

Nitrogen and phosphorus differentially control marine biomass production and stoichiometry

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Globally averaged, surface particulate nitrogen and phosphorus approximate the 16:1, N:P “Redfield ratio.” In observations, N:P ratios vary latitudinally at ranges attributable to both phytoplankton community composition and physiological acclimation, but their relative contributions to the N:P ratio remain unclear. Here, results from a 29-day mesocosm experiment highlight how inorganic nitrogen and/or phosphorus nutrient supply can affect the bulk particle stoichiometry of a North Pacific Subtropical Gyre plankton community. Nitrogen additions, with and without phosphorus, increase total productivity and diatom abundance, whereas treatments with just phosphorus additions remain similar to the no-nutrient addition control. Continual nitrogen supply without phosphorus results in higher particulate N:P ratios than expected based on the phytoplankton community present. Several P-stress markers identified in those treatments highlight the importance of acclimation in extending particulate N:P ratios beyond the Redfield ratio. Phytoplankton’s ability to maintain growth under P-stress conditions has implications for global carbon cycling.

Nearly 100 years ago, Alfred Redfield reported on the remarkably similar relationship between the molar ratios of carbon, nitrogen and phosphorus in marine particulate organic matter (POM) (106 C: 16 N: 1 P; Redfield ratio) and deep water dissolved nutrient ratios

($16\text{NO}_3^-: 1\text{PO}_4^{3-}$) across ocean basins^{1,2}. The relative constancy of the Redfield ratio has led to its use as a benchmark for identifying potential nutrient stress in marine surface waters and for modeling marine biogeochemical processes. Observed N:P ratios of surface particles,

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however, vary latitudinally³ at a range notably captured by both the N:P requirements of different phytoplankton species^{4,5} (an attribute of species adaptation to specific growth conditions) and the measured nutrient flexibility within individual taxa^{6–8} (an attribute of physiological acclimation to changing environmental conditions). Nutrient supply rates have been identified as a key regulator of particulate C:N:P ratios in field observations⁹, and inverse models similarly support a relationship between nutrient supply and particulate biomass N:P stoichiometry across ocean regimes from nutrient-rich upwelling zones to nutrient-poor oligotrophic gyres¹⁰. Biological regulation of particulate C:N:P has significant implications for the efficacy of the marine C pump^{11,12}, and thus carbon storage in the oceans. However, since nutrients influence both phytoplankton community composition, and are a major driver of cellular acclimation, it remains challenging to determine how nutrient supply dictates biological regulation of particulate C:N:P.

The relative contributions of phytoplankton community composition and acclimation on particulate C:N:P is often evaluated through field observations informed by plankton culture studies. Cultures provide the environmental control that field observations cannot, but they often lack inter-species competition and rare or slow-growing organisms critical to shaping community composition. Mixed communities as well as the presence of detritus can mask cellular-level stoichiometry changes within the POM pool. The relative timescales of community restructuring and cellular acclimation also differ, with physiological responses occurring within the lifetime of a cell and changes in community composition occurring on the longer timescale of cell growth and replication balanced by mortality and grazing¹³. If bulk particle stoichiometry is driven by phytoplankton community composition, one may expect particulate C:N:P stoichiometry to shift in tandem with community restructuring. The timescale and magnitude for which acclimation imprints on bulk particle stoichiometry in natural communities is less clear.

We used mesocosms, which provide the control of cultures and the complexity of natural systems, to explore how phytoplankton community composition and cellular acclimation influenced bulk particulate stoichiometry under the experimental conditions. An oligotrophic plankton community was collected from 15 m depth in the North Pacific Subtropical Gyre near Oahu, Hawai'i, USA and incubated in 115 L mesocosm chambers using semi-continuous culturing techniques¹⁴. The community was supplied with inorganic nutrients once daily (except days 5 and 6) at concentrations intended to mimic high and low nutrient supply regions of the ocean. The incubation lasted 29 days to capture community shifts arising from fast and slower growing organisms. This semi-continuous approach allowed us to sample for a variety of biological and chemical features of the community at maximum five times per week and at minimum once at the end of the experiment depending on the sample water requirement (Table S1). Sampling frequency was not short enough to capture instantaneous responses to the nutrient amendment, but did capture integrated responses retained in the particulate pool over time as one might encounter in field observations. This fully replicated experiment provided a unique opportunity to evaluate how resource supply rates led to distinct patterns of particulate C:N:P within the experimental chambers.

Results and Discussion

Carbon fixation and C:N:P stoichiometry were nutrient dependent

Experimental treatments consisted of four unique N:P nutrient supply ratios added in small, daily doses: -N-P (negative control, no nutrients added), -N+P (+P = 9.4 nmol phosphate L seawater⁻¹ per day), +N-P (+N = 150 nmol N L⁻¹ seawater per day added as 1:1 nitrate: ammonium), and +N+P (N:P = 16). The four macronutrient treatments consisted of

two sub-treatments run in triplicate, one with iron addition (+Fe) and the other without (-Fe; see Methods). Iron concentrations were 1.1 nM in the starting seawater¹⁴ and Fe had no significant influence on the concentration of particulate C, N, P, chlorophyll *a* (chl *a*), or gross oxygen production at any point in time within the N:P groupings (distribution of pairwise *p*-values indistinguishable from chance, Table S2). The Fe sub-treatments are differentiated in figures for transparency. The seawater used to fill the mesocosms had phosphate and nitrate plus nitrite concentrations of 67 nM and 41 nM, respectively (Fig. S1), which is within the range of concentrations measured in the region¹⁵ and in surface waters (<50 m) of the North Pacific Subtropical Gyre in August for the Hawaii Ocean Time-series using the same nutrient analytical techniques (mean ± standard deviation: 64 ± 29 nM and 23 ± 19 nM, respectively; <https://hahana.soest.hawaii.edu/hot/hot-dogs> accessed December 2024).

Adding N led to increases in chl *a* (a proxy for phytoplankton biomass) and gross oxygen production (a measure of photosynthetic rates) (Fig. S2). After an initial peak in chl *a* on day 5 (Fig. S2a), the N supply rate was such that surplus nitrate was not detected when sampled 16 hours after nutrient spikes were added (Fig. S1a). A rapid decline in chl *a* occurred after day 5, possibly because of the two-day pause in nutrient addition, before it started to accumulate again on day 7. Whether the nutrient pause influenced particle stoichiometry or the sequential timing of biological responses remains speculative with the data in hand. Low residual nitrate concentrations and increased productivity with N supply indicated that N was the nutrient proximally limiting net phytoplankton production throughout the experiment. Phosphate was drawn down by day 7 of the experiment to the method detection limit (2 nM) in the +N-P treatments (Fig. S1b), and as low as 7 nM in the +N+P treatments. A gradual depletion of phosphate was observed in the -N-P treatments to a minimum of 18 nM, while the -N+P treatments increased to > 100 nM phosphate (Fig. S1b). Chl *a* and photosynthetic rates remained unchanged in the -N-P treatments. Productivity measures (chl *a* and photosynthesis) were consistently different between the +N and -N treatments throughout the experiment according to Tukey's HSD Test (*p* < 0.05), but not within the treatment groupings (+N-P versus +N+P, and -N-P vs -N+P, *p* > 0.05; Table S3, S4) even though the +P treatments were generally more productive than their -P counterparts after day 21 of the experiment (Fig. 1, S2).

In all +N treatments, increases in particulate N (Fig. 1b, S3b) were matched by proportional increases in particulate C (Fig. 1a,d, S3a,d), attributable to new phytoplankton production stimulated by the added N. Particulate C:N molar ratios ranged from 5.5 to 14.1 across all samples analyzed (mean 9.0; median 8.9), including the starting fill water replicates (range 6.5 to 12.0; Fig. S3d). There were few significant differences in C:N ratios between treatment groups or within a treatment over time (Tukey HSD significance here defined as *p* < 0.05; details in Table S3, S5). Yet, two distinct patterns emerged. First, the +N treatments had on average higher C:N ratios than the -N treatments for all days sampled, but only significantly so on day 11 (Tukey HSD *p* < 0.001), the first analysis day when diatom abundances became dominant in the eukaryotic composition of the +N treatments. Higher C:N ratios in the +N treatments could be related to differences in their unique community composition. Second, all treatments, even those without N addition, had increasing C:N ratios until experiment day 18, after which C:N stabilized. This pattern may reflect acclimation to the relatively high light levels used for the incubations¹⁶ (Fig. 1d, S3d).

