Accumulation and enhanced cycling of polyphosphate by Sargasso Sea plankton in response to low phosphorus

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Phosphorus (P) is an essential element for all living organisms. However, P can be extremely scarce in open-ocean surface waters such as in the subtropical western North Atlantic (the Sargasso Sea), where soluble reactive P (SRP) concentrations are routinely <10 nmol-L\textsuperscript{-1} and turnover rates are on the order of hours (1). Despite this scarcity, primary production by phytoplankton in the Sargasso Sea does not appear to be limited primarily by P (2, 3), reflecting the intensity of P recycling by the microbial community (4, 5) and the exquisite adaptations of marine phytoplankton to low P conditions, which remain to be fully characterized.

Phytoplankton respond to low P by producing enzymes such as alkaline phosphatase to hydrolyze extracellular dissolved organic P molecules (4, 6, 7), increasing the affinity and rate of P uptake (8, 9) and reducing their inventory of P-containing biochemicals (1, 10). In contrast, when P is abundant, phytoplankton take up excess P and store it as a luxury reserve that is generally thought to be composed of polyphosphate (polyP) (10–12). This modulation of P-containing biochemicals results in basin-scale relationships between P availability and biomass carbon-to-phosphorus (C:P) ratios (13). Presently, the only class of molecules known to contribute to these gradients in cellular P are lipids because P stress in phytoplankton triggers substitution of non-P-membrane lipids for phospholipids, such as the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) for the phospholipid phosphatidyglycerol (PG) (1, 14). However, lipid substitution alone probably cannot account for the full range of C:P observed in the ocean, and yet an understanding of other biochemical drivers of the C:P gradient remains elusive. Further, it is unknown how changes in plankton biochemical composition influence the recycling of nutrients.

Polyphosphate (polyP), a ubiquitous inorganic P polymer of three to hundreds of residues, has diverse physiological roles and complex dynamics in microbes. It is critical for surviving nutritional stress and stationary phase (13–17) but is also important for P homeostasis: microbes produce polyP when P is more abundant than required for growth, so-called luxury uptake, and break down this polyP store upon P stress (18). Moreover, if P-stressed cells experience a spike in P availability, they overproduce polyP in excess of luxury uptake levels, the so-called “overplus” response (19). Given these complex dynamics, it has been hypothesized that polyP might be either virtually absent, or particularly abundant, in oligotrophic marine systems (20).

Results and Discussion

Sectional Measurements of P Molecules and Phytoplankton Physiology. We measured polyP in particles collected on glass fiber GF/F filters (0.7-μm nominal pore size) along a transect from the P-replete temperate western North Atlantic (TWNA) to the P-depleted Sargasso Sea. Most surface ocean particulate C, N, and P are in living biomass, with heterotrophic bacteria contributing ~30% of particulate P in the subtropical North Atlantic (13). However, GF/F filters probably capture only ~50% of heterotrophic bacteria (21, 22). Although bacteria may have

Significance

Phosphorus is scarce in many subtropical ocean regions, and phytoplankton in these regions adjust their biochemical composition such that they require less of it. We show here that polyphosphate in the ultra–low-phosphorus Sargasso Sea are enriched in polyphosphate (polyP), a phosphorus molecule hitherto thought of primarily as a luxury storage product in marine phytoplankton. We further show that polyP appears to be more readily recycled in the surface ocean than other phosphorus-containing biochemicals. Thus, the high relative levels, and fast cycling, of polyP in low-phosphorus environments may form a feedback loop that contributes bioavailable phosphorus for primary production, potentially reducing the likelihood of growth limitation by phosphorus.


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contributed to the trends we describe below, the data are likely dominated by phytoplankton community signals. This view is supported by observed changes in SQDG:PG (see following paragraph) as marine heterotrophic bacteria are PG-rich and do not substitute lipids under P stress (1). PolyP was extracted using an enzyme-based method, and fluorometric measurements were calibrated against synthetic polyP (23), which were corrected for background sample fluorescence and matrix effects, giving a relative measure of polyP concentration that is expressed as nanoequivalents per liter (neq·L⁻¹) of the standard. We present the ratio of polyP to total particulate P (polyP:TPP) although it is not a quantitative measure of the absolute molar quantity of TPP that is composed of polyP because polyP:TPP > 1 in some instances (detailed discussion of the enzymatic polyP extraction method and comparison with the more-established NaOH-based extraction are discussed in ref. 23 and SI Materials and Methods).

SRP, TPP, APase activity, and membrane lipids were measured to examine how patterns in polyP related to biogeochemical P pools and known metrics of P stress.

