

Two distinct pools of B₁₂ analogs reveal community interdependencies in the ocean

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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved November 28, 2016 (received for review May 25, 2016)

Organisms within all domains of life require the cofactor cobalamin (vitamin B₁₂), which is produced only by a subset of bacteria and archaea. On the basis of genomic analyses, cobalamin biosynthesis in marine systems has been inferred in three main groups: select heterotrophic Proteobacteria, chemoautotrophic Thaumarchaeota, and photoautotrophic Cyanobacteria. Culture work demonstrates that many Cyanobacteria do not synthesize cobalamin but rather produce pseudocobalamin, challenging the connection between the occurrence of cobalamin biosynthesis genes and production of the compound in marine ecosystems. Here we show that cobalamin and pseudocobalamin coexist in the surface ocean, have distinct microbial sources, and support different enzymatic demands. Even in the presence of cobalamin, Cyanobacteria synthesize pseudocobalamin—likely reflecting their retention of an oxygen-independent pathway to produce pseudocobalamin, which is used as a cofactor in their specialized methionine synthase (MetH). This contrasts a model diatom, *Thalassiosira pseudonana*, which transported pseudocobalamin into the cell but was unable to use pseudocobalamin in its homolog of MetH. Our genomic and culture analyses showed that marine Thaumarchaeota and select heterotrophic bacteria produce cobalamin. This indicates that cobalamin in the surface ocean is a result of de novo synthesis by heterotrophic bacteria or via modification of closely related compounds like cyanobacterially produced pseudocobalamin. Deeper in the water column, our study implicates Thaumarchaeota as major producers of cobalamin based on genomic potential, cobalamin cell quotas, and abundance. Together, these findings establish the distinctive roles played by abundant prokaryotes in cobalamin-based microbial interdependencies that sustain community structure and function in the ocean.

B₁₂ | cobalamin | Thaumarchaeota | pseudocobalamin | Cyanobacteria

Cobalamin (vitamin B₁₂) is synthesized by a select subset of bacteria and archaea, yet organisms across all domains of life require it (1–3). In the surface ocean, cobalamin auxotrophs (including most eukaryotic algae) (3) obtain the vitamin through direct interactions with cobalamin producers (3) or breakdown of cobalamin-containing cells (4, 5). Interdependencies between marine cobalamin producers and consumers are critical in surface waters where primary productivity can be limited by the availability of cobalamin and the compound is short-lived (1, 6, 7). The exchange of cobalamin in return for organic compounds is hypothesized to underpin mutualistic interactions between heterotrophic bacteria and autotrophic algae (3, 6, 8, 9). The apparent pervasiveness of cobalamin biosynthesis genes in chemoautotrophic Thaumarchaeota and photoautotrophic Cyanobacteria genomes (1, 10, 11) raises the question of whether cobalamin production by these autotrophs may underlie additional, unexplored microbial interactions.

Cobalamin is a complex molecule with a central cobalt-containing corrin ring, an α ligand of 5,6-dimethylbenzimidazole (DMB), and a β ligand of either OH-, CN-, Me-, or Ado- (12) (Fig. 1). Previous studies have shown that instead of producing cobalamin, Cyanobacteria produce pseudocobalamin (11, 13, 14), an analog of

cobalamin in which adenine substitutes for DMB as the α ligand (12) (Fig. 1). Production of pseudocobalamin in a natural marine environment has not been shown, nor have reasons for the production of this compound in place of cobalamin been elucidated.

To explore the pervasiveness of cobalamin and pseudocobalamin supply and demand in marine systems, we determined the standing stocks of these compounds in microbial communities from surface waters across the North Pacific Ocean using liquid chromatography mass spectrometry (LC-MS). Our LC-MS method (15) quantifies cobalamin and pseudocobalamin with different β ligands. We found that in the surface ocean, pseudocobalamin and cobalamin concentrations associated with organisms and detritus captured on a 0.2- μ m filter (particulate fraction) were often of equal magnitude (Fig. 2B). Pseudocobalamin had peak concentrations within the euphotic zone at each station and was not detected below the euphotic zone. In contrast, cobalamin was measurable throughout the sampled waters and maintained similar or higher concentration from the lower euphotic zone to our deepest samples (Fig. 2A and Fig. S1).

The overlapping spatial distribution of cobalamin and pseudocobalamin suggests that these cofactors are produced in each other's presence, likely with different sources and sinks. To investigate correlations between Cyanobacteria and pseudocobalamin abundance, we compared observations of Cyanobacteria carbon inferred from

Significance

Cobalamin (vitamin B₁₂)-dependent organisms span all domains of life, making procurement of the vitamin from the few prokaryotic producers an essential function in organismal interactions. Yet not all key producers of cobalamin have been identified in the ocean. We show that in the marine environment, select heterotrophic bacteria and Thaumarchaeota produce cobalamin, while Cyanobacteria, the most abundant phytoplankton on earth, supply and use pseudocobalamin. These chemically distinct cofactors support different members of the microbial community because they are not interchangeable as cofactors in enzymes. Our findings identify key organisms supporting cobalamin-based interdependencies that underpin primary production and microbial interactions in the ocean.

Author contributions: K.R.H., J.W.M., A.H.D., E.V.A., D.A.S., and A.E.I. designed research; K.R.H., W.Q., F.R., W.C.-M., and L.R.H. performed research; K.R.H. contributed new reagents/analytic tools; K.R.H., W.Q., F.R., and A.D.B. analyzed data; and K.R.H. and A.E.I. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the European Nucleotide Archive [accession no. PRJEB10943 (ERP012248) (project title "Variable Influence of light on the activity of Thaumarchaea")].

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1608462114/-DCSupplemental.

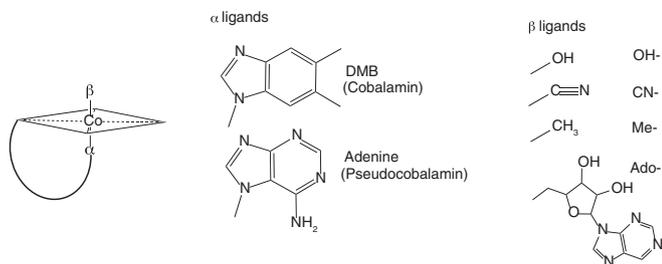


Fig. 1. General form of cobalamin analogs. Shown is a schematic of the conserved corrin ring with various upper (β) and lower (α) ligands. Structures of cobalamin analogs monitored in this study (eight total) are shown.

flow cytometry with pseudocobalamin measurements taken at the same depth. In cases where we had both continuous measurements (by SeaFlow) (16) and discrete flow cytometry data, we used the discrete measurements, as collection of these samples was closely coupled in time and space to pseudocobalamin sampling ($n = 16$ for discrete, $n = 4$ for continuous). Pseudocobalamin concentrations are statistically correlated with carbon from *Synechococcus* and *Prochlorococcus* ($R^2 = 0.71$, $P < 0.001$), both in the surface and into the subsurface ocean (Fig. 2C), suggesting a primarily cyanobacterial source. No significant correlation existed

between Cyanobacteria carbon and cobalamin concentrations (Fig. S2).