While the C:N ratios remained overall statistically similar between treatments, the C:P and N:P ratios deviated based on the N supply rate and the N:P supply ratio, with significantly higher C:P and N:P in the +N-P treatments after day 18 of the experiment (Tukey HSD *p* < 0.05; Table S3), and similarly low ratios in the other three treatment groups (Fig. 1c,e,f and S3e,f; *p* > 0.05, Table S3). Stable N:P ratios and accumulation of phosphate in the -N+P treatments suggest that adding P

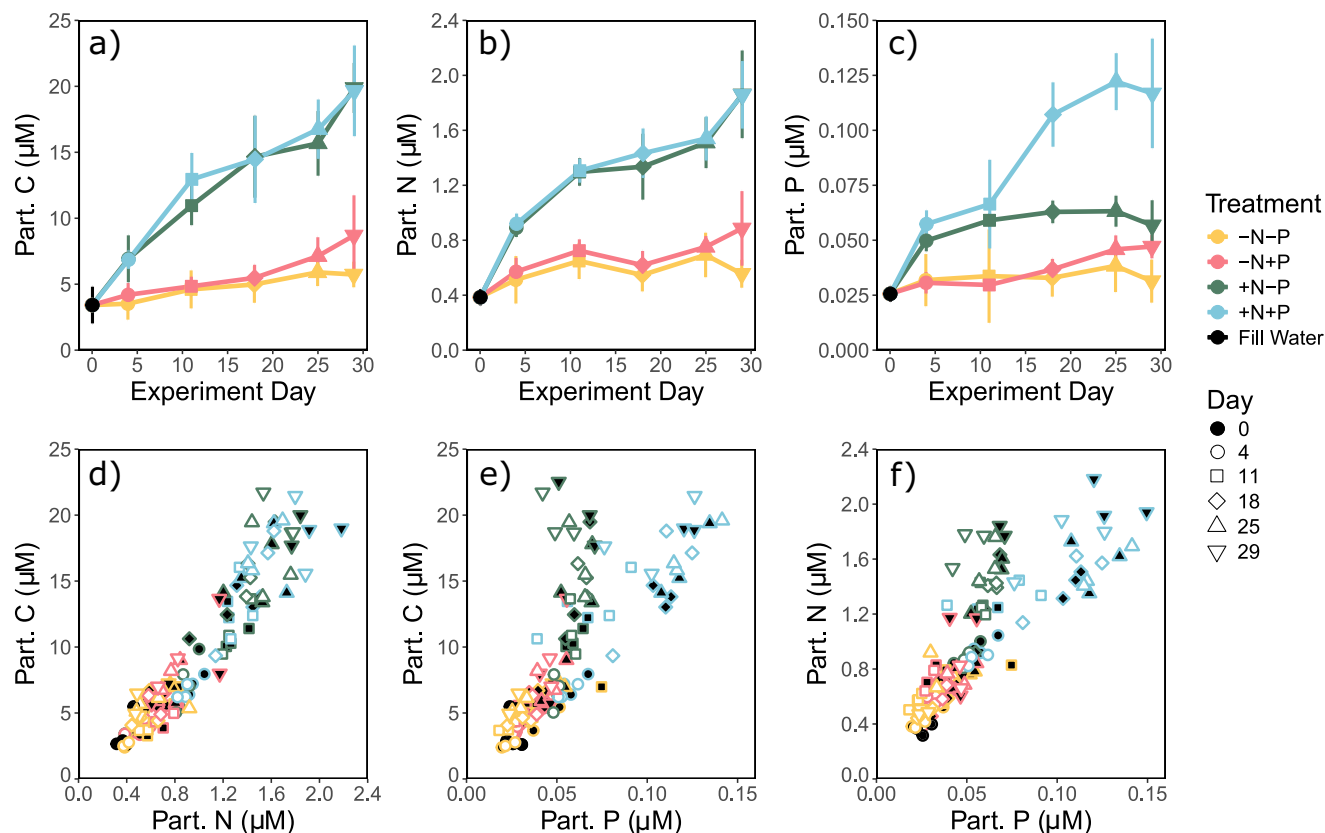


Fig. 1 | Particulate C:P and N:P are sensitive to the nutrient N:P supply ratio, while C:N is more constant. Concentrations of particulate carbon (C) (a), nitrogen (N) (b), and phosphorus (P) (c) in the incubations over time show that particulate C accumulation was stimulated by the addition of N and was equivalent whether or not P was added along with N. Error bars represent 1 σ for treatment replicates

($n = 6$, except for all -N+P, +N+P on day 11 in a–c, and -N-P on day 29 in c where $n = 5$). Also shown are relationships between particulate C and N (d), C and P (e), and N and P (f). Point shapes indicate sampling day. Sub-treatments are denoted in d–f by the point fill color; added iron (+Fe) treatments are filled black and no iron added (–Fe) treatments are filled white. Source data are provided as a Source Data file.

alone did not lead to cellular P accumulation when cells were N-limited. Our data are consistent with a non-linear, power law relationship between local phosphate concentration and particulate C:P ratios, as observed in the oceans¹⁷ (Fig. S4).

Nutrient supply influenced the final community composition

Community composition was measured weekly by 18S rRNA gene sequencing (Fig. S5) to examine relationships between N and P supply, community structure, and particulate C:N:P stoichiometry (See Table S1 for sample frequency). Most eukaryotic reads (>62 % in each sub-treatment) belonged to four major marine phytoplankton groups: diatoms, dinoflagellates, chlorophytes, and haptophytes (Fig. 2). Of these dominant groups, dinoflagellates were most abundant in the experiment fill water (Fig. S5). Metazoa, which made up roughly 21% of the starting community, were nearly absent from all treatments by day 11. Ciliates and Rhizaria had low relative abundances throughout the experiment in all treatments, whereas Syndiniales—diverse parasites—meaningfully contributed to the -N treatments until the end of the experiment (Fig. S5). Significant changes to the eukaryotic community occurred in response to N supply over time (ANOSIM; $p < 0.05$, Fig. 2a). Adding N led to an increase in the relative abundance of diatoms such that they constituted the eukaryotic majority from day 11 and onward (Fig. S5). Our ability to resolve how quickly diatoms restructured the community was set by the sampling frequency (days 0, 2, 11, 18, 25, and 29). However, the first chl *a* peak on day 5 (Fig. S2a) and dominance of diatoms by day 11 (Fig. S5) is consistent with incubation studies from temperate oligotrophic regions, which also suggest that diatoms restructure phytoplankton communities within ~6 days of resource addition^{18,19}. The maintenance of diatoms for weeks under continued N

supply in our experiment aligns with diatom community dominance in polar and coastal upwelling regions²⁰. The diatom composition was >90% *Pseudo-nitzschia* in three of the +N treatments (Fig. S6), and a mix of *Pseudo-nitzschia* and centric diatoms (primarily *Bacteriastrum* and *Chaetoceros*) in the +N-P-Fe treatment. Differences between the +N-P and +N+P treatments were statistically significant (ANOSIM $p < 0.05$). Community composition was not significantly different between the no nutrient control treatment (-N-P) and the P only treatment (-N+P; ANOSIM $p > 0.05$) suggesting P supply alone did not impact the community structure. The -N treatments were consistently dominated by dinoflagellates, as is typically observed at oligotrophic sites such as Station ALOHA²¹ (Fig. 2b, S5). Consistent changes in community composition were observed in response to the controlled nutrient supply treatments, though factors such as grazing and bottle effects may have influenced the final community structure.

