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Genomic adaptation of marine phytoplankton populations regulates phosphate uptake

Adam C. Martiny,[1,2*] **Lucas Ustick**,[2] **Catherine A. Garcia**,[1] **Michael W. Lomas**[3]

[1]Department of Earth System Science, University of California, Irvine, California
[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, California
[3]Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine

Abstract

In this study, we combined "reciprocal transplant experiments," cell-sorting, and metagenomics to understand how phytoplankton adapt to differences in phosphate availability and the implications for nutrient uptake rates. Reciprocal transplant experiments were conducted on six stations ranging from cold, nutrient-rich water in the Labrador Sea to warm, extremely P-deplete water in the Sargasso Sea. In most cases, the direct impact of environmental conditions and likely P availability was the strongest control on phosphate uptake. However, especially the transplant experiments between the northern and southern stations revealed that there are situations where changes in community composition and functional genes have an important effect on uptake rates. Phytoplankton lineages responded uniquely to changing environmental conditions. The picoeukaryotic phytoplankton P uptake response was strongly regulated by the phosphate concentration, whereas the effect of community composition was larger for *Prochlorococcus* and *Synechococcus*. In support, we found a tight negative relationship between ambient phosphate concentration and the frequency of P acquisition genes in both *Prochlorococcus* and *Synechococcus*, and such differences in genome content could be linked to lineage-specific shifts in uptake rates. Linking genes with ocean biogeochemistry is a major scientific and technical challenge and most studies rely on correlations between genotypes and environmental conditions. However, our study demonstrates how reciprocal transplant experiments are a possible tool for understanding the relative role of environmental condition vs. plankton diversity in regulating important open ocean ecosystem processes.

Phosphorus is an important biogenic element in the ocean and is commonly regarded as the ultimate limiting nutrient (Tyrrell 1999; Karl 2014). Phosphate concentrations vary between ocean regions and can become growth limiting in places like the western North Atlantic Ocean or the Mediterranean Sea (Ammerman et al. 2003; Thingstad et al. 2005). The reduced delivery of phosphate is also regarded as important for future changes to the abundance and growth of phytoplankton (Bopp et al. 2013). Thus, understanding the impact of phosphate on marine ecosystem processes is key for predicting future changes to ocean productivity.

*Correspondence: amartiny@uci.edu

Additional Supporting Information may be found in the online version of this article.

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Phytoplankton vary greatly in their ability to grow and take up phosphate at different concentrations. Broadly, the P cell quota and uptake capabilities scale with cell size (Edwards et al. 2012; Lomas et al. 2014). Thus, smaller cells are thought to be more competitive under low phosphate conditions due a large surface-to-volume ratio. In addition to an allometric scaling in P competitive capabilities, there are also considerable intraspecific differences in P acquisition. For example, strains of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus* display heterogeneity in the presence or absence of many P acquisition genes (Scanlan et al. 2009). Some strains contain genes responsible for transcriptional regulation (e.g., *pho*BR and *ptr*A), high affinity P uptake (*pst*ABCS and *pho*E), and the use of dissolved organic phosphate sources (e.g., *pho*A) (Moore et al. 2005). In contrast, many of these P acquisition genes can be absent among close relatives leading to incongruence between P uptake capabilities and phylogenetic relationship (Martiny et al. 2006). Instead, it appears that the concentration of ambient P controls the presence of P acquisition genes such that many prokaryotic cells found in ocean environments with $< 25–50$ nmol L^{-1} phosphate contain many P acquisition genes and vice-versa for high P areas (Rusch et al. 2007; Martiny et al.

2009, 2011). Less is known for eukaryotic picophytoplankton as this group is very diverse. However, some reports suggest variation in the presence of P acquisition genes in this group as well (Lin et al. 2016; Whitney and Lomas 2016). However, we do not understand yet how differences in population diversity and associated genome content translate functionally into adaptation to different P regimes in the ocean.

"Reciprocal transplant experiments" are a common tool in ecology to understand the functional and ecological impact of adaptation to specific environmental conditions (De Villemereuil et al. 2016). Reciprocal transplant experiments are based on a factorial design, whereby you move organisms or communities from each of two environments into the other—although the design can be extended to more organisms and/or environments. This experimental design was originally applied to terrestrial ecosystems and in particular plants. There are also some examples using reciprocal transplant experiments for terrestrial microorganism revealing strong evidence for an interaction between adaptation to specific environments and ecosystem processes (Reed and Martiny 2007; Martiny et al. 2017). However, due to the complexity in separating cells from seawater, a reciprocal transplant experimental design is not commonly used to understand the functional role of adaptation in open ocean plankton.

Here, we present a large-scale reciprocal transplant experiment study in the western North Atlantic Ocean. Using extremely gentle techniques, we separated cells from seawater at stations along a gradient in nutrient levels and then recombined seawater and plankton communities in a factorial design. We then asked: (1) what is the relative impact of community composition vs. environmental conditions on P uptake and (2) are population genomic alterations in P acquisition capabilities regulating different uptake rates?

Methods

Cruise and environmental data

Experiments were conducted on six stations during the North Atlantic Ocean cruise AE1319 from Bermuda to the Labrador Sea and back (15 August 2013 to 08 September 2013). An extensive description of the environmental conditions and biological communities from this cruise and region has been presented previously (Lomas et al. 2014; Baer et al. 2017; Kent et al. 2019). For this study, we first collected surface seawater (~ 5 m) from Bermuda Atlantic Time-series station (BATS). The water was stored in 20-liter acid-washed carboys and never exposed to sunlight until being used later in the experiments. Next, we sequentially occupied the Sta. 55°N, 49°N, 45°N, 39°N, 35°N, and then visited BATS (31.7°N) a second time (Table 1). The environmental conditions at BATS for the two collection times were very similar but not identical (Table 1). At all stations except BATS, we conducted two incubations (each in triplicate). The first incubation had local water (defined as everything passing through a 0.22 μm filter) plus the local community and the second incubation had water from BATS plus the local community. Thus, water but not plankton was moved between stations and all incubations consisted of a mixture of separate plankton and filtered seawater. At BATS, we combined the community from BATS with water from each station. Each incubation consisted of filtered source water (i.e., no cells) and a separate community. To make particle-free water, we filtered 500 mL per sample seawater through a 0.22-μm pore size polycarbonate filter. To obtain the microbial community, without the local water, we very gently vacuum filtered (5 mm Hg) 500 mL of seawater using a polycarbonate filter of 47 mm 0.22 μm pore size. A key detail is to make certain that the filter never dried out in order to avoid cells attaching strongly to the filter and/or rupturing due to shear stress. We then used a sterile transfer pipette to gently resuspend and mix ~ 2 mL of remaining water with retained cells. Assessed using flow cytometry counting of picophytoplankton, this procedure led to > 90% cell recovery and no detectable shifts in community composition (Casey et al. 2007; Batmalle et al. 2014). The cell suspension was then combined with 500 mL of filtered water. The bottles were incubated for 24 h in an on-deck incubator with 50% local light level and the local seawater temperature. Thus, plankton had 24 h to acclimate to the specific seawater conditions. A short incubation period was used in order to minimize any community shifts.