To identify the major producers of cobalamin and pseudocobalamin in the environment, we investigated representative marine isolates and then expanded our search into available genomes that encompass the phylogenetic diversity at our study site. As expected (1, 8), two strains of marine Alphaproteobacteria with cobalamin synthesis genes (*Sulfitobacter* sp. SA11 and *Ruegeria pomeroyi* DSS-3) produced cobalamin, whereas the gammaproteobacterium *Vibrio fischeri* ES114 (which lacks cobalamin biosynthesis genes) did not (Table S1). Four pure strains of marine chemoautotrophic Thaumarchaeota (*Nitrosopumilus* spp. SCM1, HCE1, HCA1, and PS0) also produced cobalamin (Table S1), confirming earlier suggestions based on the presence of cobalamin biosynthesis genes in Thaumarchaeota genomes (10). Like other Cyanobacteria (11, 13, 14), four axenic strains of marine Cyanobacteria (*Prochlorococcus* MED4 and MIT9313 and *Synechococcus* WH8102 and WH7803) produced pseudocobalamin (Table S1). In all of the cobalamin or pseudocobalamin producers, we detected compounds with β ligands Me-, Ado-, and OH- but not CN- (Table S1).

The observed cell quotas of cobalamin or pseudocobalamin per cellular carbon varied greatly among producers (Table S1). Laboratory cultures of Alphaproteobacteria and *Prochlorococcus* strains had lower amounts of cobalamin or pseudocobalamin (less than 1,200 nmol cobalamin analog per mole carbon) than *Synechococcus*

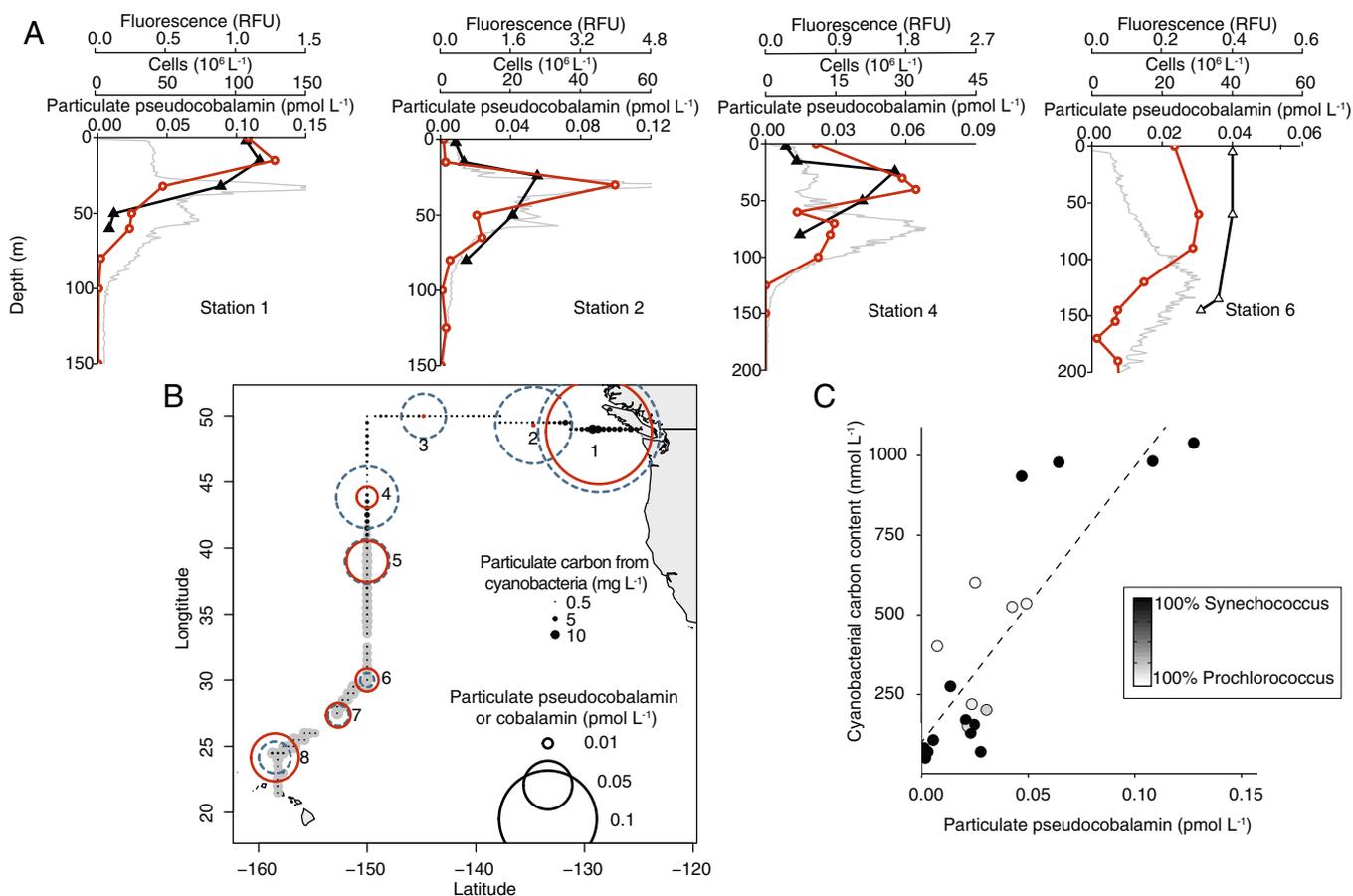


Fig. 2. Pseudocobalmin and Cyanobacteria cooccurrence. (A) Depth profiles of particulate pseudocobalamin (orange circles), in situ chlorophyll a (gray), and cell abundance of *Synechococcus* (closed triangles) or *Prochlorococcus* (open triangles), whichever was the dominant Cyanobacteria at each station. (B) Location of sampling with surface concentration of particulate cobalamin (dashed blue circles) and pseudocobalamin (orange circles). Closed circles are cyanobacterial (*Synechococcus*, black; *Prochlorococcus*, gray) carbon content calculated from cell abundance and size estimates (at 5 m via SeaFlow) (16). (C) Correlation between environmental pseudocobalamin and calculated Cyanobacteria carbon content ($n = 20$, $R^2 = 0.71$, $P < 0.001$). Cobalamin and pseudocobalamin concentrations are summed values of the detected β ligands for these compounds (Ado-, Me-, and OH- for cobalamin; Me- and OH- for pseudocobalamin); contributions of each beta ligand are provided in Dataset S2. Pseudocobalamin concentrations are presented in cobalamin equivalents (see SI Materials and Methods).