Prokaryotic community structure as determined by 16S rRNA gene sequencing was similar among the different nutrient additions, though as with eukaryotes, the -N treatment communities were significantly different from the +N treatment communities (Fig. S7, S8; ANOSIM $p < 0.05$). Masked in the NMDS ordination analysis of community structure, and in our lumping of taxonomic species, are important species-specific dynamics. Notably, all treatments saw a decline in the relative abundance of *Prochlorococcus* (Fig. S9a), which is a common observation in long-term incubation studies^{22,23}. The persistence of *Synechococcus* (Fig. S9b), SAR11, and *Rhodobacterales* throughout the incubation (Fig. S9) show that other key oligotrophic bacteria were retained. The relative abundance of diazotrophic species increased in all -N treatments, but rarely in the +N treatments except

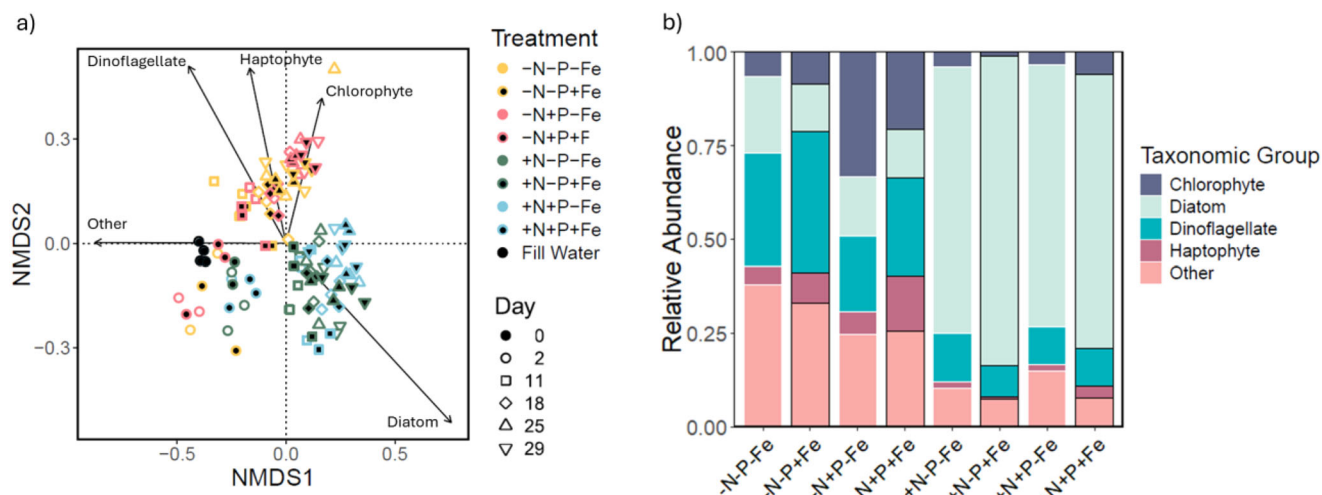


Fig. 2 | Eukaryote communities were restructured by nutrient supply. A non-metric multi-dimensional (NMDS) scaling plot of eukaryotic community structure separates based on treatment type (a), with loadings from the four major phytoplankton groupings used in this study shown in the black arrows. Community

taxonomy at the end of the experiment shows the relative abundance of the four phytoplankton groups (b). Treatments with iron added are filled black (a) or outlined in black (b). Data shown are the average of all incubation replicates for both days 25 and 29 taken together. Source data are provided as a Source Data file.

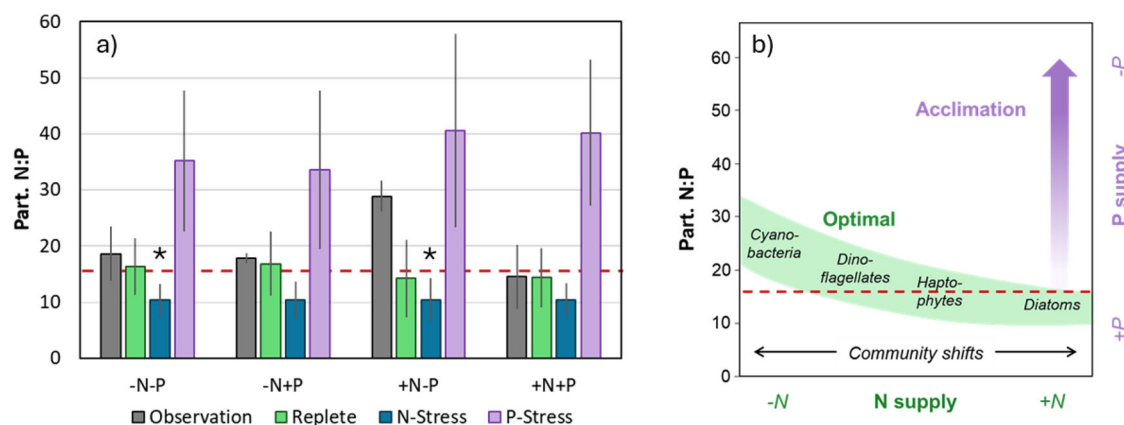


Fig. 3 | Relation between experiment results and phytoplankton dynamics. Observed N:P ratios in this study (grey, average of day 25 and 29) are compared to N:P calculated ratios based on the eukaryotic phytoplankton groups present in our incubations (average of day 25 and 29) using the meta-analysis results of Sharoni and Halevy⁵ for phytoplankton grown under nutrient replete (green), nitrogen-limited (blue), and phosphorus-limited (purple) conditions (a). Error bars represent 1 σ . Stars indicate which calculated N:P values are significantly different from the observation (Welch's t-test, $p < 0.05$, Observation $n = 12$ except for -N+P ($n = 10$) and

-N-P ($n = 11$); Replete $n = 64$; N-Stress $n = 37$; P-Stress $n = 42$). We suggest the underlying mechanisms that may drive the observed particulate N:P ratios (b) as both a function of community structure dictated by inorganic N supply to the system, and physiological acclimation mediated by P supply to the system. The optimal N:P ratios visualized by the green band were extracted from the Sharoni and Halevy⁵ meta-analysis, and the flexible N:P range visualized with the purple arrow is from Geider and La Roche⁶. The Redfield N:P ratio is shown as a red dashed line in a and b. Source data are provided as a Source Data file.

for a notable increase in *Richelia* in the +N+P-Fe treatment (Fig. S10). Even though diazotroph abundances increased in all -N treatments, only the -N+P treatments had elevated photosynthetic rates, particulate N, and chl *a* concentrations at the end of the experiment, suggestive of a N subsidy from diazotrophic N_2 fixation. Without quantitative N_2 fixation rates, we cannot confirm its influence on total productivity. We do note that the relative abundance of diazotrophs differed based on P and/or Fe supply (Fig. S10) which is important to consider for long-term nutrient regulation in the ocean²⁴.

Particle N:P stoichiometry is related to community composition

A recent study concluded that phytoplankton community composition is a good predictor of particulate stoichiometry across vastly different ocean regions, from nutrient-rich upwelling zones to oligotrophic gyres⁵. We used Sharoni and Halevy's⁵ meta-analysis of plankton grown in culture to calculate expected bulk particulate N:P

ratios for the final major phytoplankton assemblages in each treatment as if they were grown under nutrient replete, P-stress, or N-stress conditions. Only the four major eukaryotic plankton groupings listed above were included due to the absence of *Prochlorococcus* in our incubations (dinoflagellates and chlorophytes were grouped together⁵). In total, 62% to 92% of the final experimental communities were captured in the calculation (Fig. S11b). In all but the +N-P treatments, the observed ratios (which include all living organisms and detritus) resembled the calculated N:P ratios for cells grown without nutrient limitation (Fig. 3a). The +N-P treatments, where N was continuously supplied without P, exhibited a particulate N:P stoichiometry reflective of P-stressed eukaryotic plankton in culture⁵. Our incubations therefore support the hypothesis that phytoplankton community composition is indicative of particulate N:P ratios based on the stoichiometry of locally well-adapted species not expressing nutrient limitation.

To better understand the relationship between N:P and community composition, we plotted the Bray-Curtis dissimilarity of 18S ASV relative abundance between pairs of treatments as a function of the Euclidean distances of their N:P ratios (Fig. S12). This calculation considers all eukaryotes sequenced down to their lowest classification level, even zooplankton, which could contribute meaningfully to bulk particulate C:N:P ratios²⁵. A significant, positive relationship was found between the community dissimilarity and N:P Euclidean distance (Spearman $\rho = 0.26$, $p < 0.001$) as would be expected when differences in community composition are associated with differences in N:P ratios. However, there are exceptions, for example, the +N-P and +N+P treatment-comparisons which appear in the upper left quadrant of the figure. These show similar community composition with very different N:P attributable to physiological acclimation. Community composition in our experiments was thus often a good predictor of N:P, but community alone could not explain the range of particulate N:P observed unless acclimation was invoked (Fig. 3b).

Physiological acclimation to P supply drives large shifts in particle stoichiometry

Cellular phenotype is largely determined by genetic factors, hence the generalized relationship between community composition and bulk particulate stoichiometry, but it can change in response to environmental factors, including both N and P availability^{13,16}. Acclimation, the process of sensing and responding to a shift in resources, occurs on the order of minutes to hours for the microeukaryotes we focus on here²⁶, though the timescale over which the resulting acclimation response manifests as measurable changes in bulk C:N:P may be longer. To examine how physiological acclimation impacted C:N:P stoichiometry, we compared the +N-P and +N+P treatments at the final time point of the experiment. Both of these treatments had final timepoint communities dominated by diatom species (Fig. 2) with the same particulate C:N ratios (Fig. 4a), but the particulate N:P and C:P ratios without P added diverged from those with P added (Figs. 1, 4b, c, S3).