High-sensitivity phosphate, chlorophyll, particulate organic carbon, and cell counts

Environmental data were collected as previously described (Lomas et al. 2014; Baer et al. 2017; Kent et al. 2019). Briefly, nutrient samples were collected after filtration through 0.8 μm Nucleopore polycarbonate filters (Whatman, Maidstone, UK) and soluble reactive phosphorus after preparation via the magnesium-induced coprecipitation method (Karl and Tien 1992; Lomas et al. 2010). Nutrient samples were collected from the incubations after 24 h and thus represent the concentration when we measured phosphate uptake. Particulate organic carbon (POC) was determined on a Costech 4010 elemental analyzer following acidification. Empty tin capsules (Costech Analytical Technologies) were run as instrument blanks, and the filter blank was packed in another tin for quality assurance. For chlorophyll, ~ 250–500 mL seawater was filtered onto 25-mm Ahlstrom glass fiber filters (nominal pore size 0.7 μm) under low pressure (15 kpa), and frozen immediately at −80°C. Samples were extracted in 90% acetone in the dark for 14–18 h at −20°C and quantified on a Turner 10-AU fluorometer using the acidification method (Parsons et al. 1984). For cell counts, samples of whole seawater were collected in 2-mL centrifuge tubes, fixed with freshly made 0.2 -μm-filtered paraformaldehyde (0.5% v/v final concentration) for 1 h at 5°C in the dark, and counted on a FACSJazz or Influx flow cytometer (BD, Franklin Lakes, NJ, U.S.A.) utilizing a 200 mW 488 nm laser, with detectors for forward scatter, side scatter, 530 nm, and 692 nm. *Prochlorococcus* populations were discriminated based on forward scatter and red fluorescence, and a gate in orange (585 nm) discriminated for *Synechococcus*. Picoeukaryotic phytoplankton were all the red

Table 1. Overview of stations and associated environmental and biological conditions.

No.	Latitude	Longitude	Date	Depth (m)	$[P_i]$ (nmol L^{-1})	Temperature (°C)	*Prochlorococcus* (cells mL^{-1})	*Synechococcus* (cells mL^{-1})	Picoeukaryotes (cells mL^{-1})	Chl ($\mu g\ L^{-1}$)	POC ($\mu mol\ L^{-1}$)	Seqs no.
1	55.0°N	49.0°W	25 Aug 13	5	144	10.4	1.4e3	2.9e4	2.6e4	1.07	7.5	1.9e7
2	49.0°N	40.0°W	28 Aug 13	5	31	15.7	3.9e3	4.0e4	1.3e4	0.56	5.7	1.8e7
3	45.0°N	45.0°W	31 Aug 13	5	46	18.7	7.5e3	4.1e4	3.2e3	0.22	4.8	1.8e7
4	39.0°N	52.5°W	03 Sep 13	5	0.5	26.3	6.1e4	7.2e3	7.1e2	0.05	1.6	1.8e7
5	35.0°N	57.5°W	05 Sep 13	5	0.5	26.6	8.5e4	5.9e3	71	0.05	1.6	1.6e7
6	31.7°N (BATS)	64.2°W	08 Sep 13	5	0.5	27.9	4.5e4	2.7e4	6.5e2	0.05	1.4	2.2e7
—*	31.7°N (BATS)	64.2°W	15 Aug 13	5	1.1	28.2	2.9e4	0.8e4	9.5e2	0.04	2	—

*This row represents the first collection of water from BATS.

autofluorescing cells that did not fit the Cyanobacteria gating scheme with a cell size below 2–3 μm.

^{33}Phosphate incubations

The approach for ambient whole community and population-specific uptake rate measurements were done as previously published (Casey et al. 2009; Lomas et al. 2014). Duplicate aliquots of 10 mL seawater were amended with 0.15 μCi (~ 80 pmol L^{-1}) additions of $^{33}P_i$ (3000 Ci mol^{-1}; PerkinElmer, U.S.A.), and incubated for 30–60 min in subdued lighting (~ 100 μmol photons m^{-2} s^{-1}) at a fixed temperature of 23°C. The duration of each incubation varied depending on turnover time of the added isotope, such that efforts were made to keep uptake to < 25% of the tracer added. Duplicate killed control incubations were conducted for each station by amending with paraformaldehyde (0.5% final concentration) for 30 min prior to the addition of isotopic tracer and incubation. Whole community incubations were terminated by filtration onto 0.2-μm polycarbonate filters that were subsequently placed in glass scintillation vials. Population-specific ambient uptake incubations were terminated by the addition of paraformaldehyde (0.5% final concentration), and stored at 4°C until sorting (< 12 h) as described in the next section. Sta. 49°N was excluded from population-specific ambient uptake incubations. We did not synchronize the timing of the incubation between stations due to the overall schedule of the cruise. Thus, we sampled at 06:50, 11:14, 13:22, 19:06, 15:23, and 10:38 h local time for the six stations.

Cell sorting by flow cytometry

As described previously (Lomas et al. 2014), samples were sorted for *Prochlorococcus*, *Synechococcus*, and an operationally defined eukaryotic algae size fraction (picoeukaryotes < 2–3 μm) on an InFlux cell sorter (BD, Seattle, WA). A 100 mW blue (488 nm) excitation laser was used. After exclusion of laser noise gated on pulse width and forward scatter, populations were discriminated by chlorophyll fluorescence (> 650 nm), PE (585/30 nm), and granularity (side scatter). Sheath fluid was made fresh daily from distilled deionized water (Millipore, Billerica, MA) and molecular grade NaCl (Mallinckrodt Baker, Phillipsburg, NJ), prefiltered through a 0.2 μm capsule filter (Pall, East Hills, NY), and a Sterivex sterile 0.22 μm inline filter (Millipore). Mean coincident abort rates were < 1% and mean recovery from secondary sorts (n = 25) was 97.5% ± 1.1% (data not shown). Sorted cells from each sample were gently filtered onto 0.2-μm Nuclepore polycarbonate filters, rinsed with copious amounts of 0.2 μm filtered seawater, an oxalate wash (Tovar-Sanchez et al. 2003), and placed in a 7 mL scintillation vial for liquid scintillation counting. *Prochlorococcus* was found in too low of abundance for sorting at Sta. 55°N and thus excluded from the analysis of this station.