and Thaumarchaeota isolates (1,480–11,600 nmol cobalamin analog per mole carbon). Published values (17) for sea ice bacterial isolates estimated using a bioassay were highly variable (0.6–6,800 nmol cobalamin analog per mole carbon). In our environmental samples, we observed an average stoichiometry of 87 nmol pseudocobalamin per mole cyanobacterial carbon, lower than the cyanobacterial isolates (Fig. 2C). This finding suggests that the cellular stoichiometry of pseudocobalamin is variable and possibly influenced by environmental factors like nutrient availability and growth rate.

To expand the breadth of our survey beyond laboratory isolates, we inspected publically available whole genome sequences from bacteria and Thaumarchaeota for evidence of cobalamin biosynthesis. This analysis expands on previous work (18) while focusing on the phylogenetic groups present at our study site. We analyzed full genomes from the Integrated Microbial Genomes (IMG) database (<https://img.jgi.doe.gov>) from phylogenetic groups that encompassed >99.9% of the Bacterial 16S rRNA gene sequences from our environmental samples to develop a systematic inference of cobalamin synthesis capacity (3,410 genomes). Alpha- and Gammaproteobacteria are hypothesized to be major marine cobalamin producers (1, 10), and 94% of the surveyed genomes from these groups that contain genes necessary for corrin biosynthesis (i.e., *cbiA/cobB*, *cbiH/cobJ*) (2) also have genes for DMB synthesis and activation (*bluB*, *cobT*) (2, 19, 20) (Fig. 3), consistent with the synthesis of cobalamin and the results from our representative cultures. All of the eight available high-quality Thaumarchaeota genomes in the IMG database code for corrin and DMB biosynthesis genes (Fig. 3). Most of the lower quality, incomplete genomes available follow this same pattern (17/19, Dataset S1). No Thaumarchaeota genomes possess the *cobT* gene and thus must activate DMB through a different pathway. Of the 255 cyanobacterial whole genomes, 247 possessed genes for the synthesis of the corrin ring, but only one genome possessed an annotated *bluB* or *cobT* gene (Fig. 3), suggesting the vast majority of Cyanobacteria are unable to produce DMB, in agreement with a recent study that examined a subset of the available Cyanobacteria genomes (11).

All of the 49 *Prochlorococcus* genomes have genes for corrin synthesis without genes for DMB synthesis or activation, and our analysis demonstrated that *Prochlorococcus* MED4, with its highly streamlined genome (21), has maintained these genes to synthesize pseudocobalamin. We propose this originates from an ancient specialization to the production and use of pseudocobalamin in lieu of cobalamin among Cyanobacteria. Biosynthesis of the corrin ring can occur via two separate pathways: an O₂-dependent or an O₂-independent pathway (2, 18). DMB synthesis can also occur via two separate routes, the O₂-dependent BluB (19) or the O₂-independent and O₂-sensitive Bza pathway (22). Rhodobacters have the O₂-dependent corrin ring and DMB pathways (11), whereas Thaumarchaeota likely possess the O₂-independent pathway for the corrin ring and the O₂-dependent DMB pathway (10). In some bacteria, pseudocobalamin can be produced if DMB synthesis is impaired; this is due to the natural presence of adenine in cells and enzyme substrate promiscuity that allows adenine to substitute for DMB in some organisms' CobT (22–27). Cyanobacteria use the O₂-independent pathway for corrin ring synthesis and neither pathway for DMB synthesis (11, 18) (Fig. 3). The use and production of cobalamin as a cofactor predates oxygenic photosynthesis (28, 29). Possessing an O₂-independent mode for producing a cobalamin analog that is not impaired by O₂ may have been necessary for the success of oxygenic photosynthetic Cyanobacteria that were largely responsible for the rise of O₂ on earth and likely endured variable O₂ concentrations over time (30).

Cyanobacteria use pseudocobalamin as a cofactor in two enzymes: methionine synthase (MetH) and class II ribonucleotide reductase (NrdJ). The 3D structure of MetH contains three β pleated sheets and two α helices that form a pocket for the DMB ligand of cobalamin in *Escherichia coli* (31, 32). Cyanobacterial MetH is predicted to form the same pocket (13). However, conserved amino acids within this pocket in the cyanobacterial MetH differ from sequences of organisms known to use cobalamin (Figs. S3 and S4), suggesting a structure that preferentially binds pseudocobalamin in place of cobalamin as experimentally demonstrated in the Cyanobacteria *Arthrospira* (13). Many Cyanobacteria also code for O₂-independent NrdJ (33), which has limited sequence similarity to noncyanobacterial NrdJ (34, 35). Similar to pseudocobalamin biosynthesis in Cyanobacteria, NrdJ is both O₂-independent and O₂-insensitive and has been hypothesized as an important bridge between the pre- and postoxygated oceans (36). The continued maintenance of both the biosynthetic pathway and pseudocobalamin-dependent enzymes throughout the diverse Cyanobacteria phylum (from *Arthrospira* and *Synechocystis* to the highly streamlined *Prochlorococcus*) suggests the production of pseudocobalamin is an ancient relic that persists in the oxic marine environment today.

