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Microbially Mediated Transformations of Phosphorus in the Sea: New Views of an Old Cycle

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Abstract

Phosphorus (P) is a required element for life. Its various chemical forms are found throughout the lithosphere and hydrosphere, where they are acted on by numerous abiotic and biotic processes collectively referred to as the P cycle. In the sea, microorganisms are primarily responsible for P assimilation and remineralization, including recently discovered P reduction-oxidation bioenergetic processes that add new complexity to the marine microbial P cycle. Human-induced enhancement of the global P cycle via mining of phosphate-bearing rock will likely influence the pace of P-cycle dynamics, especially in coastal marine habitats. The inextricable link between the P cycle and cycles of other bioelements predicts future impacts on, for example, nitrogen fixation and carbon dioxide sequestration. Additional laboratory and field research is required to build a comprehensive understanding of the marine microbial P cycle.

1. INTRODUCTION

Phosphorus (P) is an essential element for life; indeed, the entire biosphere is built around P (De Duve 1991). The supply of P to the sea is ultimately controlled by tectonics and subsequent weathering of continental rocks, and if these processes were to cease, so too would life on Earth. P is vital for cell structure (phospholipids), the storage and expression of hereditary information (nucleic acids), cellular energy transduction (nucleotides), and many metabolic regulatory functions. Having evolved in their habitat for nearly 4 billion years, marine microorganisms have acquired many unique adaptations for optimal growth and survival, especially for P acquisition and metabolism. In the marine environment, P is present in a variety of inorganic and organic forms that are converted between dissolved and particulate pools, creating what is referred to as the microbial P cycle.

The marine environment, especially the large anticyclonic subtropical gyres that dominate our planet, is in a state of chronic phosphate (PO_4^{3-}, hereafter P_i) starvation compared with growth conditions imposed on microorganisms during primary isolation or laboratory-based physiological studies. Just as in the human condition of P deficiency (hypophosphatemia, which leads to muscle and neurological dysfunction owing to depleted ATP levels), P deficiency in natural marine microbial assemblages leads to a disruption of metabolism, growth, and essential ecosystem services. For example, the P status of an ecosystem controls solar energy capture, net primary production, fish production, and the potential for carbon dioxide (CO_2) sequestration. Although selected microorganisms can partially substitute sulfur (S) for P in membrane lipids under conditions of severe P deficiency (Van Mooy et al. 2009), P can never be completely replaced. A recent high-profile report of the substitution of arsenic (As) for P in nucleic acids (Wolfe-Simon et al. 2011) was subsequently challenged by eight independent Technical Comments (Alberts 2011); currently, P remains the obligate staff of life (Karl 2000).

The earliest investigations of P date back to 1669, when the German alchemist Hennig Brand accidentally discovered it while searching for the philosopher's stone, a mystical and magical substance that would turn base metals into gold. Phosphorus is the Greek word for "light bearer," and the name was chosen because the form of P that Brand had isolated from urine spontaneously combusted in air and glowed in the dark. In 1777, Antoine-Laurent Lavoisier—the father of modern chemistry—identified P as the 13th element (i.e., a substance that could not be further decomposed by a chemical reaction). Lavoisier's *Traité Élémentaire de Chimie* (published in 1789 and translated into English by Robert Kerr in 1790 as *Elements of Chemistry*) was the first classification of elements and a predecessor of the modern periodic table. Lavoisier concluded that P was present in both plant and animal tissues and that it was generally combined with charcoal (carbon; C), hydrogen (H), and azote (nitrogen; N). However, it was not until the late nineteenth century that P was recognized as essential for all living organisms.

