from the Bermuda Atlantic Time Series site in the Sargasso Sea from 21 to 23 August 2010. Corrected biogenic silica was calculated by subtracting 15% of the lithogenic silica measurement from the corresponding biogenic silica measurements.

possible for the Sargasso Sea given the lack of available data on diatoms; however, measurements of size-fractionated biogenic silica concentrations at the surface and in the deep chlorophyll maximum at three stations sampled in August 2010 indicate that 18 ± 10% of the biogenic silica was in the 0.4–3.0 μm fraction that would contain *Synechococcus* (Table 2). This size fraction also contains significant amounts of clay particles, 1–15% of which may dissolve during the analysis of biogenic silica[18]. When biogenic silica measurements are corrected by assuming the largest possible contribution of lithogenic silica, the picoplankton size fraction still accounts for 14 ± 3% of the total biogenic silica.

The implications of our results for marine biogeochemistry are several, some of which could be profound. Interpretations of common measurements involving pools and fluxes of Si in the ocean need to acknowledge the potential contributions of *Synechococcus*. More fundamentally, recognition of Si accumulation by *Synechococcus* may also alter our understanding of the long-term controls on oceanic diatom production, Si cycling and C sequestration. At present, it is believed that the accumulation of highly silicified diatoms in the Southern Ocean exerts a substantial control on growth of diatoms elsewhere by reducing ratios of $\mathrm{Si(OH)_4/NO_3}$ within the nutrient-rich modal and intermediate waters that supply nutrients to surface waters at more northerly latitudes[19]. However, recent studies have suggested that picocyanobacteria such as *Synechococcus* contribute substantially more to C export than previously believed[20–22]. If the Si associated with Synechococcus cells is externally bound and

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rapidly exchangeable with the surrounding water, the implications of our results for export of Si and other elements may be minimal. However, if the Si associated with Synechococcus cells is in the form of dense amorphous mineral silica contained within the cell wall, the presence of Si in these cells could help explain the export attributable to picocyanobacteria by ballasting aggregates and faecal material containing these organisms or their remains. This influence could be substantially larger if the closely related and very abundant cyanobacterium *Prochlorococcus* also accumulates substantial amounts Si. This organism accounted for 2–3-fold more biomass in the EEP, but was too small to image using the methods employed here. Irrespective of the form of Si in picocyanobacteria, their high abundance and cosmopolitan distribution suggest that they exert a diffuse, but geographically widespread, influence on the marine silica cycle.

Methods

SXRF methods. Water samples from both the Sargasso Sea and the EEP were collected and processed at sea using established protocols[23]. Cells were imaged at sea using Nomarski optics and epifluorescence to identify *Synechococcus* cells on the basis of a combination of size and presence of strong autofluorescence by phycoerythrin. The presence of low Zn and high Ni contents typical of prokaryotic cells verified these designations. SXRF analyses were conducted at the 2-ID-E beamline of the Advanced Photon Source Argonne National Laboratory using a monochromatic 10 keV X-ray beam focused to ∼0.3 µm diameter spot. The sample was moved in a stepwise raster fashion in 0.15 µm increments with respect to the focused beam and full X-ray fluorescence spectra were collected for between 4 and 9 s at each position using an energy-dispersive Ge detector. Background-corrected spectra for the cells were analysed using MAPS software[24] with reference to National Institute of Standard and Technology thin-film standards (NBS 1833 and NBS 1832). Fluorescent yields for elements not contained in the standards were estimated indirectly from regressions between observed and theoretical maximum fluorescent yields[25]. SXRF detection limits are a function of various factors[26]. For the WH7803 cells analysed with SXRF, the maximum detection limit, calculated as 3× s.d. of the analysed background regions on the grid, was 11 amol Si per cell. Fourteen of the sixteen WH7803 cells analysed with SXRF had Si quotas below the detection limit.