The transect crossed the Gulf Stream at 37.4°N, which separated the TWNA from the Sargasso Sea. In the TWNA, surface mixed layer SRP concentrations were 100–400 nmol L⁻¹, alkaline phosphatase (APase) activity was detected in just 3 out of 46 samples (1.4–2.2 nmol P·L⁻¹·h⁻¹), and the molar sulfolipid to phospholipid ratio (SQDG:PG) was 0.3–2.3. In the Sargasso Sea, SRP concentrations were 1–25 nmol L⁻¹. APase was always detected (1.3–4.3 nmol P·L⁻¹·h⁻¹), and SQDG:PG was 4–10 (Fig. 1). These data clearly indicate phytoplankton P stress in the Sargasso Sea coincident with the low SRP; very similar observations along a similar western North Atlantic transect in 2008 suggest that these patterns are representative of the region in general (Fig. S1).

The polyP:TPP ratio was approximately fivefold higher in the Sargasso Sea than in the TWNA (Fig. 1 and Table S1). In the upper 50 m across the entire transect, polyP:TPP was inversely correlated with SRP concentration, increasing sharply below ~25 nmol L⁻¹ SRP (Fig. 2) (Spearman’s rho ≥0.80, P < 0.001). Similar correlations with SRP were observed for SQDG:PG and APase on both cruises (Fig. 2 and Fig. S1) (Spearman’s rho ≥0.80, P < 0.001). The polyP:TPP therefore covaries with known responses to P stress, such that the latitudinal gradients in polyP:TPP, SQDG:PG, APase, and SRP divide the western North Atlantic into two biogeochemical provinces, characterized by distinct regimes of phytoplankton physiology and P cycling.

The latitudinal gradient in polyP:TPP was driven by ninefold lower TPP concentrations in the Sargasso Sea versus the TWNA, consistent with recent reports (13) whereas polyP concentrations were less than twofold lower (Fig S2 and Table S1). This gradient indicates that polyP is experienced by P stress in the Sargasso Sea preferentially maintaining polyP over other cellular P reservoirs. In contrast, lipid P was 10-fold lower, 15 ± 1 nmol L⁻¹ versus 1.3 ± 0.17 nmol L⁻¹, effecting a shift from 11% of the TPP to 8.4% of TPP (Table S1). However, based on our measured C:P ratios (TWNA, 106 ± 11; Sargasso Sea, 200 ± 33) and TPP concentrations, we estimate that P-sparing strategies collectively save 13 nmol L⁻¹ TPP, but that phospholipid substitution accounted for only 14% of this saving (Table S1 and SI Materials and Methods).

Therefore, predominant strategies for reducing cellular P probably involve DNA (e.g., “genome streamlining”) (24), RNA (e.g., slower growth) (25), or other P-containing biochemicals whereas polyP cannot be spared beyond a certain extent. Indeed, polyP is intimately linked to primary metabolism in both eukaryotes and prokaryotes such that the inability to synthesize polyP results in a diverse range of cellular defects (26, 27).

Deep chlorophyll maxima (DCM) were found in both the TWNA (25–40 m) and the Sargasso Sea (60–100 m) (Fig S2). In both regions, the upper part of the DCM (TWNA, 30 m; Sargasso Sea, 65 m) contained levels of polyP, TPP, and polyP:TPP ratios similar to surface values whereas the base of the DCM (TWNA, 40 m; Sargasso Sea, 90 m) contained severalfold less polyP, TPP, and much lower polyP:TPP ratios (Fig. 1 and Fig. S2). This difference might indicate a shift away from autotrophic biomass in the total particle pool at the deepest extent of the euphotic zone. In the Sargasso Sea, the higher polyP:TPP at the base of the DCM might also reflect the physiological response of phytoplankton to a supply of SRP via diapycnal mixing across the phospholine; SRP was indeed slightly elevated at
Physiological Shifts in polyP:TPP in Field and Cultured Populations of Synechococcus. The polyP:TPP gradient in surface waters is not solely due to taxonomic shifts. Using cell-sorting flow cytometry, natural populations of Synechococcus, one of the dominant taxa throughout the transect, were isolated for biochemical analysis. SQDG:PG was greater than twofold higher in cells from surface waters of the Sargasso Sea relative to surface TWNA waters, but SQDG:PG was low in cells in the Sargasso Sea DCM (Fig. 3). Although absolute concentrations of polyP in Synechococcus did not differ latitudinally, we estimate, using Synechococcus P quotas reported from the region, that polyP:TPP was approximately threefold higher in cells sorted from the Sargasso Sea (28) (Fig. 3). This difference in polyP:TPP cannot be statistically resolved because of the low sample sizes and high variability inherent to isolating a single genus from a mixed community. Nevertheless, the trend of higher polyP:TPP in Synechococcus mimics the latitudinal gradient, underscoring the physiological underpinnings of the two biogeochemical provinces.

Elevated polyP:TPP ratios may be common in planktonic microbes from the Sargasso Sea: whole-community copy numbers of polyP-related genes detected in marine metagenomic surveys were higher in low SRP settings (29). Using 31P NMR, the cyanobacterium Trichodesmium was shown to increase polyP:TPP from 0.2% in P-replete cultures to 16% in P-starved cultures, and polyP:TPP ratios in Trichodesmium colonies collected from the Sargasso Sea were similarly high (8.0–25%) (30). Furthermore, polyP:TPP also increased sevenfold in P-stressed diatom batch cultures (31), consistent with the fivefold difference in polyP:TPP of bulk particles we report here between the TWNA and the Sargasso Sea.