Phytoplankton acclimation to low P in culture studies often involves a shift from growth on phosphate to other, less easily accessible, sources of P²⁷. The enzyme alkaline phosphatase (AP) helps cells to meet their P quota by hydrolyzing phosphate from organic P sources^{27,28}, and elevated AP expression has been used to identify P-stressed cells in open ocean surface waters²⁹. Although both +N treatments were depleted in measured phosphate (below 40 nM) and had measurable AP activity, AP activity and C-normalized AP activity doubled in the +N-P treatments relative to the +N+P treatments (Welch's t-test, $p = 0.047$, Fig. 4e), consistent with a community acclimation response to low P supply. However, this acclimation signal is not necessarily expected to correspond with a change in cellular N:P ratios due to a changed cellular P quota, if sufficient P can be acquired from dissolved organic P for growth.

When insufficient P is available for growth, phytoplankton can conserve it by modulating their cellular P pools^{30,31}. Cellular P requirements are predominantly for nucleic acids and lipids, and P can be stored intracellularly as polyphosphates¹⁶. Elevated particulate N:P ratios have been associated with cellular acclimation to P deficits in culture experiments (e.g.^{6,7}) and field assessments³², though similarly elevated particulate N:P ratios could be explained by accumulation of detrital material with a high N:P signature due to the preferential regeneration of P³³. Both acclimation and preferential regeneration of P likely occur and are important to maintaining biological productivity when P availability is scarce³⁴.

Cellular DNA is generally tied to genome size³⁵ and may not be expected to decrease in response to P-stress, whereas cellular RNA can fluctuate with changing growth conditions³⁶. Here, DNA and RNA as mol P per mol C were both lower in the +N-P treatments relative to the +N+P treatments, although the difference was only statistically significant for

DNA (Welch's t-test, $p_{DNA} = 0.033$ and $p_{RNA} = 0.074$, respectively; Fig. S13b,c). A closer look at the results revealed high variability between replicates (Fig. S13b), and a particularly low RNA replicate in the +N+P treatment that likely swayed the significance of the finding. The unexpected difference in DNA P content between treatments could be tied to detritus. Concentrations of the nucleotide adenosine triphosphate (ATP) normalized to C were reduced ($p = 0.056$) in the +N-P treatments (30.1 $\mu\text{mol ATP / mol C}$) relative to +N+P (57.8 $\mu\text{mol ATP / mol C}$; Fig. 4d), but no difference was found when normalized to particulate P (Fig. S14). The same ATP normalization trends were observed in P-starved phytoplankton cultures and interpreted as a cellular reduction in ATP in response to P stress^{37,38}, though others have argued that ATP: living C ratios are inflexible and low ATP: particulate C ratios imply a decrease in percent living biomass³⁹. Additionally, the nucleotide adenosine monophosphate was significantly reduced relative to adenosine in the +N-P treatments (Welch's t-test, $p = 0.0013$, Fig. 4f), as previously demonstrated for P-stressed eukaryotic cultures and P-stressed natural communities³⁰, congruent with an acclimation response as cells try to conserve cellular P.

Cells are thought to reduce their lipid P requirements by replacing P-containing lipid headgroups with non-P headgroups, such as in betaine-lipids³¹. Here, the C-normalized total lipid P concentrations were similar between the +N+P and +N-P treatments (Fig. S13d). However, the P-containing metabolites phosphocholine and glycerophosphocholine—precursors to phospholipids—were significantly depleted relative to the non-P containing metabolite choline in the +N-P treatments compared to the +N+P treatments (Welch's t-test, $p = 0.016$ and 0.0023 , respectively; Fig. 4g,h), supporting a P acclimation response. The ratio of N-based betaine lipids to phosphatidylcholine (BL: PC), an indicator of P stress in diatoms⁴⁰, was not significantly different between the +N-P and +N+P treatments (Welch's t-test, $p = 0.065$, Fig. 4i), suggesting lipid substitution was not a dominant P-saving strategy in the diatom communities. However, BL:PC was negatively correlated with phosphate across all treatment timepoints (Fig. S15; BL: PC Spearman $\rho = -0.64$, $p < 0.001$) supporting a moderate lipidome response to the decreased P availability observed in the +N treatments.

We found that P was distributed similarly between the measured P pools (ATP, DNA, RNA, and phospholipids) for both +N+P and +N-P treatments (Fig. S14e, Welch's t-test, $p = 0.58$). However, when normalized to particulate C, the relative abundance of all P-containing molecules was higher in the +N+P treatments compared to the +N-P treatments, and the C:P ratios were significantly lower in the +N+P treatments compared to the +N-P treatments (C:P = 249.8 and 503.2, respectively; Welch's t-test, $p = 0.0091$). Thus, while it is clear that P-stress likely contributed to the elevated bulk C:P and N:P particle ratios in the +N-P treatments, the increase could not be attributed to any one specific macromolecular pool.

Bulk and community level responses as discussed above are markers of nutrient limitation that have been clearly observed in phytoplankton cultures, but become challenging to interpret in mixed communities due to the presence of detritus and the combined influence of community composition and cellular acclimation on the bulk features. To overcome this, we examined differences in the relative transcript abundance of *Pseudo-nitzschia* in the +N-P and +N+P treatments where this diatom genus was the most abundant eukaryote (Fig. S6). Consistent with the community-scale measurements, there was a significant increase in the relative abundance of *Pseudo-nitzschia* AP transcripts in the +N-P treatments relative to the +N+P treatments (Fig. 4j; DESeq, $p = 0.002$). There was also a significant increase in the relative abundance of glycerophosphoryldiester phosphodiesterase (GDPD) transcripts in the +N-P treatment relative to the +N+P treatments (Fig. 4k; DESeq, $p = 0.026$), complementing results from P-stress eukaryotic cultures^{41,42}. The enzyme GDPD participates in lipid degradation by scavenging phosphate from internal phosphodiesters, which is consistent with the lower concentrations of phosphodiesters like