For many eukaryotic algae, pseudocobalamin supports lower growth yields than cobalamin (11, 37, 38). We examined the underlying cause of this reduced growth by supplying the model diatom *Thalassiosira pseudonana* (CCMP 1335) with pseudocobalamin and tracking it into the cells. Like others, we found that growth of *T. pseudonana* is limited at 1 pM cobalamin (39), and the addition of pseudocobalamin (at 200 pM) is unable to overcome this limitation (11, 38). We observed that *T. pseudonana* takes up the inactive OH-pseudocobalamin from their growth media and converts it into the cofactor form Ado-pseudocobalamin yet is unable to recover to cobalamin-replete growth rates (Fig. 4, Fig. S5, and Table S2). The role of Ado-cobalamin in diatoms is unclear, although *T. pseudonana* does code for Ado-cobalamin-dependent methylmalonyl-CoA mutase (MCM) and actively transcribes a putative adenosylcobalamin transferase (which converts OH-cobalamin to Ado-cobalamin) under cobalamin limitation (39). Previous studies suggest that when diatoms are starved for cobalamin, low Me-cobalamin (required for MetH activity) deprives cells of S-adenosylmethionine (SAM) (7, 39). We found that when pseudocobalamin is supplied to cobalamin-limited *T. pseudonana*, they contain significantly less SAM per cell than cobalamin-replete conditions (Fig. 4, Fig. S5, and Table S2). The depressed levels of SAM and lack of detectable Me-pseudocobalamin within cells suggest that *T. pseudonana*

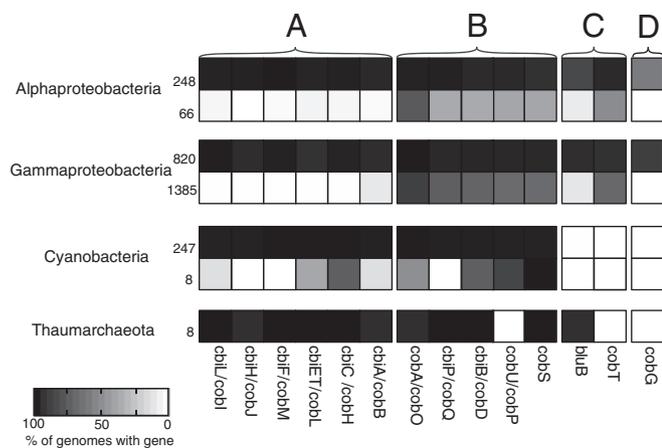


Fig. 3. Presence or absence of annotated cobalamin biosynthesis genes in full-genome representatives. Four major groups of microbes contributing cobalamin biosynthesis genes in marine surface waters (10) are shown; each group is split into potential cobalamin producers (top row) and non-cobalamin producers (bottom row), with the number of genomes in each group. All of the high-quality Thaumarchaeota genomes are potential cobalamin producers. The shade of each box indicates the percent of genomes with that gene. Genes are grouped as follows: (A) corrin ring biosynthesis genes that are common to both O₂-dependent and -independent pathways in bacteria and archaea, (B) final synthesis and repair genes, (C) genes for DMB synthesis and activation, and (D) O₂-dependent *cobG* in the O₂-dependent corrin ring biosynthesis pathway. In Archaea, *cobY* is used in place of *cobU/cobP* (10, 55). For the list of genomes in each group, see Dataset S1, summarized in Table S1.

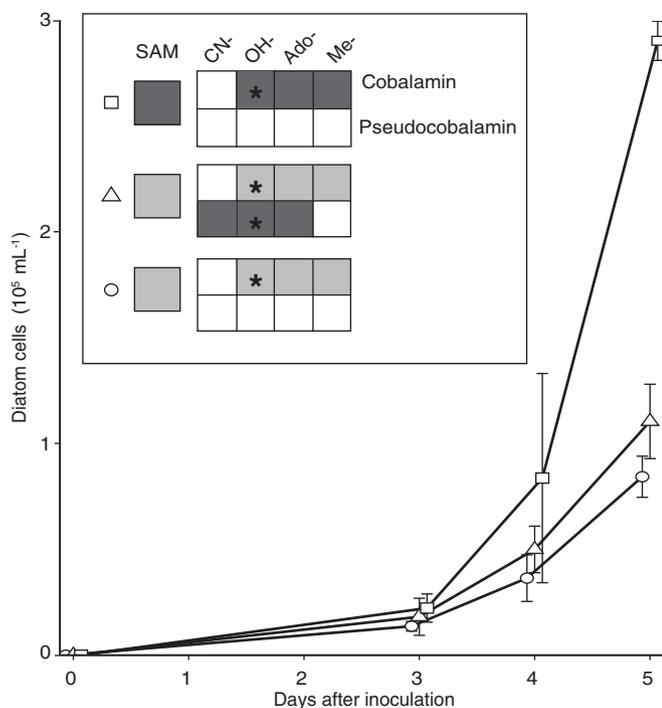


Fig. 4. Growth of the diatom *T. pseudonana* with pseudocobalamin. (A) Growth of diatoms under Control (1 pM cobalamin, circle), +Pseudocobalamin (1 pM cobalamin with 200 pM pseudocobalamin, triangle), and +Cobalamin (200 pM cobalamin, square) treatments over time; error bars, SD from three replicates. *Inset* shows the final SAM, cobalamin, and pseudocobalamin cellular contents compared with Control treatment on a per cell basis: not detected (white), similar range to control (light gray), or statistically higher than control (Student's unpaired *t* test, $P < 0.05$, dark gray). Black stars indicate what forms of cobalamin or pseudocobalamin were added to each treatment. See Fig. S5 for absolute values of cobalamins and SAM.

are unable to efficiently use pseudocobalamin in their MetH. This same phenomenon has been demonstrated physiologically in marine cobalamin-dependent bacteria: Cobalamin or methionine stimulates growth, but pseudocobalamin does not (40). These combined findings demonstrate that marine Cyanobacteria achieve both independence and a competitive advantage by producing and requiring only pseudocobalamin; they meet their own need for their preferred cofactor while also not directly supplying cobalamin to other photoautotrophs.

Corrin synthesis genes in oligotrophic surface water metagenomes are dominated by pseudocobalamin-producing Cyanobacteria (10), suggesting that de novo production of true cobalamin in these regions is limited to a small subset of heterotrophic bacteria, including clades like Rhodobacterales that are commonly associated with eukaryotic algae (9). Cobalamin auxotrophs may foster close physical or chemical relationships with these cobalamin producers, offsetting the expense of cobalamin biosynthesis to secure a consistent cobalamin source. Alternatively, organisms may employ techniques to use closely related compounds like pseudocobalamin. Many organisms without the biosynthetic capacity for de novo production of cobalamin have salvage or remodeling strategies for reactivating cobalamin analogs (11, 20, 41). A recent study has shown that some eukaryotic algae have the genetic capacity to make cobalamin when supplied with pseudocobalamin and DMB, although this phenomenon may be limited by low DMB concentrations in natural seawater (11), and DMB biosynthesis seems to be limited to bacteria and archaea. Of the 3,408 phylogenetically representative prokaryote genomes we surveyed, we found 73 genomes that did not have the biosynthetic pathway for the corrin moiety of cobalamin but did have genetic capacity for cobalamin repair as well as synthesis and

activation of DMB, which has no known role in cells beyond α ligand of cobalamin. These organisms include several heterotrophic bacteria known to occur in marine environments such as *Methylophaga* and *Marinobacter* (Dataset S1). Previous work has suggested that ligand-bound cobalt in low latitudes may be cobalamin degradation or precursor compounds, which are present at concentrations much higher than has been observed for dissolved cobalamin (1, 15, 42). In these environments, organisms capable of salvaging and repairing cobalamin degradation products or precursors could be major suppliers of cobalamin to the environment and de novo biosynthesis may take a minor role. The prevalence of cobalamin salvage pathway transcripts in marine systems (6) suggests that synthesizing DMB or remodeling cobalamin is a common strategy that plays an important role connecting the production of pseudocobalamin to the growth of cobalamin auxotrophs in the low latitude oceans where Cyanobacteria are abundant.