Phytoplankton might either depend on permanently high relative polyP quotas for critical physiological functions, perhaps linked to the P-stress response, analogous to bacterial cultures (15–17). Alternatively, if SRP fluctuates rapidly in the Sargasso Sea, cells might be in a perpetual overplus state. To test these two ideas, surface water from the Sargasso Sea was incubated in bottles spiked with 200 nmol L⁻¹ SRP. With the SRP addition, Synechococcus cells increased their polyP content approximately twofold (22 and 33 attoequivalents polyP cell⁻¹ versus 8.5 and 17 attoequivalents-cell⁻¹ in control incubations; both n = 2), which is consistent with an overplus response. This response would be unlikely if polyP were already at overplus levels and thus is suggestive of P stress, not chronic overplus in situ. Culture experiments with Synechococcus WH8102 confirmed that cells are capable of the overplus response (Fig. S3), but polyP:TPP actually decreased upon addition of P, unlike the high values found in the Sargasso Sea. In the experimental cultures, polyP depth in this region (usually ≥10 nmol L⁻¹). Thus, the vertical gradient in polyP:TPP and SRP is similar to the latitudinal gradient in surface waters.

Fig. 2. Cruise data from Fig. 1 plotted against soluble reactive phosphorus (SRP) on a log scale. (A) The molar sulfolipid-to-phospholipid ratio (SQDG:PG), (B) alkaline phosphatase activity (APase), and (C) polyP-to-total particulate P ratio (polyP:TPP) all increased sharply below ~15 nmol L⁻¹ SRP. Note that the trends were most pronounced in the upper 50 m; for all three panels, Spearman’s rho ≥0.80, P < 0.001.

Fig. 3. Sulfolipid-to-phospholipid ratio (SQDG:PG) and estimated polyP-to-total particulate P ratio (polyP:TPP) in Synechococcus cells isolated by cell-sorting flow cytometry. Mean ± SD are shown for cases where n = 3, and mean and range are shown for cases where n = 2. To estimate polyP:TPP, we used the Synechococcus cellular P quotas reported from the subtropical western North Atlantic on a previous cruise (28); for polyP measurements in the Sargasso Sea, we used the average cellular P quotas from inside an anticyclonic and a cyclonic eddy (26.5 amol P cell⁻¹) whereas, for TWNA data, we used the higher quota reported from inside a mode water eddy (50.8 amol P cell⁻¹). A twofold difference in cellular P quota between P-starved and P-replete cells is consistent with results obtained in laboratory Synechococcus cultures (50).
per cell decreased by 20% upon P stress, consistent with some of the polyP acting as a luxury store. However, the elevated polyP:TPP and elevated SQDG:PG in P-stressed cultures matched patterns observed in the Sargasso Sea whereas the patterns from replete and overplus cultures did not (32) (Fig. 4 and Fig. S3). Thus, both field and culture observations from Synechococcus appear to discount a polyP overplus response. The modest net polyP breakdown upon P-stress might mask substantial intracellular recycling: cells have multiple polyP pools with distinct physiological functions and dynamics (33, 34), and enzymes to synthesize and degrade polyP are both up-regulated in P-stressed cultures (31). Moreover, P-stressed Trichodesmium cultures have polyP:TPP ratios indistinguishable from those of natural Sargasso Sea Trichodesmium populations (30), which is consistent with our Synechococcus observations.

PolyP has many functions in addition to its role in P homeostasis, including metal detoxification and energy storage. PolyP forms complexes with metal cations and was implicated in luxury iron storage in a diatom (35). However, although polyP can help detoxify metals, in which case polyP is broken down and metal–phosphate complexes transported out of the cell (36), a role in metal storage remains entirely speculative, and it seems unlikely that microbes in the dust-rich Sargasso Sea would use the very scarce P for the primary purpose of maintaining a luxury store of metals. PolyP can be used by some microbes as an energy store in sub- and anoxic environments (37, 38), but, in these settings, >10% of the organism’s dry weight is in the form of solid polyP granules (39, 40), unlike along our transect. The far more modest polyP levels seen during P stress or stationary phase could only cover a cell’s energy needs for timescales of seconds (41).

Although it may be difficult to disentangle the effects of P stress and stationary-phase physiology on polyP accumulation in laboratory batch cultures, stationary phase is unlikely to be involved in polyP accumulation in the field because phytoplankton growth rates are not markedly reduced in the Sargasso Sea (42). Overall, the polyP:TPP gradient appears to be more strongly linked to the SRP gradient than other potential controlling factors.