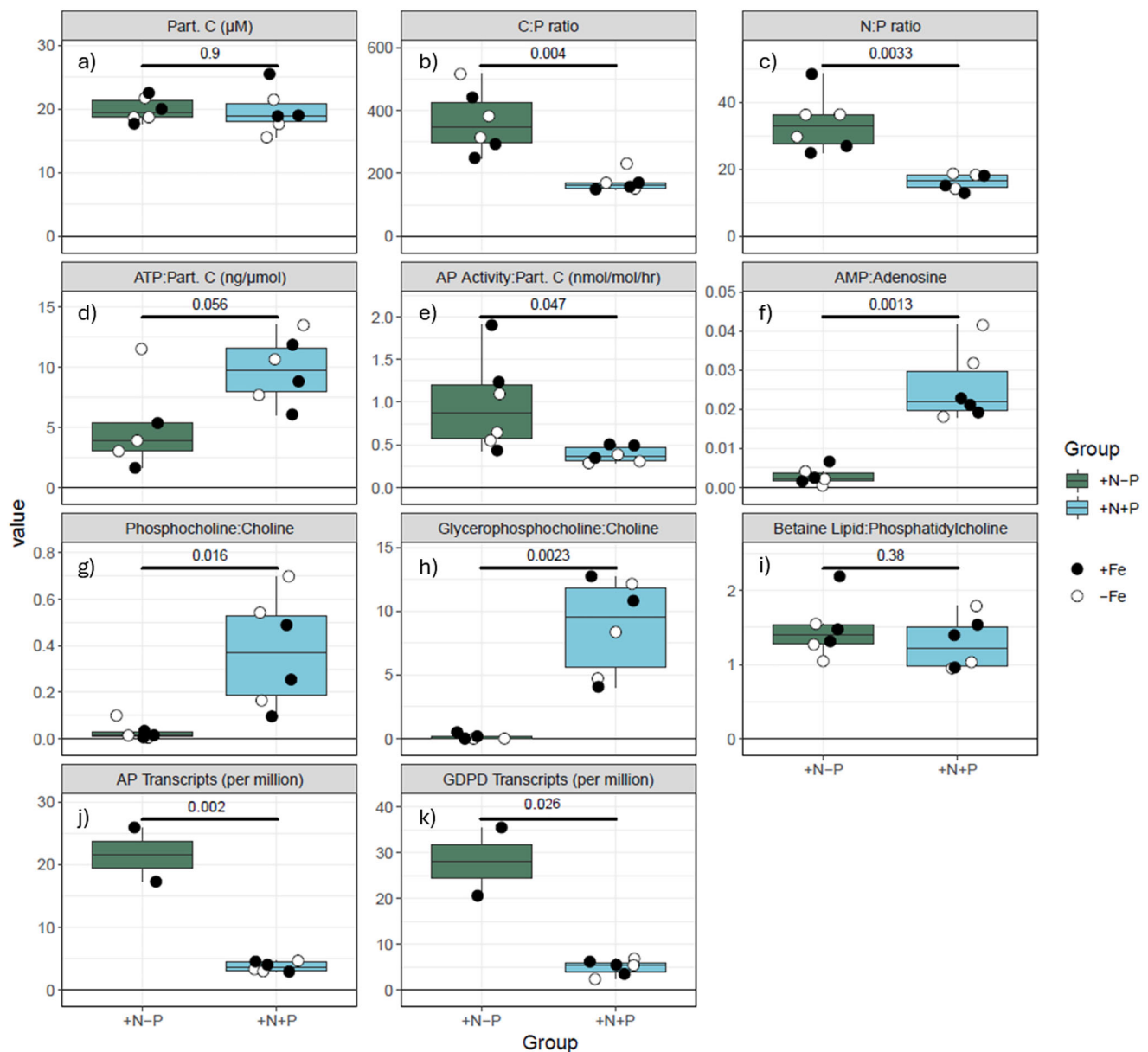


Fig. 4 | Multiple lines of evidence demonstrate that physiological acclimation to P-limitation took place in the +N-P (green) treatments and not the +N+P (blue) treatments. Plots reflect data at the final time point of the experiment, except alkaline phosphatase where the second to last time point was used due to anomalous values at the final time point. Particulate carbon (a) was similar between treatments, while particulate carbon:phosphorus (C:P) (b) and nitrogen:phosphorus (N:P) (c) ratios significantly differed. Reduced particulate adenosine triphosphate (ATP) per μmol C (d), elevated alkaline phosphatase activity (e), and reduced adenosine monophosphate (AMP) to adenosine ratios (f) have all been previously observed in P-stressed cultures. The P-containing lipid precursors phosphocholine and glycerophosphocholine both declined in the P-stressed treatment relative to choline (g, h), however, betaine lipid:phosphatidylcholine (i)

was similar in both treatments. *Pseudo-nitzschia* transcripts for the P-regulated alkaline phosphatase (j) and GDPD (k) indicate P-stress for this genus in the +N-P treatment. *Pseudo-nitzschia* was not abundant at the final timepoint in +N+P-Fe and was therefore excluded from the transcript analysis. Significance values have been calculated for each measured variable using Welch's t-test (for a–i) or DESeq2 (for j and k) as described in the “Statistical Approaches” section of the Methods. The box plots represent the data median (middle line), first quartile (lower hinge), and third quartile (upper hinge). Box whiskers extend to the smallest and largest data values within 1.5 times the inter-quartile range from the hinges. Significance between the treatment pairs was determined using Welch's t-test, and the resulting *p* values are printed above the horizontal black lines denoting the pairs. Source data are provided as a Source Data file.

glycerophosphocholine observed in our community-scale analysis (Fig. 4h). Both AP and GDPD are commonly up-regulated in response to low P in diatom cultures^{42–44}, and their expression patterns recapitulate the community responses in a single genus. These transcriptional patterns, as well as past evidence of elevated C:P ratios in P-stressed *Pseudo-nitzschia* cultures⁸, support the role of physiological acclimation as a control on particle stoichiometry. Taken together, the high bulk particulate C:P and N:P ratios measured when phosphate concentrations were low, combined with multiple indicators of cellular

acclimation to P-stress from the community level and within a single species, point toward the role of physiological acclimation as a contributor to changes in particle stoichiometry under conditions of P-stress.

Mesocosm insights into phytoplankton regulation of particulate C:N:P stoichiometry

Mesocosm incubations such as those presented here, where complex communities can be monitored under controlled inorganic nutrient

perturbations, provide a way to bridge the gap between culture experiments, short-term incubation studies, and the natural environment in two key ways. First, longer timeframes compared to typical incubations allowed us to explore how responses varied over days and weeks. Second, larger sample volumes allowed us to simultaneously track many types of responses. For example, the immediate response to inorganic N addition in our mesocosm experiment was consistent with shorter incubation studies from both the North Pacific^{22,45} and North Atlantic^{18,46} Subtropical Gyres, which find that added N is key to stimulating productivity. As we observed, N supply alone generally stimulated growth similar to N and P co-additions in prior experiments, including deep seawater addition⁴⁵, except in cases when the starting water was low in phosphate⁴⁶. We thus support that N is the 'proximal' limiting nutrient in much of the surface ocean as described by Tyrrell²⁴. With time, we captured the development of P-limited growth conditions, first noted as a decline in phosphate below 10 nM by day 7, followed by increased AP activity first detected day 11, then elevated N:P ratios detected after day 11. The resulting particulate N:P ratios are consistent with summertime observations made in the Bermuda Atlantic Time-series Study³⁴ (Fig. S4) and are within range of N:P observed when phytoplankton are grown in P-limited media⁵⁶. If the timescales of response are similar in our treatments to the natural environment, we may expect P-stress acclimation responses to manifest in the bulk particulate pool within a week of the onset of P-limitation.

We found that inorganic N supply in the absence of inorganic P supply drove bulk particulate stoichiometry far from the canonical Redfield ratio towards higher N:P (40:1 at the end of the experiment for +N-P treatments) without significantly slowing photosynthetic growth. Higher N:P and C:P ratios persisted for at least two weeks before the 29-day experiment was terminated, whereas the C:N ratio was relatively constant between treatments. Global climate models predict decreasing surface P concentrations due to changing ocean circulation patterns^{47,48}. Maintaining similar phytoplankton productivity under this scenario would require flexibility in cellular N:P akin to what was observed in our experiments. Further, stoichiometric flexibility is important because even small changes in global ocean C:N:P export ratios will lead to dramatic changes in the amount of carbon stored in the oceans¹¹, with implications for global climate. Our work thus illustrates the ways in which phytoplankton communities might respond to future changes in nutrient supply rates and N:P ratios and motivates further work exploring how nitrogen fixation and denitrification couple to return the oceans to a 16:1 ratio over timescales longer than the duration of our experiment.

Methods

Experiment implementation

Data were collected during PERI-FIX (Pelagic Ecosystem Research Incubator- Fe Incubation eXperiment), an experiment conducted at the University of Hawai'i Marine Center in Honolulu, HI, USA from August 9, 2021 to September 7, 2021, according to methods which are detailed in Seelen et al.¹⁴. Seawater for the incubations was collected between 2 AM and 5 AM HST south-west of Oahu (21.089° N, 158.074° W) in oligotrophic waters generally representative of the North Pacific Subtropical Gyre, such as Station ALOHA⁴⁹, the Hawaii Ocean Time-series study site⁵⁰. Unfiltered seawater was pumped from 15 m depth using an ARO non-metallic, polytetrafluoroethylene 1.5" air-operated double diaphragm (AODD) pump aboard the vessel *Hukī Pau* into 1 m³ high density polyethylene water totes. Prior to use, the totes were rinsed sequentially with 1% citranox, 1% HCl, and 4x with distilled water, and then rinsed 2x with seawater on station before filling. After water collection, the totes were covered in opaque, insulated shipping jackets to maintain dark and cool conditions during transport back to shore. Approximately nine metric tons of water were collected to fill the incubation tanks at the beginning of the experiment and to

replace water removed as samples were collected during the experiment.

Incubations were performed in twenty-four, 115 L PERIcosm incubation tanks that we designed with all plastic components, enclosed to minimize trace-metal contamination, and fitted with ports for sampling suspended, dissolved, and settled material¹⁴. The PERIcosms were operated inside a warehouse bay, within a tented enclosure cooled with portable air conditioning units to temperatures near 25 °C (25.4 ± 1.4 °C standard deviation). At the start of the experiment, unfiltered seawater was transferred from the totes to the PERIcosms using a 1/2" all-PFA AODD pump at a rate of ~9 L/minute. All PERIcosms were filled the same day seawater was collected. Light was supplied to each PERIcosm with a Kessil A360X refugium light set to 75% blue/ 25% red light at 100% brightness for 12-hour day/night cycles. The daily photon flux of ~8 mol quanta m⁻² d⁻¹ was chosen to match the light flux at approximately 45 m depth at Station ALOHA⁵¹.