Metagenomic library preparation and analysis

As described previously (Bostrom et al. 2004; Kent et al. 2019), 4 L of seawater were prefiltered on a GF/D glass-fiber filter before being collected on a 0.22 μm Sterivex filter (Millipore,

Burlington, MA, U.S.A.) and preserved with 1.62 mL TES buffer (50 mmol L^{-1} Tris-HCl pH 7.6, 20 mmol L^{-1} EDTA pH 8.0, 400 mmol L^{-1} NaCl, 0.75 mol L^{-1} sucrose) at –20°C until further processing. DNA was extracted using lysozyme (180 μL of 50 mg mL^{-1} at 37°C for 30 min) and then proteinase K (180 μL of 1 mg mL^{-1}) plus sodium dodecyl sulfate (100 μL of 10%) and incubated at 55°C overnight. DNA was pelleted using 3 mol L^{-1} sodium acetate (pH 5.2) and cold isopropanol. Finally, DNA was purified using a genomic DNA Clean and Concentrator kit (Zymo Corp., Irvine, CA, U.S.A.) and stored at –20°C. The DNA concentration was quantified with Qubit dsDNA HS assay kit (Life Technologies, Carlsbad, CA, U.S.A.) and subsequently diluted in Tris buffer (10 mmol L^{-1}, pH 8.0). Using 0.5 ng of DNA, each library was prepared using Nextera XT barcodes (Illumina, San Diego, CA) and an Illumina Nextera library prep kit with a modified PCR amplification mixture. For PCR amplification, 20 μL of master mix was added consisting of 0.5 μL Phusion High Fidelity buffer (New England Biolabs, Ipswich, MA), 0.5 μL dNTPs (New England Biolabs, Ipswich, MA), 0.25 μL Phusion High Fidelity polymerase (New England Biolabs, Ipswich, MA), and 14.25 μL of PCR water. Equimolar samples were pooled and the quality was checked and quantified on a Bioanalyzer (Agilent, Santa Clara, CA). The pooled library was sequenced on a HiSeq-4000 (Illumina, San Diego, CA) producing paired end reads (2 × 150 bp) at the UC Davis Genome Center leading to 1.6e7–2.2e7 quality filtered sequences per sample (Table 1). The sequences were submitted to the Sequence Read Archive with accession number PRJNA517745.

Adaptors and low-quality reads were removed from the raw sequences using trimmomatic-0.35 (Bolger et al. 2014) and PhiX contamination was filtered using bbduk (BBTools v 37.50, DOE JGI, Walnut Creek, CA). Sequences were then recruited using Bowtie2 (Langmead and Salzberg 2012) to all available *Prochlorococcus* and *Synechococcus* genomes (Biller et al. 2014) and summarized using Anvi'o (Eren et al. 2015; Delmont and Eren 2018). The clustering of orthologous genes were initially done using Anvi'o but further curated by hand based on past genomic analyses (Martiny et al. 2006; Kettler et al. 2007; Scanlan et al. 2009; Biller et al. 2014) to more accurately capture all copies of each orthologous cluster. P acquisition gene cluster (Supporting Information Table S1) frequencies were normalized to the median frequency of all single-copy core genes in *Prochlorococcus* and *Synechococcus*, respectively.

Results

To quantify the biogeochemical impact of adaptation in plankton communities, we did a reciprocal transplant experiment across six stations in the North Atlantic Ocean (Fig. 1A). The reciprocal transplant experiment consisted of the pair-wise comparison between five local stations and the well-studied Bermuda Atlantic Time-series station (BATS, Fig. 1). The stations spanned a gradient in environmental and biological conditions (Table 1). The northern most station (55°N) had the lowest temperature and highest phosphate concentration. Furthermore, the phytoplankton community consisted of high picoeukaryotic phytoplankton and *Synechococcus* abundance but a low abundance of *Prochlorococcus*. Progressively, the temperature increased, phosphate concentrations decreased, the particulate organic carbon and chlorophyll concentrations decreased, and *Prochlorococcus* became more abundant toward the southern stations. Hence, the three northern stations (55°N, 49°N, and 45°N) all had near or above 30 nmol L^{-1} phosphate, whereas phosphate was almost completely drawn down to the south (39°N, 35°N, and BATS). Furthermore, the phosphate levels in the incubations were close to ambient levels (Lomas et al. 2014). Thus, there was a clear shift in the environmental and biological conditions between the stations.

We observed a significant impact of seawater origin and associated environmental conditions on whole community phosphate uptake (Figs. 2–3). Phosphate uptake was considerably higher when the same community was exposed to seawater from one of the three northern stations vs. BATS water (Fig. 2A–C). In contrast, we saw a smaller difference in uptake rates among the three southern stations (Fig. 2D–F). In addition, there was a clear gradient in uptake rate, when plankton from BATS was exposed to each of the local waters (Fig. 2F). Here, the observed variation in uptake rates appeared to vary as a function of the phosphate concentration of the water

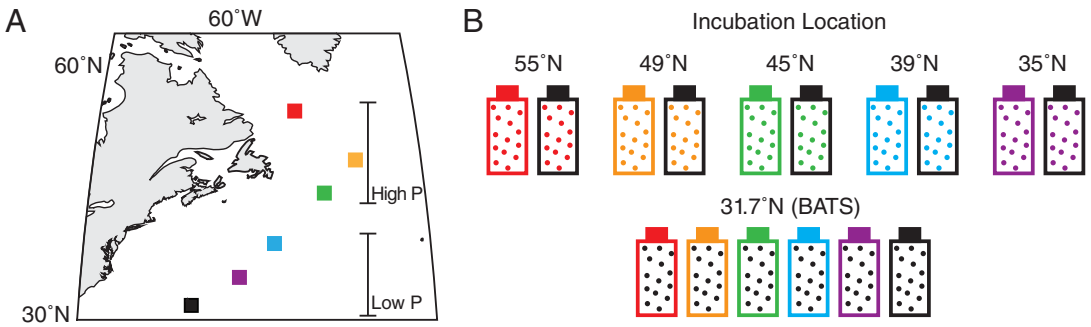

Fig. 1. Reciprocal transplant experiment design. (**A**) Sampling and incubation locations during the AE1319 cruise in 2013. (**B**) Factorial design consisting of a mixture of seawater (illustrated by bottles) and plankton communities (illustrated by dots) incubated at local conditions. The design covered combinations of local communities and water from each station pair-wise reciprocally transplanted with communities or water from BATS.