**Fate of PolyP and Significance for P and N Cycling.** To assess the biogeochemical fate of polyP in the western North Atlantic, TPP and polyP were measured in sinking particles collected in sediment traps deployed at 150 m for three successive 24-h periods in both the TWNA and the Sargasso Sea. In the TWNA, polyP:TPP values for sinking and for bulk surface particles were indistinguishable (0.34 ± 0.05 versus 0.39 ± 0.17 neq-nmol⁻¹, t = 0.85, df = 11.6, P = 0.412). In contrast, in the Sargasso Sea, polyP:TPP was significantly lower in sinking particles than in bulk surface particles (0.69 ± 0.21 versus 2.0 ± 0.68 neq-nmol⁻¹, t = 4.52, df = 7.8, P = 0.002). Although our sediment-trap observations are limited in time and space, the data indicate that polyP produced by P-stressed Sargasso Sea phytoplankton is discriminated against during the biogeochemical processing of living biomass into sinking particles captured by our sediment traps at 150 m, and thus P from polyP is preferentially retained in shallower waters in the Sargasso Sea (Fig. 4). The exact mechanisms behind this discrimination are not known. However, because the majority of polyphosphate biomass is remineralized before it escapes surface waters as sinking particles, we speculate that regulation of hydrolytic enzyme activities, such as APase, by external dissolved P concentrations might effectively prime the

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**Fig. 4.** Conceptual model highlighting the biogeochemical differences between the two North Atlantic provinces in phytoplankton phosphorus physiology. In the temperate western North Atlantic, high external P concentrations provide enough P for cells to synthesize phospholipids, carry high total intracellular P quotas, and high polyphosphate (polyP) levels due to luxury storage. However, polyP is overall a modest component of total cellular P. As dead particles sink out of the euphotic zone and are degraded and reprocessed by microbes and zooplankton, polyP is lost in proportion to total P, so the polyP:TPP ratio does not change with depth. Consequently, the contribution of polyP to overall P cycling and primary production is minor. In the subtropical western North Atlantic (Sargasso Sea), ultra-low ambient P concentrations induce a cellular P-stress response, leading to substitution of non-P lipids for phospholipids, production of the alkaline phosphatase enzyme, a strong reduction in total cellular P, but retention of a significant polyP pool. Sinking particles preferentially lose polyP relative to total P, possibly due to preferential remineralization, allowing P from polyP to be preferentially retained in the upper ocean. Consequently, polyP is a significant, previously unrecognized pool of bioavailable P in this oligotrophic system, which likely supports a larger fraction of primary production and carbon export than in nutrient-replete waters.
microbial community for preferential polyP recycling in low-P surface waters.

The flux of TPP in sediment traps in the Sargasso Sea has increased markedly over the last decade (43), which may potentially indicate changing annual patterns in P export relative to other systems. Consistent with this observation, our sediment-trap data suggest that a greater fraction of the surface pools of TPP and polyP were exported in the Sargasso Sea than in the TWNA (Table S1). However, we estimate that the reduction in polyP:TPP between the surface and the sediment traps in the Sargasso Sea reduced by a factor of three the percentage of the polyP stock exported (SI Materials and Methods); thus, preferential polyP recycling mitigates TPP export in the Sargasso Sea to a significant degree. Preferential retention of polyP in surface water provides an additional potential explanation for why relative nitrate-to-phosphate ratios (the so-called N*/P) tracer increase with depth in the Sargasso Sea, which cannot be accounted for solely by nitrogen fixation (44).

The sediment trap results also suggest considerable variation across different ocean provinces in the extent to which polyP is either recycled in surface waters or exported to depth. Further work is required to assess preferential polyP remineralization in the deep-sea and in marine sediments, which, if confirmed, has the potential to impact water-column phosphorus inventories and nutrient stoichiometries on global scales, particularly because export of polyP to sediments can promote long-term P burial by promoting apatite formation (11).

Dissolved polyP is an inorganic polymer but would nonetheless compose part of the operationally defined dissolved organic P (DOP) pool (45). If intact polyP is preferentially released from sinking particles into surface waters, then polyP may contribute more to DOP in the Sargasso Sea than the ~10% reported from P-replete regions (11, 12, 46). Dissolved polyP, but not all DOP, is readily bioavailable to phytoplankton (12, 47), and most primary production in the Sargasso Sea is based on nutrient recycling and DOP utilization (4, 43). As polyP is apparently both mobile and available to phytoplankton, we speculate that polyP forms the basis for a feedback loop that retains P in surface waters, rendering P limitation by P less likely and maintaining high fluxes of nitrogen and carbon export per unit P via the biological pump. Our data are beginning to shed light on the long-standing question (20, 48) of whether polyP plays a biogeochronologically significant role in oligotrophic ocean regions.