We inspected genomes of marine prokaryotes to identify likely cobalamin producers in our study site. We combined bacterial 16S rRNA gene sequence-derived phylogenetic data with current knowledge of corrin biosynthesis genes (including our analysis)

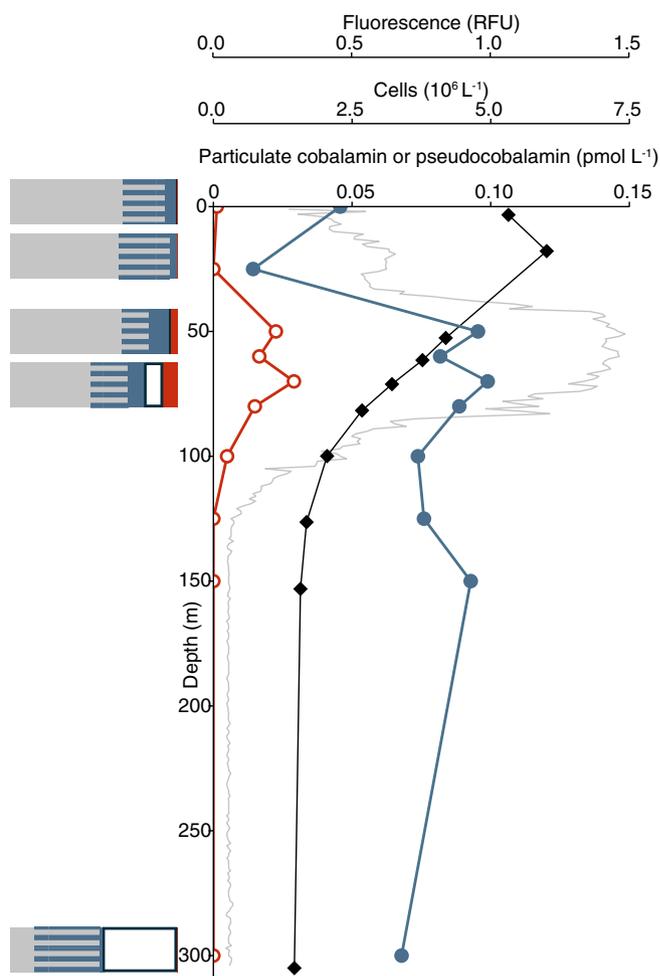


Fig. 5. Pseudocobalamin and cobalamin producers at station 3. Shown are particulate cobalamin (closed blue circles), pseudocobalamin (orange open circles), prokaryote cell abundance (black diamonds), and in situ fluorescence (gray). Bar graphs show prokaryote population with the following predicted cobalamin strategies: unlikely producers (gray), bacteria with unknown cobalamin biosynthesis capacity (gray/blue striped), bacteria that are likely to produce cobalamin (blue), Thaumarchaeota (likely to produce cobalamin, white), and Cyanobacteria (likely to produce pseudocobalamin, orange) at the corresponding depths. Cobalamin and pseudocobalamin concentrations are as in Fig. 2.

across different phylogenetic groups to infer the cobalamin biosynthetic capacity of organisms in the microbial communities at our study site as likely, unknown, or unlikely (Table S3). We quantified Thaumarchaeota by quantitative PCR (qPCR) (43, 44) and calculated their contribution to the microbial population by comparing this value to direct counts of prokaryotic cells determined for each sample. Our analysis at five targeted locations in the North Pacific suggested that Thaumarchaeota represent 30–80% of prokaryotes having likely or unknown genetic capacity to synthesize cobalamin in the lower euphotic zone and deeper (Fig. 5 and Fig. S1). High cobalamin contents on a per carbon basis in cultured Thaumarchaeota implicate them as a major source of cobalamin in deeper waters (Table S1).

Like *Prochlorococcus*, marine Thaumarchaeota have maintained several genes committed to the biosynthesis of cobalamin in their small genomes. Detection of MCM, NrdJ, BluB, and cobalamin biosynthesis proteins in the proteome of an oceanic thaumarchaeote with a highly streamlined genome, “*Candidatus Nitrosopelagicus brevis*,” implies that cobalamin is actively produced and used in these microbes (45). In Thaumarchaeota, the cobalamin-dependent MCM catalyzes a key step in their exceptionally energy-efficient pathway for carbon fixation (46–48). The scarcity of dissolved cobalamin in the water column (often <1 pM as assessed by bioassay) (1) and enzymatic demands like MCM may have necessitated that Thaumarchaeota retain the ability to synthesize cobalamin. Thaumarchaeota likely play a critical role in the microbial loop in the lower euphotic zone and deeper by providing an essential nutrient to cobalamin auxotrophs. In turn, the auxotrophs provide substrates that promote Thaumarchaeota growth (49, 50)—most critically the ammonia required by the ammonia-oxidizing Thaumarchaeota (46).

Although Thaumarchaeota and select heterotrophic bacteria synthesize cobalamin, undoubtedly benefiting from being their own source of their preferred cofactor, the production of dissimilar cobalamin analogs by marine Cyanobacteria is likely a result of their distinct ecological niches, enzymatic demands, and interactions with other cobalamin-dependent organisms. Producers of cobalamin and related compounds thus play distinct roles in cobalamin-based microbial interdependencies that sustain primary productivity and shape marine community structure.

Materials and Methods

Environmental samples for cobalamin and pseudocobalamin, phytoplankton abundance, archaeal gene quantification, prokaryotic cell abundance, and DNA for 16S rRNA sequencing were collected in August 2013 along the historical transect Line P to ocean station papa (our station 3), then following

150 W to the south into the North Pacific subtropical gyre sampling from the surface down to a maximum depth of 300 m, as shown in Fig. 2. Culture and environmental samples were analyzed for cobalamins, pseudocobalamins, and SAM using an organic solvent extraction (51) paired with LC-MS (15), both modified as described in *SI Materials and Methods* (Figs. S6 and S7 and Table S4). Phytoplankton abundance was monitored continuously using SeaFlow (16), in addition to discrete samples taken at depth and analyzed by flow cytometry.