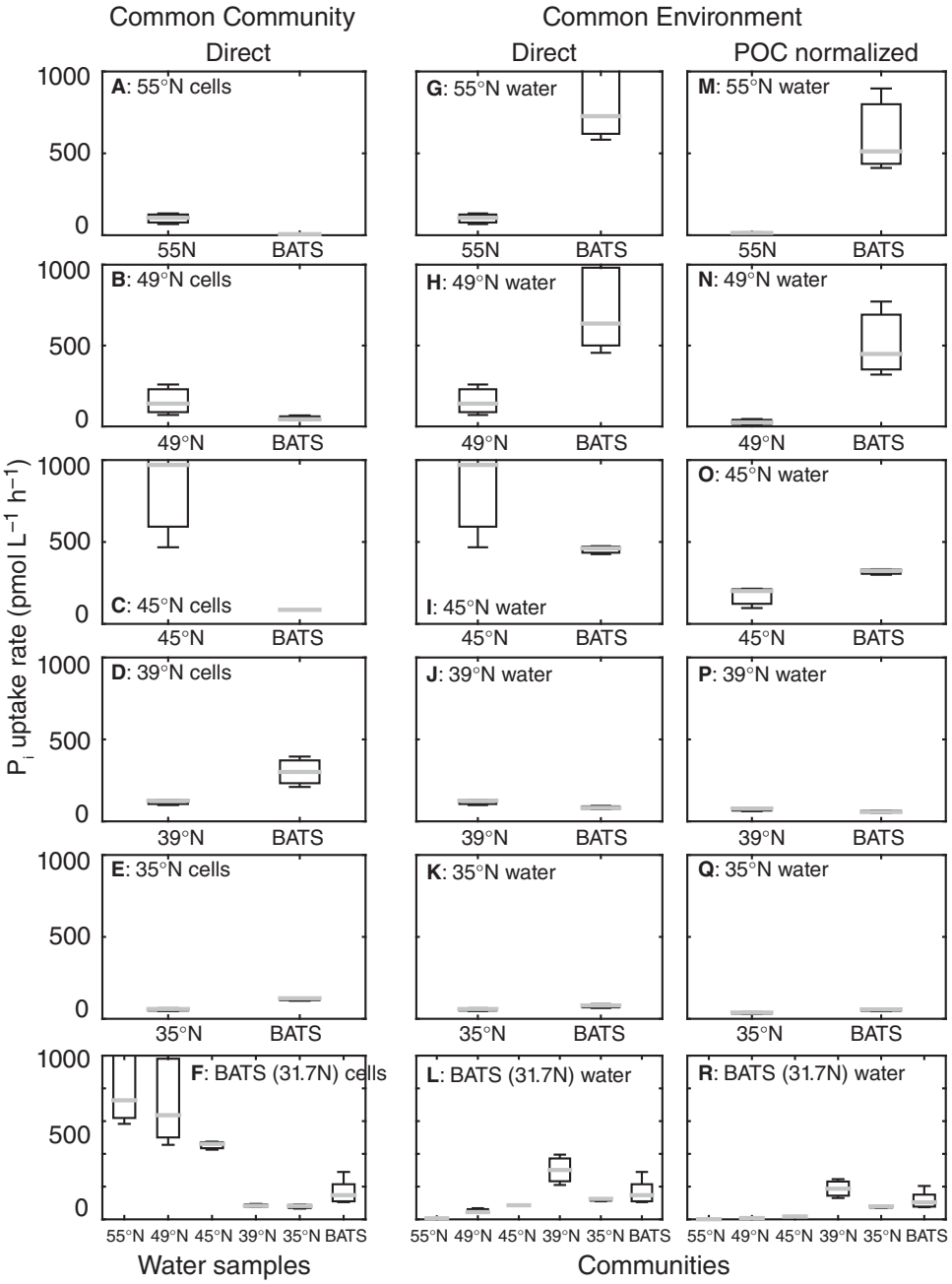

Fig. 2. Impact of seawater origin and community composition on whole community phosphate uptake using a reciprocal transplant experiments. Impact of different seawater sources on whole community P uptake (**A–F**), impact of different community composition on whole community phosphate uptake (**G–L**), and impact of different community composition on whole community phosphate uptake normalized to total POC (μmol L^{-1}) (**M–R**).

source. Hence, uptake was considerably higher for BATS plankton when exposed to water from the northern compared to the southern stations. The only exception was that the BATS community had higher rates in BATS water compared to other low phosphate water sources. Supported by an ANOVA (Fig. 3), there was a significant seawater origin effect on whole community phosphate uptake.

We also saw a significant impact of community origin on phosphate uptake rates (Figs. 2–3). At Sta. 55°N and 49°N, plankton from BATS had higher uptake rates compared to local cells when exposed to water from these two stations (Fig. 2G,H). We also normalized to overall biomass using POC (Fig. 2M,N) to roughly account for changes in overall biomass levels. However, this normalization did not change the community impact on uptake rates at the two most northern stations. At 45°N, the local community had higher rates of uptake than plankton from BATS (Fig. 2I), but this effect largely disappeared when normalizing to POC (Fig. 2O). Thus,

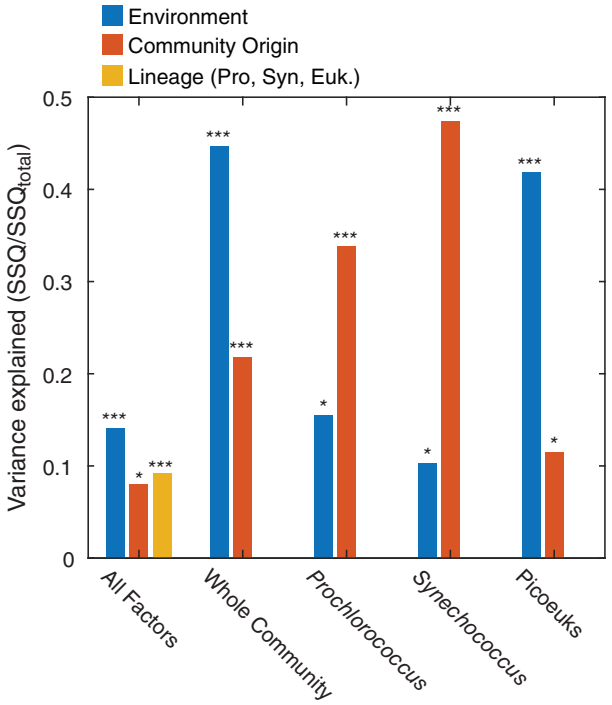

Fig. 3. Impact of environment, community origin, and lineage identity on variance in P uptake. The variance is estimated using an ANOVA with two or three factors. Environment and community origin each have six levels whereas lineage has three levels.

on per biomass level 45°N and BATS seemed to do equally well. The communities from the southern stations outperformed the northern stations when exposed to BATS water (Fig. 2L,R), whereas we did not see much difference in uptake rate among the southern stations (Fig. 2J,K). An ANOVA supported that community composition had a significant effect on total P uptake rates (Fig. 3). Overall, the results suggest that plankton from low phosphate in comparison to high phosphate environments have higher uptake rates when exposed to the same environmental conditions.

We next quantified the lineage-specific uptake rates and found unique impacts of source water on *Prochlorococcus*, *Synechococcus*, and picoeukaryotic phytoplankton populations (Fig. 4). At Sta. 45°N, local *Prochlorococcus* cells had a much higher uptake rate in their local environment compared to BATS water (Fig. 4A). However, this pattern flipped at Sta. 39°N and 35°N, where higher rates were observed in BATS water compared to local water (Fig. 4B,C). Finally, cells from BATS had highest uptake rates in water the southern stations and generally did not thrive in water from the northern stations (albeit with one exception at 49°N) (Fig. 4D). An identical pattern was observed for *Synechococcus* with higher rates in local water at the northern stations (Fig. 4E,F), higher rates in BATS water at the southern stations (Fig. 4G,H), and cells from BATS exhibiting highest uptake rates in water from the southern stations (Fig. 4I). Picoeukaryotic phytoplankton shared some of these patterns but also displayed some unique effects. Again, we saw higher rates at 55°N (Fig. 4J) and highest uptake rates in BATS water at the southern stations (Fig. 4L,M). However, the picoeukaryotic phytoplankton population from BATS responded strongly to increased P availability leading to higher rates in northern vs. southern waters for BATS cells (Fig. 4N). As a result, there are clear source water effects on the P uptake from all three phytoplankton lineages, but the specific effects were lineage-specific (Fig. 3).

We observed a significant effect of within-lineage diversity on cellular P uptake among *Prochlorococcus*, *Synechococcus*, and picoeukaryotic phytoplankton (Fig. 5). *Prochlorococcus* and *Synechococcus* showed similar variation in P uptake (Fig. 5A–I). At the northern stations, the local populations generally outperformed cells from BATS (Fig. 5A,E,F) whereas the effect in 35°N and 39°N waters was mixed (Fig. 5B–D,G–I). In BATS water, cells from the southern stations clearly had higher rates than cells from the northern stations (Fig. 5D,I). Picoeukaryotic populations from the southern stations consistently had higher uptake rates compared to cells from the northern stations (Fig. 5J–N). Within the southern stations, the signal was more mixed (Fig. 5L–N). In sum, there was a clear geographic origin effect on P uptake across all the analyzed phytoplankton lineages (Fig. 3).