Materials and Methods

Full methods are described in SI Materials and Methods. Particles were collected with Niskin bottles and wide-aperture net sediment traps between West and Bermuda and-Guadalupe Islands. Lipids were analyzed by liquid chromatography mass spectrometry using authentic standards after solvent extraction (49); we report the ratio of sulfoquinovosyldiacylglycerol (SQDG) to phosphatidylglycerol (PG). Total particulate phosphorus was quantified using the molybdate blue method after K2S2O8 wet oxidation. Particulate organic carbon was determined on a stable isotope mass spectrometer after fuming the samples with concentrated hydrochloric acid. PolyP was determined fluorometrically after boiling and enzymatic digestion, accounting for background fluorescence and matrix effects (23). Comparison with a synthetic polyP standard allows relative quantification expressed as nanoequivalents of P in the standard. A different enzymatic digestion based on snake-venom phosphodiesterase yielded identical polyP values (Fig. S4) whereas a NaOH leach gave lower absolute polyP values but showed the same temporal trend in polyP:TPP (Fig. S5). Alkaline phosphatase activity was measured in unfiltered seawater using the substrate 4-methylumbelliferyl phosphate (2008 cruise) and on filtered particles using the substrate 6,6-difluoro-4-methylumbelliferyl phosphate (2008 cruise). Soluble reactive phosphorus was measured using standard methods. Samples for cell-sorting flow cytometry were filtered, formaldehyde-fixed, and flash-frozen (32); after sorting, Synechococcus were filtered and flash-frozen. Additional particle samples for lipid analysis were collected on 0.2 μm PVDF filters; SQDG:PG ratios in PVDF-filtered samples were converted to GF/F equivalent values using the relationship in Fig. S6 (SI Materials and Methods). Synechococcus WH8102 was grown in SN medium using standardized methods. Samples for lipid analyses and cell counts (flow cytometry) were taken daily once fluorescence in P-limited cultures stopped increasing. P was resupplied to Refuel cultures to 45 μmol L−1 24 h before final sampling.

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Supporting Information

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SI Materials and Methods

Field Sampling. Field samples were collected during two cruises, OC443 along 65°W (April 2008) and BLATZ-II from Woods Hole to Bermuda (April 2012). Seawater for bulk particle chemical measurements was collected with Niskin bottles on a conductivity-temperature-depth (CTD) rosette equipped with a fluorometer: 1–4 L were filtered for lipid analysis (OC443, 0.2-μm PVDF filters; BLATZ-II, ~0.7-μm glass fiber GF/F filters and 0.2-μm PVDF filters), and total particulate P (TPP) and polyphosphate (polyP) (on BLATZ-II only, GF/F). Filters were flash-frozen in LN₂ and stored frozen (lipid samples at ~80 °C) until analysis. Two to sixteen liters were filtered onto 0.2-μm polycarbonate filters for cell sorting (BLATZ-II only), fixed in 1% formaldehyde for 20 min, flash-frozen, and stored at ~80 °C. Soluble reactive P (SRP) samples were filtered (0.2 μm Sterivex) in the temperate western North Atlantic (TWNA) but not the Sargasso Sea, stored at ~20 °C, and analyzed using standard methods; south of the Gulf Stream, additional samples for quantification after MAGIC preconcentration (1) were taken. Wide-aperture, surface-tethered net sediment traps (2) were deployed on BLATZ-II to collect sinking particles; they are designed to collect representative samples of the flux rather than quantify it exactly, but we note that our TPP fluxes are consistent with reports from a nearby time-series of the flux rather than quantify it exactly, but we note that our TPP fluxes are consistent with reports from a nearby time-series site (3). Three traps were deployed successively for 24 h each in both the TWNA and the Sargasso Sea. Trap samples were screened through 563-μm Nitex to remove live zooplankton, split in a rotary splitter, and filtered onto GF/F filters.

Flow-Cytometry Cell Sorting. Samples were thawed at room temperature, gently inverted to resuspend cells off the filter, and prescreened through a 40-μm mesh. They were then sorted on a Becton Dickinson (formerly Cytopeis) Influx Mariner cell sorter. Samples were excited with a 200-mW 488-nm Coherent laser. Cellular chlorophyll (692/40 band pass) and phycoerythrin (572/27 band pass) fluorescence were quantified and used in conjunction with forward light scatter to identify Synechococcus populations using standard methods (4–6). Sorted aliquots were stored at 4 °C until the entire sample was sorted, and then combined and syringe-filtered onto 0.2-μm polycarbonate (polyP) or 0.2-μm PVDF (lipids) filters, flash-frozen, and stored at ~80 °C until analysis. Before filtering, a small aliquot of the sorted cells was removed to confirm cell enumeration in sorted samples; cell abundances were used to calculate polyP per cell. Procedural blanks for polyP and lipid analysis were prepared by filtering seaweed fluid collected at the outflow of the flow cytometer through the appropriate filter type, but our target analytes were not detected. To calculate polyP:TPP, the Synechococcus cellular P quotas were estimated based on reports from the subtropical western North Atlantic (7); for polyP measurements in the Sargasso Sea, we used the average cellular P quotas from inside an anticyclonic and a cyclonic eddy whereas, for TWNA data, we used the twofold higher cellular P quota reported from inside a mode-water eddy.