Water column inventory estimates. Water column inventories of Si in *Synechococcus* were calculated by multiplying mean cellular Si contents (as determined by SXRF) by the number of *Synechococcus* cells integrated through the euphotic zone[27], which spanned the surface to the depth to which 0.1% of surface light penetrated (94–112 m). Si in diatom biomass was determined by first multiplying the average diatom cell volume for that station[27] by an EEP-wide average Si/vol ratio of 0.8 fg µm^{-3} (ref. 7). The resulting estimate of Si per cell was then multiplied by the euphotic zone inventory of diatom cells at each station.

Culture experiments. Three strains of *Synechococcus* that represent ecologically distinct lineages were cultured in the laboratory to determine their propensity to accumulate Si under controlled conditions. WH5701 was grown at Stony Brook at 23 °C under an irradiance of ∼90 µE in m^{-2} d^{-1} and a 14 h:10 h light/dark cycle. WH7803 was cultured at Bigelow Ocean Sciences Laboratory at 23 °C under a 12 h:12 h light/dark cycle of 14 µE in m^{-2} d^{-1} irradiance using cool white fluorescent bulbs. CC9311 was cultured at the University of California at Santa Barbara at 18–20 °C at 120 µE in m^{-2} d^{-1} under a 12 h:12 h light/dark cycle.

Cells were grown in polycarbonate flasks using either nutrient-amended sea water or artificial media. Low $Si(OH)_4$ concentrations in seawater-based media were achieved in two ways. In the experiments with WH5701, Sargasso sea water with initially low amounts of $Si(OH)_4$ (∼1 µM) was used. In the experiments with WH7803, a diatom (*Thalassiosira weissflogii*) was allowed to grow in north Atlantic sea water (collected near the Bahamas, 25° 38′N 77° 29′W), after which the diatoms were removed by filtration through a 0.2-µm filter. Across two experiments, this procedure resulted in initial $Si(OH)_4$ concentrations of 0.25 µM and 0.73 µM. For all experiments that employed seawater-based media, nutrients other than $Si(OH)_4$ were added at f/2 levels (f/2 − Si; ref. 28). In experiments that employed the artificial media Aquil (ref. 9), unamended silicic acid concentrations typically ranged between 0.3 and 0.5 µM owing to contamination from salts. In all cases, high $Si(OH)_4$ treatments were produced by adding appropriate amounts of aqueous sodium metasilicate.

Brucite particles ($MgOH_2$) that precipitate from sea water at high pH (>9.6) can scavenge silicic acid from solution, potentially leading to the false conclusion that algae are accumulating Si (ref. 12). The magnesium silicate sepiolite is unlikely to form at the pH and silicate concentrations observed *in situ*, but can form in sea water at pH > 8.6 when silicic acid concentrations are 100 µmol l^{-1} (ref. 12). To prevent formation of these minerals, cultures were sterilized by microwaving or by filtration through sterile 0.2-µm filters. Strains also were bubbled with ambient air passed through a bacterial filter to keep the pH below 8.6.

Cultures were transferred 1–2 times into fresh media before analysis of cellular Si content. Cells were collected after seven divisions but during exponential growth. Cells were removed by filtration onto 0.2-µm polycarbonate filters that were then rinsed with $Si(OH)_4$-free NaCl solution. Dissolved $Si(OH)_4$ in the media was determined by colorimetry and biogenic silica on the filters determined by the same technique after incubation of filters containing cells with hot NaOH described in ref 29, but using Teflon instead of polypropylene tubes, which provide low and stable blank values. Cells were enumerated by Coulter Counter (Multisizer III) or epifluorescence microscopy.

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Author contributions

S.B.B. and B.S.T. were responsible for the initial idea for the paper, and both were most involved in the writing and figure preparation, with substantial input from M.A.B. and J.W.K. All SXRF analyses were conducted by B.S.T. and S.B.B. S.V. provided logistics at the 2-ID-E microprobe at the Advanced Photon Source and oversaw analysis for X-ray fluorescence spectra. S.B.B., B.S.T., J.W.K. and M.A.B. all oversaw the various culture experiments. D.A. and H.M. were both involved in designing, conducting and analysing the culture experiments. J.W.K. and M.A.B. provided analyses of biogenic and lithogenic silica for the Sargasso samples that were collected by B.S.T.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.B.B.

Competing financial interests

The authors declare no competing financial interests.

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