We investigated 11 axenic strains of marine prokaryotes for demonstrable evidence of cobalamin or pseudocobalamin production: four strains of *Nitrosopumilus* spp. (SCM1, HCA1, HCE1, and P50), two strains of *Prochlorococcus* (MED4 and MIT9313), two strains of *Synechococcus* (WH7803 and WH8102), *Sulfitobacter* sp. SA11 (52), *R. pomeroyi* DSS-3, and *V. fischerii* ES114. We used the model diatom *T. pseudonana* to investigate the fate of pseudocobalamin in eukaryotic algae by culturing it under three conditions: cobalamin limited, cobalamin replete, and cobalamin limited with pseudocobalamin. To compare cobalamin- and pseudocobalamin-binding sites in MetH, we gathered MetH amino acid sequences from organisms known to use true cobalamin or pseudocobalamin as their cofactor. We then aligned the sequences and used a known crystal structure (32, 53) to visualize the binding pocket.

We used publically available full genomes from the IMG database that phylogenetically represent organisms at our study site and searched for cobalamin biosynthesis genes in these genomes (genomes and functions we used are listed in Dataset S1). We quantified Thaumarchaeota via qPCR and performed direct cell counts to quantify total prokaryotes as previously described (43, 44, 54). DNA for 16S rRNA sequencing was extracted, amplified, and sequenced as described in *SI Materials and Methods*. Operational taxonomic units (OTUs) were called using a 97% nucleotide identity threshold, and taxonomic inference was based on the SILVA rRNA gene database (<https://www.arb-silva.de>). This yielded phylogenetic information we combined with the current knowledge of cobalamin-biosynthesis capacity (from the literature and our whole genome analysis) to estimate the contribution of Thaumarchaeota to the prokaryotic community with the potential for cobalamin biosynthesis capacity at our sampling sites. Further details on all aspects of the methods are given in *SI Materials and Methods*. Environmental data are supplied in Dataset S2.

ACKNOWLEDGMENTS. We thank L. T. Carlson, R. Lionheart, A. Weid, R. Morales, and D. Rico for laboratory assistance; L. T. Carlson, D. French, R. Horak, A. Nelson, S. Amin, H. van Tol, and the captain and crew of the *R/V Kilo Moana* for sampling support; S. Chisholm, J. Becker, and S. Biller for axenic *Prochlorococcus* cells; and B. Durham for axenic *Synechococcus* cells. This work was supported by National Science Foundation (NSF) Awards OCE1228770 (to A.E.I. and D.A.S.) and OCE1046017 (to A.E.I., E.V.A., D.A.S., A.H.D., and J.W.M.). This work was supported by the Simons Foundation SCOPE Award ID 329108 (to A.E.I., E.V.A., and S.C.) and NSF Research Experience for Undergraduates Award OCE-1046017AM004 (to W.C.M.). K.R.H. was partially supported by an NSF Graduate Research Fellowship Program and University of Washington's Program on Climate Change graduate student fellowship.

- Sañudo-Wilhelmy SA, Gómez-Consarnau L, Suffridge C, Webb EA (2014) The role of B vitamins in marine biogeochemistry. *Annu Rev Mar Sci* 6(1):339–367.
- Warren MJ, Raux E, Schubert HL, Escalante-Semerena JC (2002) The biosynthesis of adenosylcobalamin (vitamin B₁₂). *Nat Prod Rep* 19(4):390–412.
- Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B₁₂ through a symbiotic relationship with bacteria. *Nature* 438(7064):90–93.
- Droop MR (2007) Vitamins, phytoplankton and bacteria: Symbiosis or scavenging? *Plankton Res* 29(2):107–113.
- Azam F (1998) Microbial control of oceanic carbon flux: The plot thickens. *Science* 280(5364):694–696.
- Bertrand EM, et al. (2015) Phytoplankton-bacterial interactions mediate micro-nutrient colimitation at the coastal Antarctic sea ice edge. *Proc Natl Acad Sci USA* 112(32):9938–9943.
- Bertrand EM, Allen AE (2012) Influence of vitamin B auxotrophy on nitrogen metabolism in eukaryotic phytoplankton. *Front Microbiol* 3:375.
- Durham BP, et al. (2015) Cryptic carbon and sulfur cycling between surface ocean plankton. *Proc Natl Acad Sci USA* 112(2):453–457.
- Cooper MB, Smith AG (2015) Exploring mutualistic interactions between microalgae and bacteria in the omics age. *Curr Opin Plant Biol* 26:147–153.
- Doxey AC, Kurtz DA, Lynch MDJ, Sauder LA, Neufeld JD (2015) Aquatic metagenomes implicate Thaumarchaeota in global cobalamin production. *ISME J* 9(2):461–471.
- Helliwell KE, et al. (2016) Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B₁₂. *Curr Biol* 26(8):999–1008.
- Renz P (1999) Biosynthesis of the 5, 6-dimethylbenzimidazole moiety of cobalamin and of the other bases found in natural corrinoids. *Chemistry and Biochemistry of B₁₂*, ed Banerjee R (John Wiley and Sons, New York), pp 557–575.
- Tanioka Y, et al. (2010) Methyladeninylcobamide functions as the cofactor of methionine synthase in a Cyanobacterium, *Spirulina platensis* NIES-39. *FEBS Lett* 584(14):3223–3226.
- Tanioka Y, et al. (2009) Occurrence of pseudovitamin B₁₂ and its possible function as the cofactor of cobalamin-dependent methionine synthase in a cyanobacterium *Synechocystis* sp. PCC6803. *J Nutr Sci Vitaminol (Tokyo)* 55(6):518–521.
- Heal KR, et al. (2014) Determination of four forms of vitamin B₁₂ and other B vitamins in seawater by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 28(22):2398–2404.
- Swalwell JE, Ribalet F, Armbrust E (2011) SeaFlow: A novel underway flow-cytometer for continuous observations of phytoplankton in the ocean. *Limnol Oceanogr Methods* 9(10):466–477.
- Taylor GT, Sullivan CW (2008) Vitamin B₁₂ and cobalt cycling among diatoms and bacteria in Antarctic sea ice microbial communities. *Limnol Oceanogr* 53(5):1862–1877.
- Rodionov DA, Vitreschak AG, Mironov AA, Gelfand MS (2003) Comparative genomics of the vitamin B₁₂ metabolism and regulation in prokaryotes. *J Biol Chem* 278(42):41148–41159.
- Taga ME, Larsen NA, Howard-Jones AR, Walsh CT, Walker GC (2007) BluB cannibalizes flavin to form the lower ligand of vitamin B₁₂. *Nature* 446(7134):449–453.
- Escalante-Semerena JC (2007) Conversion of cobinamide into adenosylcobamide in bacteria and archaea. *J Bacteriol* 189(13):4555–4560.
- Partensky F, Garczarek L (2010) *Prochlorococcus*: Advantages and limits of minimalism. *Annu Rev Mar Sci* 2:305–331.
- Hazra AB, et al. (2015) Anaerobic biosynthesis of the lower ligand of vitamin B₁₂. *Proc Natl Acad Sci USA* 112(34):10792–10797.
- Crofts TS, Seth EC, Hazra AB, Taga ME (2013) Cobamide structure depends on both lower ligand availability and CobT substrate specificity. *Chem Biol* 20(10):1265–1274.