Chemical Analyses. Lipids were extracted and quantified by liquid chromatography triple quadrupole mass spectrometry as described (8). On BLATZ-II, lipid samples were taken on PVDF and GF/F filters north of 38°N and at 33°N; samples in between were taken only on PVDF filters. However, where samples were taken on both filters, the sulfoquinovosyl diacylglycerol (SQDG):phosphatidylglycerol (PG) ratio was linearly related (Fig. S6). We used this relationship to calculate a GF/F equivalent value for all SQDG:PG measurements from PVDF samples taken on BLATZ-II; Fig. 1 shows these GF/F equivalent data. The offset in SQDG:PG between filter types was much smaller than the latitudinal variation and was probably caused by GF/F filters not capturing (PG-rich) heterotrophic bacteria quantitatively (9).

TPP was analyzed by K2SO3 wet oxidation followed by standard colorimetric measurement (10). Complete oxidation efficiency was confirmed using glucose-6-phosphate.

Particulate organic carbon (POC) was measured on a Finnigan MAT DeltaPlus stable isotope ratio mass spectrometer at Woods Hole Oceanographic Institution using anatase as an external standard.

Alkaline phosphatase activity was measured on particles collected onto 0.2-μm filters (2008 cruise), or in unfiltered seawater samples (2012 cruise). Samples were incubated in 96-well plates with saturating concentrations of either the substrate 6,8-difuoro-4-methylumbelliferyl phosphate (2008 cruise) or 4-methylumbelliferyl phosphate (2012 cruise). Fluorescence was measured on a microwell plate reader over the linear range of the assay against a standard curve to calculate maximal rates of uptake (11).

PolyP was determined fluorometrically with 2,6-diamidino-2-phenyindole, after boiling and enzymatic digestion (DNase+RNase, proteinase K) of a subsample from each filter, and expressed as equivalents of the molar amount of P in a synthetic polyP standard (12). Fluorescence was measured at 550 nm upon excitation at 415 nm. Matrix effects were accounted for by standard addition to each sample. We consider this method to give a relative, not absolute, measure of polyP concentration because the synthetic standard (total P content confirmed by lab methods and P determination) appears to fluoresce less brightly than natural polyP in our environmental plankton samples. Thus, samples from the Sargasso Sea invariably contained more equivalents of the standard than the molar concentration of TPP, yielding ratios greater than 1. Analysis of fresh subsamples using snake venom phosphodiesterase (Sigma Aldrich) in place of DNase+RNase yielded a 1:1 relationship to the data presented here (Fig. S4), suggesting that residual nucleic acid–polyP complexes are unlikely to be responsible (13). Analysis of a third set of subsamples using 0.25 M NaOH extraction (14) yielded lower values, but the same latitudinal trend reported above (Fig. S5). However, we found this method unsuitable for high-sensitivity analysis of small sample quantities because the strong base must be neutralized exactly with HCl and then diluted with neutral buffer to ensure a suitable pH and salt concentration for staining. Fluorescence signals in NaOH extracts were therefore lower, especially between 38°N and 34°N, where only very small subsamples could be analyzed. More importantly, standard addition indicated more substantial and erratic matrix effects in the NaOH-extracted samples than with enzyme digestion, probably because of insufficient dilution and/or pH adjustment. NaOH extraction was therefore out of the question for flow-sorted samples. It is probably more suitable for analysis of larger sample quantities that allow for greater dilution, such as the sediments for which the NaOH method was developed (14). However, it is unlikely that alkali extraction alone quantitatively extracts all polyP (15), which might partly account for the lower values we obtained using this method. Overall, it seems likely that our enzymatic method at present fails to break down some form of complex between polyP and another macromolecule that has enhanced fluorescence in the presence of DAPI compared with just polyP. The high fluorescence is certainly not due to a matrix effect; matrix effects were accounted for in every sample by...
standard addition, and in fact were responsible for a 10–20% reduction in fluorescence compared to the pure standard. Neither is the high fluorescence likely to be due to fluorescence from DAPI interacting with other constituents of biomass, because deep chlorophyll maxima (DCM) samples, containing much biomass as surface samples, contained very little polyP. Likewise, biomass-rich coastal samples contained low levels of polyP. Moreover, we can rule out any background fluorescence, as background fluorescence was measured in each sample in the absence of DAPI and subtracted.