We detected a distinct shift in the ecotype composition of *Prochlorococcus* and *Synechococcus* populations. The frequency of *Prochlorococcus* and *Synechococcus* reads in the metagenomic libraries vs. using cell counts by flow cytometry were significantly correlated ($R = 0.98$, $p_{\text{pearson}} < 0.001$). This suggested limited bias in the metagenomic coverage of populations and their associated gene frequencies in our analysis. In concordance with previous studies, the low temperature, high nutrient adapted ecotypes of *Prochlorococcus* (HLI) and *Synechococcus* (Clades I + IV) dominated the northern stations (Zwirglmaier et al. 2008; Kent et al. 2019) (Fig. 6). A clear shift occurred between 45°N and 39°N and the low nutrient, high temperature adapted ecotypes (HLII for *Prochlorococcus* and clade II + III for *Synechococcus*) dominated at the southern stations.

Concurrently, we observed clear differences in P acquisition genes among *Prochlorococcus* and *Synechococcus* populations that could be linked to uptake rates. In *Prochlorococcus*, most P acquisition genes could be detected across populations from all stations (Fig. 7A). However, some genes became less frequent in populations from the northern stations including *pho*B, *ptr*A, and several genes of unknown function. Conversely, we saw little change in genes directly responsible for inorganic phosphate uptake transport (i.e., *pst*ABCS). There were also clear differences in the presence of P acquisition genes in *Synechococcus* populations across stations (Fig. 7B). In particular, we observed genes annotated as alkaline phosphatase (*pho*A and *pho*X) at all the southern stations but not the northern stations. Furthermore, there was a negative relationship between ambient phosphate concentration and the frequency of P acquisition genes in both *Prochlorococcus* and *Synechococcus* (Fig. 7C,D) although only significant in *Synechococcus* ($R_{\text{Pro}} = -0.67$, $p_{\text{spearman}} = 0.16$, $R_{\text{syn}} = -0.84$, $p_{\text{spearman}} = 0.04$). In relation to the reciprocal

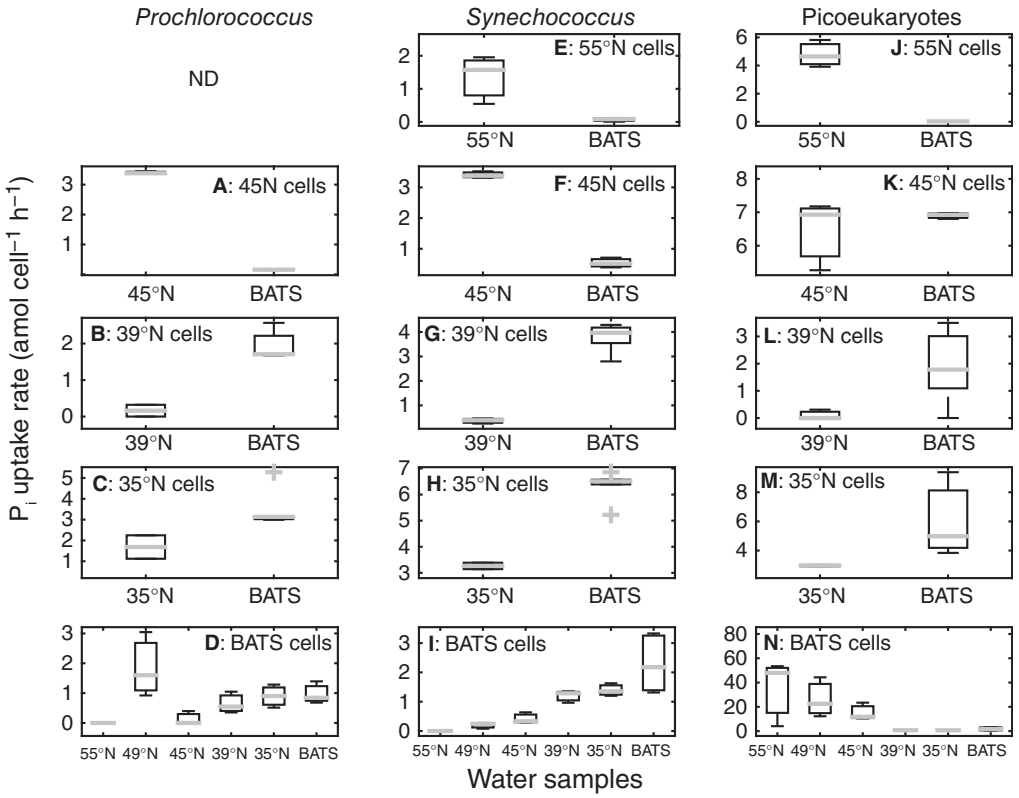

Fig. 4. Impact of seawater sources on lineage-specific cellular phosphate uptake rates using reciprocal transplant experiments. Impact of different seawater sources on phosphate uptake for *Prochlorococcus* (**A–D**), *Synechococcus* (**E–I**), and picoeukaryotic phytoplankton (**J–N**). There were not enough *Prochlorococcus* cells at Sta. 55°N and lineage-specific phosphate uptake rates were not quantified for Sta. 49°N, so these samples were excluded from the analysis. We quantified phosphate uptake for specific phytoplankton lineages using cell sorting.

transplant experiment, *Prochlorococcus* populations from the three southern stations did not differ much in genome content and this corresponded to very similar P uptake rates (Fig. 5B–D). In contrast, the northern *Prochlorococcus* populations had lower P acquisition gene frequencies as well as lower P uptake rate (Fig. 5A). For *Synechococcus*, there were significantly higher frequencies of P acquisition genes in the southern vs. northern populations, which corresponded well with higher P uptake rates given the same environmental conditions (Fig. 5E–I). Thus, the combination of metagenomic sequencing and the reciprocal transplant experiment demonstrated how differences in genome content are related to P uptake capabilities among phytoplankton field populations.

Discussion

We observed a significant impact of both environmental conditions and plankton diversity on P uptake rates. We find that phosphate availability (1) directly regulates physiologically uptake rates and (2) selects for communities with different frequencies of P acquisition genes. In most cases, the direct impact of environmental conditions and likely phosphate levels were the strongest control on uptake (Fig. 3). However, the transplant experiments between the northern and southern stations revealed that there are situations where changes in community composition and associated functional genes can have an important effect on P uptake. Thus, P uptake is regulated by the product of environmental variation and adaptation.