Eight different nutrient amendment treatments were tested during the experiment. The nutrients included N, P, and Fe, and were added every afternoon of the experiment after sampling was complete, except days 5 and 6 which were skipped entirely. The +N treatments received 75 nmol of NH₄⁺ and 75 nmol NO₃⁻ per L of seawater (1:1 mol N), the +P treatments received 9.4 nmol PO₄³⁻ per L of seawater, and the +Fe treatments received 1 nmol FeCl per L seawater each day. The starting seawater Fe concentrations exceeded 1 nM¹⁴ and were likely not limiting at the start or throughout the experiment. The treatments were labeled according to nutrient addition (e.g., +N-P-Fe signifies only N was added). The treatments were reduced to four groups based on their N and P additions and include -N-P, -N+P, +N-P, and +N+P. Each treatment group consisted of 6 PERIcosm incubations except -N+P which included 5 PERIcosm incubations. Within that treatment, one -N+P-Fe tank started with outlying high C, N, P, and chl *a* concentrations on Day 4, and decreased through the remaining experiment therefore displaying an opposite pattern to the remaining -N treatments. That tank was removed from analyses, and the data are not presented. Small volume sampling for nutrients and chl *a* took place almost daily, negligibly decreasing the total incubation volume. Larger sampling events took place twice per week, removing either ~6% or ~20% of the tank volume to meet the water demand of our sampling regime. Sampling frequency is presented in Table S1. The volume removed was replenished using filtered seawater via the AODD pumping system with a Pall Acropak™ 0.2/0.8 µm cartridge filter connected in-line.

Samples were collected from a sampling port installed near the top of the PERIcosms after they were mixed by hand with a customized magnet-controlled paddle (see Seelen et al.¹⁴ for more details). Although not measured, we estimate the internal flow rate due to mixing is less than 1 m/sec. It is assumed that these samples represent the suspended particulate composition at the time of sampling. Each PERIcosm was connected from the sampling port to a peristaltic pump head allowing for rapid and simultaneous sample collection from all 24 tanks. For samples processed by vacuum filtration, seawater from the PERIcosms was collected in clean bottles and filtered off-line. Filtered samples could be directly acquired from the PERIcosm using filter cartridges. Sampling and analytical protocols for each sample type are detailed below as well as in a separate PERIcosm method paper¹⁴.

Detailed sampling protocol and analytical procedures

Particulate C, N, P, and ATP. The particulate C and N sampling took place mid-afternoon Hawaii Standard Time (HST). Depending on biomass levels, 1 to 2 L of sample water was removed from the PERIcosm and filtered onto a combusted 25 mm diameter glass fiber filter (Whatman GF/F) using a vacuum filtration manifold. The filters were then wrapped in combusted foil and stored at ~20 °C until they could be dried at 60 °C, pelleted, and analyzed at the University of Southern California on a 4010 Costech Elemental Analyzer. Acetanilide (C:N = 6.84) was used as a reference material to calibrate the system for

carbon and nitrogen at the start of the analytical run and after every 12 sample measurements⁵². Duplicate samples collected on day 25 of the experiment were analyzed with and without filter acidification to determine the contribution of particulate inorganic carbon in the particulate C measurements. On average, the particulate C concentrations increased with acidification by $20\% \pm 14\%$ ($n=19$), and particulate N by $7.5\% \pm 14\%$ ($n=19$). The cause of the variable results post acidification is unknown, potentially linked to when the filter duplicates were collected, but ultimately unable to be tested due to insufficient samples. The final particulate C and N values reported here were not corrected for this difference.

Sampling for particulate P took place between 7 and 9 am HST. One liter of water was removed from each PERLcosm and equally divided to provide paired particulate P (measured as particulate phosphate) and ATP samples. For particulate P, 0.5 L was filtered onto a combusted, acid washed (10% HCl) GF/F. The filters were stored in microcentrifuge tubes at room temperature until analysis. The filters were transferred to acid-clean, borosilicate tubes and ashed at 450 °C for 4.5 hours. The particulate P filters were subsequently leached in 10 mL 0.15 N HCl for one hour, mixed by vortexing, and centrifuged to remove filter debris. A 5 mL subsample of the supernatant was transferred into new polypropylene tubes and 0.5 mL of reagent mixture was added⁵³; see Hawaii Ocean Time-series analytical protocol at <https://hahana.soest.hawaii.edu/hot/protocols/protocols.html>. After one hour, the samples were analyzed by spectrophotometry at 880 nm using a 10 cm pathlength cell. For ATP, 0.5 L was filtered onto a 47 mm diameter GF/F and immediately extracted in 5 mL of boiling Tris buffer. The filters were extracted for 5 minutes, allowed to cool and then stored frozen (−20 °C) until analyzed using the firefly bioluminescence method⁵⁴; see Hawaii Ocean Time-series analytical protocol at <https://hahana.soest.hawaii.edu/hot/protocols/protocols.html>.

Statistics were run between sub-treatments and the treatment groups for all time-points of the study. The number of datapoints used was typically $n=3$ for sub-treatments and $n=6$ for the treatment groups. Exceptions are for -N+P-Fe for which $n=2$. Further, missing data-points result in $n=2$ for +N+P+Fe on day 11 (missing C, N, P, and ATP) and for -N+P+Fe on day 29 (missing P), which impacts their treatment groups on those days.

Dissolved nutrients

Dissolved nutrient samples were either obtained from the amplicon sequencing filtrate or by Pall AcropakTM filtration (0.8/0.2 µm Supor) directly from the PERLcosm. The filtered water was stored frozen at −20 °C in VWR[®] metal-free polypropylene centrifuge tubes or HDPE bottles until analyzed at the University of Hawai'i, Mānoa on a Seal Analytical AA3 HR Nutrient Autoanalyzer. Dissolved inorganic nitrogen was determined as the sum of nitrate + nitrite using a diazo reaction modified from Armstrong et al.⁵⁵ and Hansen and Koroleff⁵⁶. Phosphate was measured colorimetrically as orthophosphate using the automated procedure adapted from Murphy and Riley⁵³. The nutrient calibration standards were made in low nutrient seawater collected from the HOT 316 cruise in October 2019 using concentrated stocks of ACS grade sodium nitrite (NaNO₂) and monopotassium phosphate (KH₂PO₄) as detailed in the Hawaii Ocean Time-series analytical protocols (<https://hahana.soest.hawaii.edu/hot/protocols/protocols.html>). A method detection limit for phosphate and nitrate + nitrite was calculated as three standard deviations above the average concentration of the low nutrient seawater blank sample. The detection limit for phosphate was determined to be 0.004 µM, and samples below that concentration (10% of samples) were set to equal half the detection limit (0.002 µM). The nitrate + nitrite detection limit was 0.018 µM, and samples with concentrations below the detection limit (35% of samples) were set to a concentration of 0.009 µM. We note, however, that a high sensitivity chemiluminescence method for nitrate is more typically used for

seawater samples with less than 0.05 µM nitrate as detailed in the Hawaii Ocean Time-series analytical protocols.

16S and 18S rRNA gene sequencing

Samples for amplicon sequencing were collected between 11 am and 2 pm HST by filtering 2 L of sample water onto 0.2 µm pore size Supor disc filters using a peristaltic pump directly from the PERLcosms. The loaded filters were flash-frozen in liquid nitrogen and stored at −80 °C. DNA was extracted from the filters at the University of California Santa Cruz using a modified Qiagen DNeasy protocol⁵⁷ after 10 ng of Takara *Thermus thermophilus* HB8 genomic DNA was added to each sample. Amplicon libraries were prepared at the University of Southern California using 515Y-926R primers ([dx.doi.org/10.17504/protocols.io.vb7e2rn](https://doi.org/10.17504/protocols.io.vb7e2rn)), which simultaneously amplify regions of both 16S and 18S SSU rRNA genes to capture prokaryotic and eukaryotic taxa from the same sample^{58–60}. Agencourt AMPure XP beads ([dx.doi.org/10.17504/protocols.io.rfvd3n6](https://doi.org/10.17504/protocols.io.rfvd3n6)) were used to purify the PCR products before the samples were normalized, pooled, and sequenced on 2 lanes of a MiSeq v3 2 × 300 paired-end sequencing platform at the University of Southern California Molecular Genomics Core. The resulting sequences were processed through the bioinformatic pipeline described by Yeh and Fuhrman⁵⁹; [dx.doi.org/10.17504/protocols.io.vi9e4h6](https://doi.org/10.17504/protocols.io.vi9e4h6). The raw sequences were demultiplexed and rid of primers using cutadapt⁶¹ before separating the 16S and 18S reads using the bbtools package (<http://sourceforge.net/projects/bbmap/>) and a custom 16S and 18S database⁵⁹. Low quality reads were removed from the forward and reverse 16S reads by truncating at 210 and 180 base pairs (bp), respectively, and the resulting sequences were passed through DADA2 in the QIIME2 software to create ASVs^{62,63}. Taxonomic identification for the 16S bacterial and archaeal ASVs was assigned using the SILVA database (v. 138⁶⁴), and for the 16S chloroplast ASVs using the PhytoREF database (from PR2 v. 4.12⁶⁵). The 18S results were truncated at 210 and 170 bp for the forward and reverse reads, respectively, concatenated using BBduk within the bbtools package, and then used to generate ASVs using DADA2 in QIIME2^{62,63}. The 18S eukaryotic ASVs were assigned taxonomy using the PR2 database (v. 4.12⁶⁶) with default QIIME2 parameters. Contaminant ASVs were isolated and removed from the final ASV tables using the decontam package in R⁶¹.