24. Cheong C-G, Escalante-Semerena JC, Rayment I (2001) Structural investigation of the biosynthesis of alternative lower ligands for cobamides by nicotinate mononucleotide: 5,6-dimethylbenzimidazole phosphoribosyltransferase from *Salmonella enterica*. *J Biol Chem* 276(40):37612–37620.
25. Cheong C-G, Escalante-Semerena JC, Rayment I (1999) The three-dimensional structures of nicotinate mononucleotide:5,6-dimethylbenzimidazole phosphoribosyltransferase (CobT) from *Salmonella typhimurium* complexed with 5,6-dimethylbenzimidazole and its reaction products determined to 1.9 Å resolution. *Biochemistry* 38(49):16125–16135.
26. Anderson PJ, et al. (2008) One pathway can incorporate either adenine or dimethylbenzimidazole as an α -axial ligand of B₁₂ cofactors in *Salmonella enterica*. *J Bacteriol* 190(4):1160–1171.
27. Keck B, Renz P (2000) *Salmonella typhimurium* forms adenylcobamide and 2-methyladenylcobamide, but no detectable cobalamin during strictly anaerobic growth. *Arch Microbiol* 173(1):76–77.
28. Benner SA, Ellington AD, Tauer A (1989) Modern metabolism as a palimpsest of the RNA world. *Proc Natl Acad Sci USA* 86(18):7054–7058.
29. Lazcano A (2012) Planetary change and biochemical adaptation: Molecular evolution of corrinoid and heme biosyntheses. *Hematology* 17(Suppl. 1):s7–s10.
30. Holland HD (2006) The oxygenation of the atmosphere and oceans. *Philos Trans R Soc Lond B Biol Sci* 361(1470):903–915.
31. Ludwig ML, Evans PR (1999) X-ray crystallography of B₁₂ enzymes: Methylmalonyl-CoA mutase and methionine synthase. *Chemistry and Biochemistry of B₁₂*, ed Banerjee R (John Wiley and Sons, New York), pp 595–632.
32. Drennan CL, Matthews RG, Ludwig ML (1994) Cobalamin-dependent methionine synthase: The structure of a methylcobalamin-binding fragment and implications for other B₁₂-dependent enzymes. *Curr Opin Struct Biol* 4(6):919–929.
33. Sintchak MD, Arjara G, Kellogg BA, Stubbe J, Drennan CL (2002) The crystal structure of class II ribonucleotide reductase reveals how an allosterically regulated monomer mimics a dimer. *Nat Struct Biol* 9(4):293–300.
34. Lundin D, Gribaldo S, Torrents E, Sjöberg B-M, Poole AM (2010) Ribonucleotide reduction - Horizontal transfer of a required function spans all three domains. *BMC Evol Biol* 10(1):383.
35. Gleason FK, Olszewski NE (2002) Isolation of the gene for the B₁₂-dependent ribonucleotide reductase from *Anabaena* sp. strain PCC 7120 and expression in *Escherichia coli*. *J Bacteriol* 184(23):6544–6550.
36. Poole AM, Logan DT, Sjöberg B-M (2002) The evolution of the ribonucleotide reductases: Much ado about oxygen. *J Mol Evol* 55(2):180–196.
37. Guillard RRL (1968) B12 specificity of marine centric diatoms(1, 2). *J Phycol* 4(1):59–64.
38. Provasoli L, Carlucci AF (1974) Vitamins and growth regulators. *Algal Physiology and Biochemistry, Botanical Monographs*, ed Stewart WDP (University of California Press, Berkeley), pp 741–787.
39. Bertrand EM, et al. (2012) Influence of cobalamin scarcity on diatom molecular physiology and identification of a cobalamin acquisition protein. *Proc Natl Acad Sci USA* 109(26):E1762–E1771.
40. Ayers WA (1960) Specificity of the vitamin B₁₂ requirement in certain marine bacteria. *J Bacteriol* 80(6):744–752.
41. Seth EC, Taga ME (2014) Nutrient cross-feeding in the microbial world. *Front Microbiol* 5:350.
42. Saito MA, Rocap G, Moffett JW (2005) Production of cobalt binding ligands in a *Synechococcus* feature at the Costa Rica upwelling dome. *Limnol Oceanogr* 50(1):279–290.
43. Horak REA, et al. (2013) Ammonia oxidation kinetics and temperature sensitivity of a natural marine community dominated by Archaea. *ISME J* 7(10):2023–2033.
44. Urakawa H, Martens-Habbena W, Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. *Appl Environ Microbiol* 76(7):2129–2135.
45. Santoro AE, et al. (2015) Genomic and proteomic characterization of “*Candidatus Nitrosopelagicus brevis*”: An ammonia-oxidizing archaeon from the open ocean. *Proc Natl Acad Sci USA* 112(4):1173–1178.
46. Urakawa H, Martens-Habbena W, Stahl DA (2011) Physiology and genomics of ammonia-oxidizing archaea. *Nitrification*, eds Ward BB, Klotz MG, Arp DJ (ASM Press, Washington, DC), pp 117–156.
47. Walker CB, et al. (2010) *Nitrosopumilus maritimus* genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. *Proc Natl Acad Sci USA* 107(19):8818–8823.
48. Könneke M, et al. (2014) Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO₂ fixation. *Proc Natl Acad Sci USA* 111(22):8239–8244.
49. Qin W, et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. *Proc Natl Acad Sci USA* 111(34):12504–12509.
50. Kim J-G, et al. (2016) Hydrogen peroxide detoxification is a key mechanism for growth of ammonia-oxidizing archaea. *Proc Natl Acad Sci USA* 113(28):7888–7893.
51. Rabinowitz JD, Kimball E (2007) Acidic acetonitrile for cellular metabolome extraction from *Escherichia coli*. *Anal Chem* 79(16):6167–6173.
52. Amin SA, et al. (2015) Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522(7554):98–101.
53. Datta S, Koutmos M, Patridge KA, Ludwig ML, Matthews RG (2008) A disulfide-stabilized conformer of methionine synthase reveals an unexpected role for the histidine ligand of the cobalamin cofactor. *Proc Natl Acad Sci USA* 105(11):4115–4120.
54. Lunau M, Lemke A, Walther K, Martens-Habbena W, Simon M (2005) An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environ Microbiol* 7(7):961–968.