Changes in the composition of *Prochlorococcus* and *Synechococcus* ecotypes and P acquisition genes provide evidence for populations that are adapted to local conditions, including the concentration of phosphate. In contrast, we know less about the diversity of such genes across picoeukaryotic phytoplankton. Picoeukaryotic phytoplankton are a very diverse group (Moon-van der Staay et al. 2001), making it challenging to link genotype and phenotypes in field populations for this lineage. Furthermore, we did not quantify P uptake among larger phytoplankton although larger but rare cells could make important contributions to nutrient cycling. The observed genomic changes matched earlier studies of *Prochlorococcus* demonstrating that adaptation to low phosphate conditions primarily occur via the gain or loss of genes responsible for gene regulation (*pho*BR and *ptr*A), the uptake of dissolved organic phosphate (e.g., *pho*A or *pho*X), and several genes of unknown function. In contrast, genes directly responsible for inorganic phosphate uptake were always present (Martiny et al. 2006, 2009). We observed an analogous pattern among

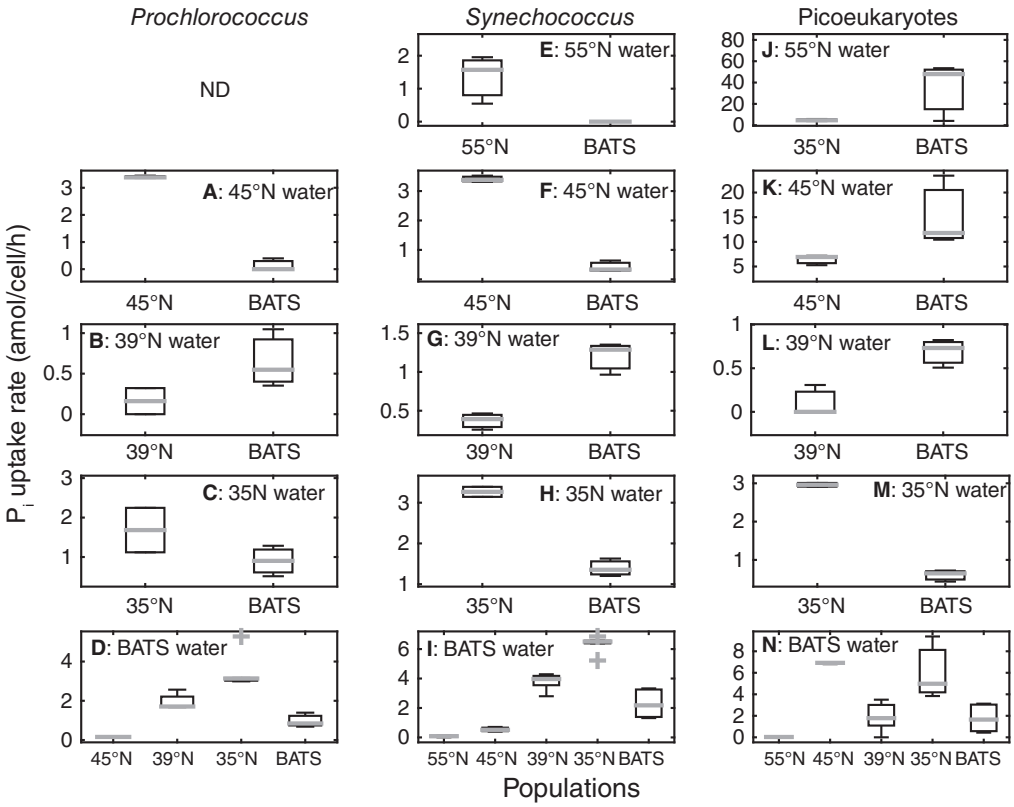

Fig. 5. Impact of community composition on population-specific cellular phosphate uptake rates using a reciprocal transplant experiments. Impact of community origin on P uptake for *Prochlorococcus* (**A–D**), *Synechococcus* (**E–I**), and picoeukaryotic phytoplankton populations (**J–N**). There were not enough *Prochlorococcus* cells at Sta. 55°N and lineage-specific phosphate uptake rates were not quantified for Sta. 49°N, so these samples were excluded from the analysis.

Synechococcus field populations confirming analyses of genomes from cultures (Moore et al. 2005; Scanlan et al. 2009). We primarily observed gene gain and loss as the response to phosphate nutrient stress, and thus our work builds on prior work and adds more evidence to the theory that lateral gene gain and gene loss are important for picophytoplankton adaptation to environments with different phosphate concentrations.

Fig. 6. Distribution of *Prochlorococcus* and *Synechococcus* ecotypes across the stations.