**Synechococcus Cultures.** Axenic *Synechococcus* WH8102 was obtained from John Waterbury (Woods Hole Oceanographic Institution) and grown in glass Erlenmeyer flasks in SN medium with 45 μM KH₂PO₄ at 30 μmol photons·m⁻²·s⁻¹ and 23 °C. The SN medium base was 0.2 μm filtered surface seawater from the Sargasso Sea mixed in a 3:1 ratio with deionized (MilliQ) water. Replicate cultures (three each for –P and Refeed, two for +P) were established from a log-phase inoculum. +P cultures started with 45 μmol·L⁻¹ P, P, and Refeed cultures with 1 μmol·L⁻¹. Fluorescence was assessed daily starting on day 11. Samples for flow cytometry (in 1% formaldehyde) and for polyP, TPP, and lipid analysis (filtered onto GF/F filters) were taken daily for growth (as judged by fluorescence) in P-limited cultures had ceased. Samples were flash-frozen and stored at −80 °C. K₂HPO₄ was added to Refeed cultures to a final concentration of 45 μmol·L⁻¹ 24 h before the last set of samples were taken. Procedural blanks for chemical measurements were prepared by filtering blank 45 μmol·L⁻¹ P medium through GF/F filters. These procedural blanks contained no lipids or polyP but did contain P carried over from the medium. These P blanks were subtracted from +P and Refeed cultures, but we subtracted only the lower reagent P blank from –P cultures because carryover of dissolved P in P-limited cultures would have been very low. TPP might thus have been overestimated in –P cultures, which would have led us to underestimate their polyP:TPP ratio. Because the –P cultures had the highest polyP:TPP, this underestimate does not impact our conclusions. Cells were enumerated on a Millipore Guava EasyCyte flow cytometer, and chemical measurements were performed as described above. Cellular lipid, polyP, and TPP quotas were stable over the final four sampling days, so measurements taken during this period were averaged for each individual replicate. These averages were then used to calculate the overall treatment mean ± SD, which is thus based on n = 3 (–P cultures) or n = 2 (+P cultures). For Refeed cultures, only the measurements taken on the final day of sampling (i.e., 24 h after P addition) are reported; data from the Refeed cultures before P addition were indistinguishable from –P cultures.

**Estimating Lipid Contribution to C:P Gradient.** In the TWNA, mixed-layer TPP averaged 130 ± 17 nmol·L⁻¹, with P-lipids contributing 15 ± 1.1 nmol·L⁻¹, or 11%. In the Sargasso Sea, mixed-layer TPP averaged 15 ± 1.1 nmol·L⁻¹, with P-lipids contributing 1.3 ± 0.17 nmol·L⁻¹, or 8.4%. Further, C:P was 200 in the Sargasso Sea, with 3.0 μmol·L⁻¹ POC, but only 106 in the TWNA, with 14 μmol·L⁻¹ POC (Table S1). Without P-sparing strategies, such that Sargasso Sea C:P was also 106, TPP would be 28 nmol·L⁻¹, given 3.0 μmol·L⁻¹ POC. Because observed TPP is only 15 nmol·L⁻¹, the latitudinal C:P gradient is responsible for a 13 nmol·L⁻¹ reduction in Sargasso Sea TPP. Without lipid substitution, P-lipids contribute 11% of TPP at a C:P of 106 so, without lipid substitution, one might expect a P-lipid concentration in the Sargasso Sea of 11% × 28 = 3.1 nmol·L⁻¹, indicating that lipid substitution spares 1.8 nmol·L⁻¹ in the Sargasso, accounting for 14% of the total TPP reduction. This rough estimate of the role of lipids is in keeping with culture experiments showing that lipid substitution can spare phytotoplankton 5–40% of cellular P (9).

**Estimating the Percentage of TPP and polyP Stocks Exported per Day.** Standing stocks of polyP and TPP were calculated by trapezoidal integration between the surface and 150 m at both sites. We used the observed TPP flux in the Sargasso Sea to calculate the polyP flux that would have been observed if the polyP:TPP of exported material was identical to the surface polyP:TPP (2.0 neq·mol⁻¹). We found that polyP flux would have been 11 ± 4 μg·m⁻²·d⁻¹ in the Sargasso Sea, meaning that, in the absence of preferential polyP recycling, 0.45% ± 0.16% of the 0–150 m mP standing stock would have been exported per day, which is threefold higher than the actually observed value of 0.14% ± 0.02%. Thus, although it is possible that a greater percentage of the 0–150 m standing stock of TPP and polyP were exported in the Sargasso Sea than in the TWNA, we estimate that the apparent preferential recycling of polyP would have played a significant role in mitigating P loss via sinking particles. However, caution is advised in making quantitative comparisons of this kind between the sediment traps and the surface standing stocks: possible collection biases of this type of sediment trap are unknown, and, because of the high seasonality especially in the TWNA, our samples probably do not represent annual mean conditions.

Fig. S1. Sectional data from an April 2008 cruise along 65°W. As on the 2012 cruise, a strong southward decrease in (A) SRP, and a concomitant rise in (B) SQDG:PG and (C) alkaline phosphatase activity were identified. There were significant inverse correlations between both (D) SQDG:PG and (E) APase against SRP. Because lipid samples were filtered onto 0.2 μm PVDF filters on this cruise, the SQDG:PG ratios were overall lower than in the GF/F-filtered samples collected during April 2012. This difference is most probably because GF/F filters do not quantitatively retain the PG-rich but SQDG-poor heterotrophic bacteria (2).
Fig. S2. Sectional data from an April 2012 cruise from Woods Hole to Bermuda, showing the absolute concentrations of (A) particulate polyP, (B) total particulate phosphorus, and (C) relative fluorescence as measured by the fluorometer on the CTD rosette.