Flow cytometry

Unfiltered samples for flow cytometry were collected mid-afternoon HST. The samples were kept in the dark and quickly transferred to cryovials. The samples were then fixed with 0.25% glutaraldehyde before flash freezing in liquid nitrogen and storage at −80 °C until analyzed at the University of Hawai'i, Mānoa on a Mariner Influx flow cytometer. We used the same flow cytometry analytical methods as reported for the Hawaii Ocean Time-series, which are adapted from Monger and Landry⁶⁷. Briefly, the forward light scatter, red fluorescence (692 nm), and orange fluorescence (580 nm) of each sample was detected to resolve the abundance of *Prochlorococcus*, *Synechococcus*, and picoeukaryotic algae. The results were processed using FlowJo software (Tree Star).

Gross oxygen production

Sampling to determine gross oxygen production by the ¹⁸O-H₂O method, as described by Ferrón et al.⁶⁸, occurred between 8 and 11 am HST. Water from each PERLcosm was sub-sampled into 1 L polycarbonate bottles and then siphoned into two 130 mL Pyrex[®] bottles with ground-glass stoppers. The incubation bottle was spiked with 650 µL of H₂¹⁸O (Medical Isotopes, 97% isotopic purity) to a final ¹⁸O-H₂O enrichment of ~2400‰, and then incubated for 5 to 7 hours in a water bath with similar temperature and light conditions as the PERLcosms. The incubation was terminated by pipetting 100 µL of saturated mercuric chloride solution to stop all biological activity. The stopper was then replaced with one that allowed the bottles to be closed without introducing bubbles. The

samples were inverted three times to mix and stored in the dark, immersed in water until analysis by membrane inlet mass spectrometry within 48 hours. The other bottle sampled from each treatment was fixed in the same way at the start of the incubation to preserve the initial conditions. Gross oxygen production was determined by the isotopic enrichment of the dissolved O₂ pool, following Ferrón et al.⁶⁸. More details can be found in Salazar Estrada⁶⁹.

Macromolecules

Bulk particulate RNA and DNA samples were collected late afternoon and filtered onto Nuclepore Track-Etched 47 mm diameter, 0.2 µm pore size polycarbonate filters by vacuum filtration. After filtration, the samples were flash frozen in liquid nitrogen and stored at −80 °C until analysis at Dalhousie University. The samples were processed and quantified from the same filter using a modified version of the fluorochrome SYBR Green II method^{70,71}. Bulk particulate lipid samples were collected late afternoon and filtered onto 47 mm diameter pre-combusted GF/F by vacuum filtration. The samples were preserved by flash freezing in liquid nitrogen and stored at −80 °C until they were analyzed at Dalhousie University. The RNA and DNA concentrations were converted to P concentrations using the following equations⁶: DNA-P (µM) = DNA (µg/L) * 0.091/30.97 and RNA-P (µM) = RNA (µg/L) * 0.095/30.97.

Metabolomics

Samples for metabolomics processing were collected between 6 and 7 pm HST by filtering 2 L of sample water onto 47 mm diameter, 0.2 µm pore size Durapore disc filters using a peristaltic pump. The loaded filters were flash-frozen in liquid nitrogen and then stored at −80 °C until analysis at the University of Washington. Samples were extracted using a modified Boysen et al. method⁷² and analyzed using HILIC chromatography on a Waters Acquity I-Class UPLC with injection volumes of 2 µl coupled to a Q Exactive Orbitrap mass spectrometer (MS). For HILIC, a SeQuant ZIC-pHILIC column (5 µm particle size, 2.1 mm × 150 mm, from Millipore) was used with 10 mM ammonium carbonate in 85:15 water to acetonitrile (Solvent A) and 10 mM ammonium carbonate in 85:15 acetonitrile to water (Solvent B) at a flow rate of 0.15 mL/minute. The column—maintained at 30 °C—was held at 100% B for 2 minutes, ramped to 64% A over 18 minutes, ramped up to 100% A over 1 minute, held at 100% A for 7 minutes, and equilibrated at 100% B for 22 minutes (total time is 50 minutes). The mass spectrometer was calibrated weekly in positive and negative modes using solutions provided by the manufacturer. For HILIC, a full scan method employing positive and negative switching was used with a scan range of 60 to 900 *m/z* and a resolution of 60,000. The capillary temperature was 320 °C, the H-ESI spray voltage was 3.5 kV, and the auxiliary gas heater temperature was 90 °C. The S-lens RF level was 65. Sheath gas, auxiliary gas, and sweep gas flow rates were maintained at 16, 3, and 1, respectively. To minimize retention time drift, the LC was kept running with the same solvents for the entire run, and Optima-grade LCMS water blanks were run after every 3–4 samples. For the quality control pooled samples, both high-resolution MS scans and data dependent MS/MS scans were collected and separate injections were done for positive and negative ion modes (30,000 resolution and a dynamic exclusion time of 20 seconds for MS/MS data, top five most abundant ions in each cycle were fragmented, collision energies of 20, 35, and 50 eV). The raw files were converted to *mzML* formats and centroided via ProteoWizard's *msconvert*⁷³. Chromatograms were then extracted using the *RaMS* package⁷⁴ in the R programming language with a 5 ppm *m/z* error tolerance and integrated using a trapezoidal Riemann sum without baseline subtraction.

Lipidomics

One liter of water for lipidomics was collected in polycarbonate bottles between 9 and 11 am HST. The samples were filtered using glass

vacuum filtration units onto 47 mm diameter, 0.22 µm pore-size Durapore PVDF membrane filters. Filters were wrapped in combusted aluminum foil, flash frozen in liquid nitrogen, then stored at −80 °C. The filters were shipped on dry ice to Woods Hole Oceanographic Institution for analysis, stored in liquid nitrogen until extraction, then extracted via a modified Bligh and Dyer procedure⁷⁵ according to Pependorf et al.⁷⁶. Samples were then analyzed on a Q-Exactive MS (ThermoFisher Scientific, Waltham, MA, USA) using an Agilent 1200 (Agilent, Santa Clara, CA, USA) high performance liquid chromatography system, following a modified method⁷⁷ originally described by Hummel et al.⁷⁸. Lipidomic compound annotation and analysis were all performed using ThermoFisher Scientific Xcalibur software and the R programming language, aided by the tidyverse data science package⁷⁹. Raw data files were converted to the *mzXML* format with the ProteoWizard Toolkit⁷³. Mass spectral features were then grouped and annotated using the R-based *xcms*^{80–82}, *CAMERA*⁸³, and *LOBSTASHS*⁷⁷ packages. Lipids were corrected for recovery using an internal dinitrophenolphosphatidylethanolamine (DNPPE) standard, and quantified using external standard curves of deuterated standards⁸⁴. Phospholipids were quantified using individual standards for each phospholipid class, and a diacylglycerol-trimethyl-homoserine (DGTS) standard was used for all N-based lipids; glycolipids were quantified using a glucosylceramide standard. Phosphatidylglycerol, DNPPE, and DGTS standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA); phosphatidylethanolamine, phosphatidylcholine, and glucosylceramide standards were purchased from Cayman Chemical (Ann Arbor, MI, USA). All samples were corrected for possible contamination by subtracting the average value of four procedural blanks from each identified lipid compound. Total lipid P was determined as the sum of all measured phospholipids in each sample.

Alkaline phosphatase activity

Sampling to determine alkaline phosphatase activity (APA) occurred between 9 and 11 am HST. Approximately 125 ml of sample was removed from each PERIcosm and vacuum filtered onto a 0.2 µm pore size, 47 mm diameter polycarbonate filter. The final volume filtered was recorded, and the filter was transferred to a 47 mm Petri dish and stored at −20 °C until analysis. Activity was assayed as previously described²⁸ using the fluorogenic phosphatase substrate 6,8-difluoro-4-methylumbelliferyl phosphate.