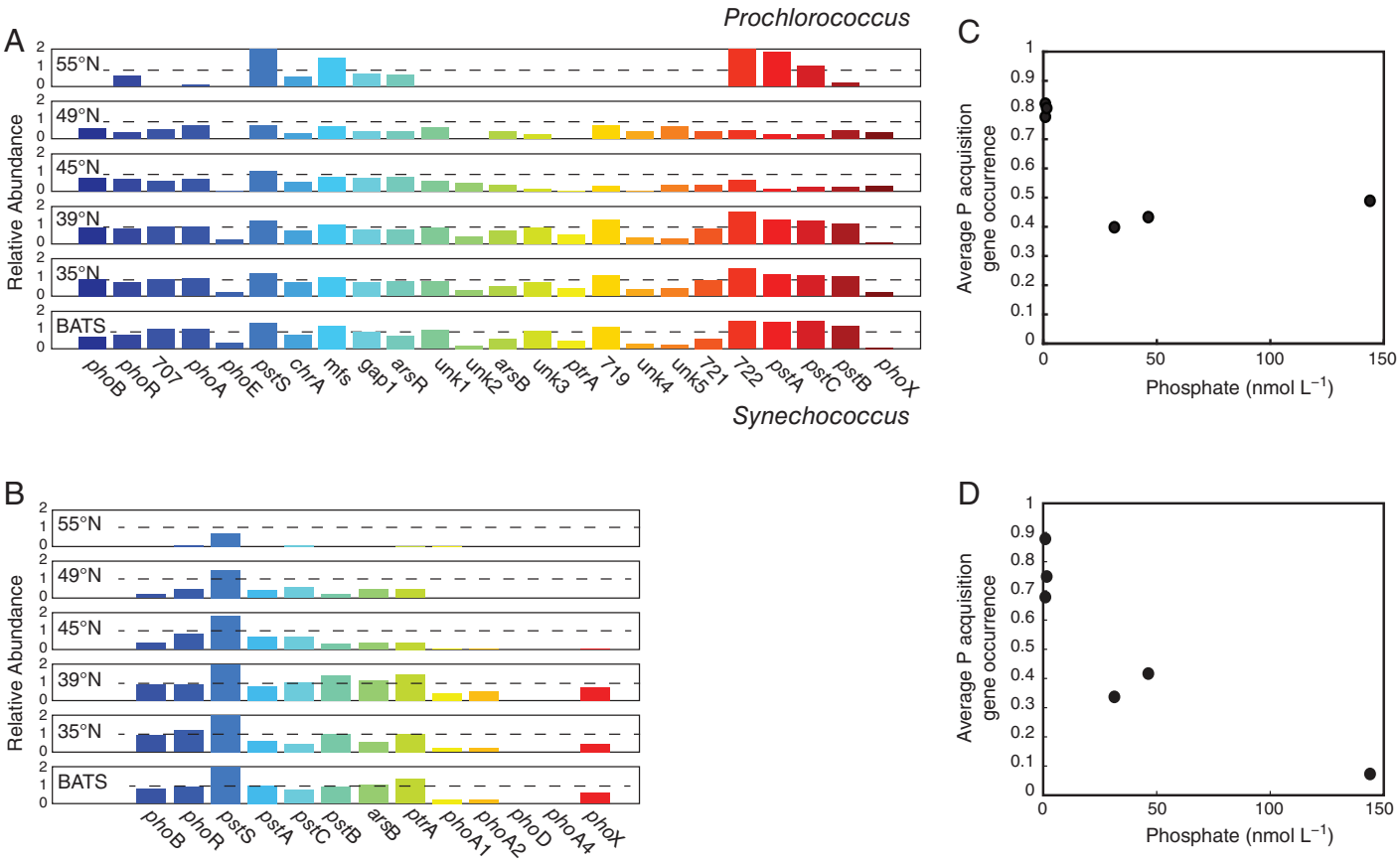

Fig. 7. Occurrence of P acquisition genes in *Prochlorococcus* and *Synechococcus* populations. (**A**) Frequency of P acquisition genes in *Prochlorococcus* (colored uniquely). Orthologs of these genes are upregulated under P stress in *Prochlorococcus* MED4 (Martiny et al. 2006) with exception of *pho*X which is simply predicted to be part of P acquisition in *Prochlorococcus* (Kathuria and Martiny 2011). (**B**) Frequency of P acquisition genes in *Synechococcus* (colored uniquely). The genes have previously been proposed as important for P acquisition including four alkaline phosphatases orthologous to WH8102_2390, WH8102_0196, WH7803_1802, and RS9916_40496 (Scanlan et al. 2009). The frequency of each gene is estimated as the ratio of average occurrence of gene orthologs affiliated with *Prochlorococcus* or *Synechococcus* divided by the median occurrence of core genes for each lineage. (**C**) The relationship between average gene occurrence of P acquisition genes in *Prochlorococcus* and ambient phosphate concentration. (**D**) The relationship between average gene occurrence of P acquisition genes in *Synechococcus* and ambient phosphate concentration. A detailed description of the genes is listed in Supporting Information Table S1.

The experiments revealed that phytoplankton lineages respond distinctively to changing environmental conditions. There was often a mismatch in response of picoeukaryotic phytoplankton vs. *Prochlorococcus* and *Synechococcus*. Picoeukaryotic phytoplankton uptake rates followed the community response including a strong stimulation of uptake by available phosphate. In contrast, *Prochlorococcus* and *Synechococcus* showed a strong effect of local adaptation. These divergent responses could suggest that some lineages (including picoeukaryotic phytoplankton) are better adapted to responding to pulses of higher nutrient water, whereas the small Cyanobacteria respond slowly but with high affinities for nutrient uptake (Fawcett et al. 2011; Lomas et al. 2014; Kretz et al. 2015). Thus, the divergent responses of Cyanobacteria vs. picoeukaryotic phytoplankton may be indicative of their unique ecological role in ocean nutrient cycling.

It is worth noting that reciprocal transplant experiments generally do not lead to a perfect recreation of the transplanted environments. In our experiment, important caveats are the disruption of trophic interactions, temperature levels, and the nearly month-long separation between the two occupations of BATS. First, our separation approach included microzooplankton, whereas viral particles were expected to pass through the filter and thus associated with the water phase. A study from the North Pacific Subtropical Gyre found that marine Cyanobacteria respond differently to unfiltered (particles, competitors, and grazers) vs. filtered deep-water (nutrients and viruses) additions (Robidart et al. 2018). Such biological interactions are challenging to capture in their entirety. Second, the temperature of the on-deck incubations matched the local environment (and thus not any reciprocal environments). Otherwise, the plankton would become thermally stressed, leading to cell death. To partially address any thermal kinetics effects (Aksnes and Egge 1991), we always did the actual P uptake measurements (i.e., after adding the radio-label) at a fixed temperature. However, we cannot rule out that there could be unaccounted covariates. Third, we occupied BATS at two separate time points. The

environmental conditions (extremely low phosphate) and phytoplankton composition (high Cyanobacteria abundances) were similar. However, the conditions were not identical as phosphate concentration and picoeukaryotic phytoplankton abundance were slightly elevated during the initial sampling. We do not expect these three caveats to have a major impact on the observed P uptake rates, but we are unable to constrain such uncertainties.

Earth System Models generally agree upon a future decline in the vertical nutrient supply due to thermally driven stratification leading to wide-spread decreases in net primary production (Bopp et al. 2013). As additional nitrogen can be supplied via nitrogen fixation, phytoplankton may increasingly become P stressed. Our and earlier work show that phytoplankton have a large adaptive potential to at least partially overcome P stress (Moore et al. 2005; Martiny et al. 2006; Lomas et al. 2014). However, adaptation is rarely considered in biogeochemical models for how marine phytoplankton will respond to climate change. Higher nutrient uptake affinity and utilization of alternative organic forms of P may at least partially compensate for the growth response to future lower P supply. Thus, we may currently overestimate the decline in net primary production due to nutrient stress.

Linking genes and community diversity with ocean biogeochemical cycles is a major scientific and technical challenge, and most studies rely on correlations. However, a reciprocal transplant experiment is a possible tool for quantifying the relative role of acclimation vs. adaptation in regulating important open ocean ecosystem processes. Furthermore, the addition of cell-sorting and metagenomics allows us to quantify how specific lineages contribute to biogeochemical fluxes in marine environments. Thus, we would like to advocate for more development of field experimental tools in marine microbiology and biogeochemistry in order to further quantify the role of microbial diversity in regulating ocean biogeochemical cycles.

References

Aksnes, D. L., and J. K. Egge. 1991. A theoretical model for nutrient uptake in phytoplankton. Mar. Ecol. Prog. Ser. **70**: 65–72. doi:10.3354/meps070065

Ammerman, J. W., R. R. Hood, D. A. Case, and J. B. Cotner. 2003. Phosphorus deficiency in the Atlantic: An emerging paradigm in oceanography. Eos Trans. AGU **84**: 165. doi:10.1029/2003EO180001

Baer, S. E., M. W. Lomas, K. X. Terpis, C. Mouginot, and A. C. Martiny. 2017. Stoichiometry of *Prochlorococcus, Synechococcus*, and small eukaryotic populations in the western North Atlantic Ocean. Environ. Microbiol. **19**: 1568–1583. doi:10.1111/1462-2920.13672

Batmalle, C. S., H. I. Chiang, K. Zhang, M. W. Lomas, and A. C. Martiny. 2014. Development and bias assessment of a method for targeted metagenomic sequencing of marine cyanobacteria. Appl. Environ. Microbiol. **80**: 1116–1125. doi:10.1128/AEM.02834-13

Biller, S. J., and others. 2014. Genomes of diverse isolates of the marine cyanobacterium *Prochlorococcus*. Sci. Data **1**: 140034. doi:10.1038/sdata.2014.34

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics **30**: 2114–2120. doi:10.1093/bioinformatics/btu170

Bopp, L., and others. 2013. Multiple stressors of ocean ecosystems in the 21st century: Projections with CMIP5 models. Biogeosciences **10**: 6225–6245. doi:10.5194/bg-10-6225-2013

Bostrom, K. H., K. Simu, A. Hagstrom, and L. Riemann. 2004. Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limnol. Oceanogr.: Methods **2**: 365–373. doi:10.4319/lom.2004.2.365