Fig. S3. (A) Sulfolipid-to-phospholipid ratio, (B) polyphosphate-to-total particulate phosphorus ratio, and (C) polyphosphate-per-cell content in batch cultures of the cyanobacterium *Synechococcus* WH8102. +P, P-replete cultures; –P, P-limited cultures; Refeed, P-limited cultures resupplied with P 24 h before sampling. Bars are means of three (–P and Refeed) or two replicate cultures (+P). For –P and Refeed, error bars indicate one SD; for +P, the data from each replicate are indicated by the points.
**Fig. S4.** Using two different enzymatic digestions to determine polyphosphate in the April 2012 cruise samples (venom phosphodiesterase digestion versus DNase+RNase digestion) yielded a relationship indistinguishable from a 1:1 line with only modest scatter.

**Fig. S5.** Using an alternative method to measure polyP based on NaOH extraction yielded lower polyP values, but a latitudinal trend identical to that observed with our enzymatic method. Note that measurements using NaOH between 37°N and 34°N were probably biased low by the very small sample quantities available, which yielded signals very close to blank levels. These data were therefore excluded from the regressions in the two Lower panels.
Fig. S6. Relationship between the sulfo-to-phospholipid ratio (SQDG:PG) measured in samples filtered onto 0.2-μm PVDF filters and onto GF/F filters across the April 2012 transect. Data are from temperate and subtropical waters.

Table S1. Surface concentrations of POC, TPP, lipid-P, and polyP in the temperate western North Atlantic (TWNA, 39°N, 0–30 m average) and the Sargasso Sea (33°N, 0–65 m average), integrated water-column inventories of TPP and polyP (0–150 m), and downward fluxes of TPP, polyP, and POC at 150 m

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>TWNA, 39°N 69°W</th>
<th>Sargasso Sea, 33°N 66°W</th>
</tr>
</thead>
<tbody>
<tr>
<td>POC, μmol L⁻¹⁻¹</td>
<td>14 ± 1.4</td>
<td>3.0 ± 0.46</td>
</tr>
<tr>
<td>TPP, nmol L⁻¹⁻¹</td>
<td>130 ± 17</td>
<td>15 ± 1.1</td>
</tr>
<tr>
<td>POC:TPP, mol:mol⁻¹</td>
<td>106 ± 11</td>
<td>200 ± 33</td>
</tr>
<tr>
<td>Lipid-P, nmol L⁻¹⁻¹</td>
<td>15 ± 1.1</td>
<td>1.3 ± 0.17</td>
</tr>
<tr>
<td>PolyP, neq L⁻¹⁻¹</td>
<td>49 ± 18</td>
<td>30 ± 9.4</td>
</tr>
<tr>
<td>Lipid-P, percent of TPP</td>
<td>11 ± 1.4</td>
<td>8.4 ± 1.0</td>
</tr>
<tr>
<td>PolyP:TPP, neq nmol⁻¹</td>
<td>0.39 ± 0.17</td>
<td>2.0 ± 0.68</td>
</tr>
<tr>
<td>TPP integrated stock, μmol m⁻²</td>
<td>8,690 ± 1,540</td>
<td>2,230 ± 330</td>
</tr>
<tr>
<td>PolyP integrated stock, μeq m⁻²</td>
<td>2,170 ± 490</td>
<td>2,490 ± 410</td>
</tr>
<tr>
<td>Trap TPP flux, μmol m⁻² d⁻¹</td>
<td>6.2</td>
<td>7.2</td>
</tr>
<tr>
<td>Trap polyP flux, μeq m⁻² d⁻¹</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Trap polyP:TPP, neq nmol⁻¹</td>
<td>0.34</td>
<td>0.29</td>
</tr>
<tr>
<td>% of TPP stock exported d⁻¹</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>% of polyP stock exported d⁻¹</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Trap POC flux, mmol C m⁻² d⁻¹</td>
<td>1.43 ± 0.69</td>
<td>0.86 ± 0.20</td>
</tr>
</tbody>
</table>

Concentrations and water-column inventories (mean ± SD) were calculated from two to three replicate profiles taken at the same station as the trap deployments. The absolute values of fluxes should be interpreted with caution because collection biases of the surface-tethered, wide-aperture net traps used in this study are unknown. Moreover, the fluxes do not represent the full range of seasonal variability, especially not in the TWNA. Note that POC fluxes were measured on additional deployments of identical traps at each site so only average POC fluxes are reported; μeq, microequivalents; neq, nanoequivalents; POC, particulate organic carbon; polyP, polyphosphate; TPP, total particulate P.