55. Woodson JD, Peck RF, Krebs MP, Escalante-Semerena JC (2003) The cobY gene of the archaeon *Halobacterium* sp. strain NRC-1 is required for de novo cobamide synthesis. *J Bacteriol* 185(1):311–316.
56. Könneke M, et al. (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature* 437(7058):543–546.
57. Qin W, et al. (2015) Confounding effects of oxygen and temperature on the TEX86 signature of marine Thaumarchaeota. *Proc Natl Acad Sci USA* 112(35):10979–10984.
58. Moore LR, et al. (2007) Culturing the marine cyanobacterium *Prochlorococcus*. *Limnol Oceanogr Methods* 5(10):353–362.
59. Morris JJ, Kirkegaard R, Szul MJ, Johnson ZI, Zinser ER (2008) Facilitation of robust growth of *Prochlorococcus* colonies and dilute liquid cultures by “helper” heterotrophic bacteria. *Appl Environ Microbiol* 74(14):4530–4534.
60. Berube PM, et al. (2015) Physiology and evolution of nitrate acquisition in *Prochlorococcus*. *ISME J* 9(5):1195–1207.
61. Saito MA, Moffett JW, Chisholm SW, Waterbury JB (2002) Cobalt limitation and uptake in *Prochlorococcus*. *Limnol Oceanogr* 47(6):1629–1636.
62. Jeffrey Morris J, Zinser ER (2013) Continuous hydrogen peroxide production by organic buffers in phytoplankton culture media. *J Phycol* 49(6):1223–1228.
63. Thompson AV, Huang K, Saito MA, Chisholm SW (2011) Transcriptome response of high- and low-light-adapted *Prochlorococcus* strains to changing iron availability. *ISME J* 5(10):1580–1594.
64. DuRand MD, Olson RJ, Chisholm SW (2001) Phytoplankton population dynamics at the Bermuda Atlantic Time-series station in the Sargasso Sea. *Deep Sea Res Part II Top Stud Oceanogr* 48(8):1983–2003.
65. Zimmerman AE, Allison SD, Martiny AC (2014) Phylogenetic constraints on elemental stoichiometry and resource allocation in heterotrophic marine bacteria. *Environ Microbiol* 16(5):1398–1410.
66. Sachs JP, Kawka OE (2015) The influence of growth rate on 2H/1H fractionation in continuous cultures of the coccolithophorid *Emiliania huxleyi* and the diatom *Thalassiosira pseudonana*. *PLoS One* 10(11):e0141643.
67. Montagnes DJ, Berges JA, Harrison PJ, Taylor F (1994) Estimating carbon, nitrogen, protein, and chlorophyll a from volume in marine phytoplankton. *Limnol Oceanogr* 39(5):1044–1060.
68. Beam JP, et al. (2016) Ecophysiology of an uncultivated lineage of Aigarchaeota from an oxic, hot spring filamentous ‘streamer’ community. *ISME J* 10(1):210–224.
69. Altschul SF, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* 25(17):3389–3402.
70. Altschul SF, et al. (2005) Protein database searches using compositionally adjusted substitution matrices. *FEBS J* 272(20):5101–5109.
71. Lengyel P, Mazumder R, Ochoa S (1960) Mammalian methylmalonyl isomerase and vitamin B(12) coenzymes. *Proc Natl Acad Sci USA* 46(10):1312–1318.
72. Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: Improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33(2):511–518.
73. Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics* 22(2):195–201.
74. Pettersen EF, et al. (2004) UCSF Chimera—A visualization system for exploratory research and analysis. *J Comput Chem* 25(13):1605–1612.
75. Soule MCK, Longnecker K, Johnson WM, Kujawinski EB (2015) Environmental metabolomics: Analytical strategies. *Mar Chem* 177(2):374–387.
76. Watanabe F, et al. (1999) Pseudovitamin B(12) is the predominant cobamide of an algal health food, spirulina tablets. *J Agric Food Chem* 47(11):4736–4741.
77. Allen RH, Stabler SP (2008) Identification and quantitation of cobalamin and cobalamin analogues in human feces. *Am J Clin Nutr* 87(5):1324–1335.
78. Juzeniene A, Nizauskaite Z (2013) Photodegradation of cobalamins in aqueous solutions and in human blood. *J Photochem Photobiol B* 122:7–14.
79. Ribalet F, et al. (2015) Light-driven synchrony of *Prochlorococcus* growth and mortality in the subtropical Pacific gyre. *Proc Natl Acad Sci USA* 112(26):8008–8012.
80. Martens-Habbena W, et al. (2015) The production of nitric oxide by marine ammonia-oxidizing archaea and inhibition of archaeal ammonia oxidation by a nitric oxide scavenger. *Environ Microbiol* 17(7):2261–2274.
81. Mincer TJ, et al. (2007) Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre. *Environ Microbiol* 9(5):1162–1175.
82. Caporaso JG, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7(5):335–336.
83. Bokulich NA, et al. (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat Methods* 10(1):57–59.
84. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26(19):2460–2461.
85. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215(3):403–410.
86. Tripp HJ, et al. (2008) SAR11 marine bacteria require exogenous reduced sulphur for growth. *Nature* 452(7188):741–744.
87. Dupont CL, et al. (2012) Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. *ISME J* 6(6):1186–1199.
88. Crosby LD, Criddle CS (2003) Understanding bias in microbial community analysis techniques due to rrn operon copy number heterogeneity. *Biotechniques* 34(4):790–794, 796, 798 passim.
89. Vetrovsky T, Baldrian P (2013) The variability of the 16S rRNA gene in bacterial genomes and its consequences for bacterial community analyses. *PLoS One* 8(2):e57923.
90. Stieglmeier M, Alves RJE, Schleper C (2014) The phylum Thaumarchaeota. *The Prokaryotes*, eds Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (Springer), pp 347–362.
91. Iverson V, et al. (2012) Untangling genomes from metagenomes: Revealing an uncultured class of marine Euryarchaeota. *Science* 335(6068):587–590.
92. Karner MB, DeLong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* 409(6819):507–510.