Casey, J. R., M. W. Lomas, J. Mandecki, and D. E. Walker. 2007. *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum. Geophys. Res. Lett. **34**. doi:10.1029/2006GL028725

Casey, J. R., M. W. Lomas, V. K. Michelou, S. T. Dyhrman, E. D. Orchard, J. W. Ammerman, and J. B. Sylvan. 2009. Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic. Aquat. Microb. Ecol. **58**: 31–44. doi:10.3354/ame01348

De Villemereuil, P., O. E. Gaggiotti, M. Mouterde, and I. Till-Bottraud. 2016. Common garden experiments in the genomic era: New perspectives and opportunities. Heredity **116**: 249–254. doi:10.1038/hdy.2015.93

Delmont, T. O., and A. M. Eren. 2018. Linking pangenomes and metagenomes: The *Prochlorococcus* metapangenome. PeerJ **6**: e4320. doi:10.7717/peerj.4320

Edwards, K., M. Thomas, C. A. Klausmeier, and E. Litchman. 2012. Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. Limnol. Oceanogr. **57**: 554–566. doi:10.4319/lo.2012.57.2.0554

Eren, A. M., Ö. C. Esen, C. Quince, J. H. Vineis, H. G. Morrison, M. L. Sogin, and T. O. Delmont. 2015. Anvi'o: An advanced analysis and visualization platform for 'omics data. PeerJ **3**: e1319. doi:10.7717/peerj.1319

Fawcett, S. E., M. W. Lomas, J. R. Casey, B. B. Ward, and D. M. Sigman. 2011. Assimilation of upwelled nitrate by small eukaryotes in the Sargasso Sea. Nat. Geosci. **4**: 717–722. doi:10.1038/ngeo1265

Karl, D. M. 2014. Microbially mediated transformations of phosphorus in the sea: New views of an old cycle. Ann. Rev. Mar. Sci. **6**: 279–337. doi:10.1146/annurev-marine-010213-135046

Karl, D. M., and G. Tien. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol. Oceanogr. **37**: 105–116. doi:10.4319/lo.1992.37.1.0105

Kathuria, S., and A. C. Martiny. 2011. Prevalence of a calcium-based alkaline phosphatase associated with the marine cyanobacterium *Prochlorococcus* and other ocean bacteria.

Environ. Microbiol. **13**: 74–83. doi:10.1111/j.1462-2920.2010.02310.x

Kent, A. G., S. E. Baer, C. Mouginot, J. S. Huang, A. A. Larkin, M. W. Lomas, and A. C. Martiny. 2019. Parallel phylogeography of *Prochlorococcus* and *Synechococcus*. ISME J. **13**: 430–441. doi:10.1038/s41396-018-0287-6

Kettler, G. C., and others. 2007. Patterns and implications of gene gain and loss in the evolution of *Prochlorococcus*. PLoS Genet. **3**: e231. doi:10.1371/journal.pgen.0030231

Kretz, C. B., D. W. Bell, D. A. Lomas, M. W. Lomas, and A. Martiny. 2015. Influence of growth rate on the physiological response of marine *Synechococcus* to phosphate limitation. Front. Microbiol. **6**: 85. doi:10.3389/fmicb.2015.00085

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods **9**: 357–359. doi:10.1038/nmeth.1923

Lin, S., R. W. Litaker, and W. G. Sunda. 2016. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. J. Phycol. **52**: 10–36. doi:10.1111/jpy.12365

Lomas, M. W., A. L. Burke, D. A. Lomas, D. W. Bell, C. Shen, S. T. Dyhrman, and J. W. Ammerman. 2010. Sargasso Sea phosphorus biogeochemistry: An important role for dissolved organic phosphorus (DOP). Biogeosciences **7**: 695–710. doi:10.5194/bg-7-695-2010

Lomas, M. W., J. A. Bonachela, S. A. Levin, and A. C. Martiny. 2014. Impact of ocean phytoplankton diversity on phosphate uptake. Proc. Natl. Acad. Sci. USA **111**: 17540–17545. doi:10.1073/pnas.1420760111

Martiny, A. C., M. L. Coleman, and S. W. Chisholm. 2006. Phosphate acquisition genes in *Prochlorococcus* ecotypes: Evidence for genome-wide adaptation. Proc. Natl. Acad. Sci. USA **103**: 12552–12557. doi:10.1073/pnas.0601301103

Martiny, A. C., Y. Huang, and W. Li. 2009. Occurrence of phosphate acquisition genes in *Prochlorococcus* cells from different ocean regions. Environ. Microbiol. **11**: 1340–1347. doi:10.1111/j.1462-2920.2009.01860.x

Martiny, A. C., Y. Huang, and W. Li. 2011. Adaptation to nutrient availability in marine microorganisms by gene gain and loss, p. 269–276. *In* F. De Bruijn [ed.], Handbook of molecular microbial ecology II. John Wiley & Sons. doi:10.1002/9781118010549.ch26

Martiny, J. B. H., and others. 2017. Microbial legacies alter decomposition in response to simulated global change. ISME J. **11**: 490–499. doi:10.1038/ismej.2016.122

Moon-van der Staay, S. Y., R. De Wachter, and D. Vaulot. 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature **409**: 607–610. doi:10.1038/35054541

Moore, L. R., M. Ostrowski, D. J. Scanlan, K. Feren, and T. Sweetsir. 2005. Ecotypic variation in phosphorus-acquisition mechanisms within marine picocyanobacteria. Aquat. Microb. Ecol. **39**: 257–269. doi:10.3354/ame039257

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press.

Reed, H. E., and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiol. Ecol. **62**: 161–170. doi:10.1111/j.1574-6941.2007.00386.x

Robidart, J. C., J. D. Magasin, I. N. Shilova, K. A. Turk-Kubo, S. T. Wilson, D. M. Karl, C. A. Scholin, and J. P. Zehr. 2018. Effects of nutrient enrichment on surface microbial community gene expression in the oligotrophic North Pacific Subtropical Gyre. ISME J. **13**: 374–387. doi:10.1038/s41396-018-0280-0

Rusch, D. B., and others. 2007. The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through eastern tropical Pacific. PLoS Biol. **5**: e77. doi:10.1371/journal.pbio.0050077

Scanlan, D. J., and others. 2009. Ecological genomics of marine picocyanobacteria. Microbiol. Mol. Biol. Rev. **73**: 249–299. doi:10.1128/MMBR.00035-08

Thingstad, T. F., and others. 2005. Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. Science **309**: 1068–1071. doi:10.1126/science.1112632

Tovar-Sanchez, A., S. A. Sanudo-Wilhelmy, M. Garcia-Vargas, R. S. Weaver, L. C. Popels, and D. A. Hutchins. 2003. A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. Mar. Chem. **82**: 91–99. doi:10.1016/S0304-4203(03)00054-9

Tyrrell, T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. Nature **400**: 525–531. doi:10.1038/22941

Whitney, L. P., and M. W. Lomas. 2016. Growth on ATP elicits a P-stress response in the picoeukaryote *Micromonas pusilla*. PLoS One **11**: e0155158. doi:10.1371/journal.pone.0155158

Zwirglmaier, K., and others. 2008. Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. Environ. Microbiol. **10**: 147–161. doi:10.1111/j.1462-2920.2007.01440.x

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Conflict of Interest

None declared.

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