Metatranscriptomics

Metatranscriptome sampling took place prior to the genomic sampling, from 8 am to 11 am HST. Samples were collected directly from the PERIcosms using a peristaltic pump to filter 20 L of seawater per sample through 47 mm diameter, 5 µm pore size polycarbonate filters, which were placed in cryovials and flash-frozen and stored in liquid nitrogen until they were extracted. Total RNA was extracted from individual filters using a Qiagen RNeasy Mini Kit with a modified lysis step. Briefly, lysis buffer and Biospec zirconia/silica beads (0.5 mm) beads were added to each filter and vortexed for 1 minute, placed on ice for 30 seconds, and vortexed again for 1 minute. Lysate from each filter was removed with a pipette and pooled into a single 5 mL microcentrifuge tube. Synthetic standards (Ambion ERCC RNA Spike-in mix) were then added based upon estimated total RNA (using extraction values from prior samples collected in this region⁸⁵). The remainder of the Qiagen RNeasy Mini Kit protocol was then followed according to the manufacturer's instructions and incorporating the on-column DNase digestion step using a Qiagen RNase-free DNase kit. The resulting total RNA was eluted with RNase-free water and then purified and concentrated with a Qiagen RNeasy MinElute kit according to the manufacturer's instructions. The quantity and quality of extracted total RNA were assessed on an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA).

Treatment samples from the final sampling time point (day 29) and tote samples (day 0) were extracted and sequenced. Extracted samples were split into two equal volumes of total RNA, where one volume was sequenced after a polyA pull-down step (Illumina®TruSeq library preparation kit) to enrich for eukaryotic mRNA (analyzed herein). Illumina®TruSeq libraries were sequenced with an Illumina NovaSeq6000 at the JP Sulzberger Columbia Genome Center following Center protocols. RNA targeting just eukaryotic mRNA signals (selected reads) and RNA targeting both eukaryotes and prokaryotes (unselected reads) were sequenced to produce 80 million 100-bp, paired-end reads. Sequence reads are available at the Sequence Read Archive through the National Center for Biotechnology Information under BioProject ID PRJNA1062801.

Metatranscriptomic mapping pipeline is described in Harke et al.⁸⁶. Pre-processing and trimming of selected reads were done with FastQC (v 0.11.9)⁸⁷, Trimmomatic (v 0.3.9)⁸⁸, and bbmap (v 39.01) (<https://sourceforge.net/projects/bbmap/>) to remove ERCC standards using the bbdup function. Selected reads were aligned with BWA (v 1.1.4, parameters -k 10 -aM)⁸⁹ and counted with HTSeq (v 2.0.3, parameters -a 1 -m intersection-strict -s no)⁹⁰. Metatranscriptomic reads were mapped against a curated reference database including *Pseudo-nitzschia fraudulenta* (MMETSP0851, NCBI SRA SRX551153), *Braarudosphaera bigelowii* (assembled from Suzuki et al.⁹¹ using Trinity v 2.11.0⁹²), *Chaetoceros* sp. (MMETSP0200, SRA SRS619007), *Epithemia pelagica* (NCBI GCA_946965045.1), *Haptolina brevifila* (MMETSP1094, SRA SRS621505), *Hemiaulus sinensis* (NCBI GenBank GAUA00000000.1), and *Rhizosolenia setigera* (MMETSP0789, SRA SRS618823). Functional annotations were derived by combining available MMETSP Pfam annotations with EggNOG-mapper annotations (v 2.1.9)^{93,94}. All subsequent analysis was done in R (v 4.2.1). DESeq2 was used to determine the significant differential expression of genes between +N-P treatments (+Fe replicates only) and +N+P treatments (all +Fe and -Fe replicates). The +N-P-Fe treatments were not dominated by *Pseudo-nitzschia* at day 29 and were excluded from this comparison. One +N-P+Fe replicate was also excluded from analyses as it was an outlier in read recruitment. For visualization, reads mapped to genes characteristic of the diatom P-stress response^{42,43} were normalized to total mapped *Pseudo-nitzschia* reads, and expressed as tags per million (tpm).

Community based N:P calculations

We estimated particulate N:P ratios for the 8 sub-treatments and 4 treatment groups as if the eukaryotic communities present (average of days 25 and 29 of the experiment) were grown under replete, N-stress, and P-stress nutrient conditions. The major phytoplankton groups and their relative abundances were determined by adding the relative abundances of all ASVs that were annotated as Bacillariophyta (diatoms), Haptophyta (haptophytes), Chlorophyta (chlorophytes), and Dinophyceae (dinoflagellates). Chlorophytes and dinoflagellates were summed into one group for the N:P calculation as reported in Sharoni and Halevy⁵. The N:P ratios for each plankton group were extracted from the SI of Sharoni and Halevy⁵ using their classifier of growth condition (Replete, N-stress, and P-stress) and used to calculate a weighted mean with the community composition as the weights. Differences between the estimated N:P ratios for the three growth conditions and the observed N:P value for each treatment were tested using Welch's t-test and a weighted standard error.

Statistical Approaches

We used parametric pairwise comparisons to determine differences between treatments and timepoints. Differences between treatments in chl *a*, gross oxygen production, and particulate C, N, and P, as well as C:N, C:P, and N:P ratios were tested separately at each timepoint via

one-way ANOVA followed by two-tailed Tukey's HSD post-hoc test (Table S3). All 8 treatments were also compared independently (Table S4). We also compared this same set of measured variables across timepoints throughout the experiment again using a one-way ANOVA and Tukey's post-hoc test to determine the day of the experiment on which these variables began to significantly differ (Table S5). Statistics were performed using R's stats library, version 4.3.1. Differences between the +N-P and +N+P treatment groups in Fig. 3 were tested using unpaired, two-tailed Welch's two-sample t-tests using the ggpubr R package, version 0.6.0. The same approach was used to test significance in Figures S13 and S14.

The relative abundances of ASVs that were categorized as diatoms, dinoflagellates, haptophytes, and chlorophytes were summed for each day, replicate, and treatment. These summed relative abundances were visualized and used to evaluate broad shifts in plankton community composition over time. The ASV relative abundance data were also used to calculate the Bray-Curtis dissimilarity between every pair of samples in the dataset, and non-metric multidimensional scaling (NMDS) was used to visualize differences in Bray-Curtis dissimilarity over time and between treatments. Finally, analysis of similarities (ANOSIM) tests were carried out using the Bray-Curtis dissimilarities to determine whether specific treatment groups had significantly different 16S and 18S communities. All Bray-Curtis dissimilarity calculations, NMDS analyses, and ANOSIM analyses were conducted using the vegan package in R⁹⁵.

To determine whether there was a relationship between changes in plankton community composition and changes in the particulate N:P ratio, the Euclidean distances between the N:P ratio measurements were plotted as a function of the Bray-Curtis dissimilarity between every pair of samples collected on day 25 and day 29 of the experiment. Then, Spearman correlation tests were used to determine whether there was a significant correlation between community dissimilarity and the Euclidean distances of the N:P ratios. The distance metrics (Euclidean distances and Bray-Curtis dissimilarity) were calculated using the vegan package in R (Oksanen et al.⁹⁵, R Core Team 2024), and all Spearman correlation tests were carried out using the stats package in R (R Core Team 2024).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The metatranscriptome, 16S, and 18S ASV gene sequencing data generated in this study have been deposited in the NCBI BioProject database under accession code [PRJNA1062801](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1062801). All remaining data generated in this study are provided in the Supplementary Information and Source Data files. Source data are provided with this paper.

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Author contributions

EAS, SGJ, and ET designed the incubation experiment with input from co-authors. EAS and ET managed the experiment and executed it with

assistance from XB, KPK, KMB, AESE, SGJ, and DMK. The data were analyzed and interpreted as follows: particulate C and N by ET, nutrients by ET, particulate P and adenosine triphosphate by KMB and DMK, gross oxygen production by SF and AESE, macromolecules by ZVF, YYH, and AJI, gene sequencing by SJG, KTK, and DAC, flow cytometry by EAS, metabolites by WK and AEI, lipidomics by DL and BVM, alkaline phosphatase activity by STH and STD and metatranscriptomics by HSA, STD, STH, and JCT. EAS wrote the paper with SGJ and contributions from all co-authors.

Competing interests

The authors declare no competing interests.

Additional information

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