Several path-finding scientific studies, especially those conducted during the first half of the twentieth century, provided the foundation for contemporary investigations of the marine P cycle. In 1903, Donald J. Matthews began a systematic study of the English Channel that led to an ecological understanding of the fundamental links between P_i, phytoplankton, and fish production in the sea. Matthews (1916, 1917) is also credited with making the first reliable estimates of P_i in seawater and with the discovery of oceanic dissolved organic P (DOP). Atkins & Harvey (1925) revealed the basic features of the oceanic P cycle in their publication of the first vertical profile of P_i concentrations in the open sea. The depletion of near-surface P_i concentrations resulting from net microbial uptake and production of particulate organic matter (POM) and the net regeneration of P_i from sinking POM at greater depths were recognized as essential P-cycle processes (see Section 2).

In preparing this review, I have benefited from these historical accounts and from an increasingly sophisticated understanding of the global P cycle, as presented in several recent authoritative

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reviews (Benitez-Nelson 2000, Canfield et al. 2005, Paytan & McLaughlin 2007, Slomp 2011, Ruttenberg 2013). Recent discoveries—including the distribution of specific P-cycle genes, gene transcription patterns and proteins, new metabolic pathways, and an unexpected P redox cycle in nature—have ignited a general interest in the contemporary marine microbial P cycle, with an emphasis on open-ocean ecosystems. Here, I endeavor to review, synthesize, and extend analyses by Dyhrman et al. (2007), Karl (2007a), and White & Metcalf (2007) while presenting new views of an old cycle.

2. PHOSPHORUS-CYCLE SCOPE AND SCALES: FROM MANTLE TO MICROBES

Despite the fundamental role of P in the sea, which ranges from genomes to biomes, very few comprehensive studies of P have been made relative to the much larger knowledge base for the C, N, and S cycles. For example, the benchmark monograph *Marine Microbiology* (ZoBell 1946) dedicated just 3 of its 208 pages to the P cycle, and the most recent edition of *Microbial Ecology of the Oceans* (Kirchman 2008) mentioned the microbial P cycle on only 1 of its 568 pages (Church 2008, p. 364).

Although P is the 11th-most-abundant element on Earth, most of it is contained in the lithosphere, where it is not readily available to support life. Consequently, the weathering of continental rocks ultimately controls the pace of the global P cycle. There are at least three nested P cycles to consider, each with a unique set of transformations, time and space scales, and controls (**Figure 1**): the tectonic-lithogenic P cycle (Wilson cycle, after J. Tuzo Wilson), the oceanic P cycle (Broecker cycle, after Wallace S. Broecker), and the marine microbial P cycle (Redfield cycle, after Alfred C. Redfield).

Over very long timescales (millions of years), the delivery of P to the global ocean is controlled by the combined influences of continental weathering and fluvial discharge (Froelich et al. 1982, Meybeck 1982). This input is balanced by P burial on the continental shelves and in deep-sea sediments and by subsequent tectonic uplift or assimilation into the mantle (**Figure 1**). During the past 160 million years, rates of P accumulation in deep sediments of the major ocean basins have varied by more than an order of magnitude around a mean of approximately 2.35×10^{12} g P y^{-1}, with enhanced continental weathering during periods of climate warming (Föllmi 1996). However, our understanding of the pace of the oceanic P cycle is rapidly changing. Recent estimates of the oceanic residence time are on the order of 10,000–20,000 years (Ruttenberg 2013), compared with "textbook" estimates that are greater by an order of magnitude. This implies that, given our current understanding of the dynamics of the ocean conveyor belt, an "average" P atom circulates 10 times throughout the world ocean before it is eventually removed.

The P contained in uplifted marine sediments and continental rocks is relatively stable, and because the delivery of P to the ocean via atmospheric deposition is inefficient compared with riverine inputs (<10% of the total; Froelich et al. 1982), surface-ocean habitats far removed from the continental shelf must obtain their P from local sources. Lateral fluxes of P, mostly as DOP, can supplement other sources of bioavailable P (BAP) for microbial assemblages in the nutrient-starved surface waters of subtropical ocean gyres (Abell et al. 2000, Mahaffey et al. 2004), but the significance of this potential transport mechanism is not well understood. Consequently, the most important source of P_i is thought to be the delivery of P-enriched seawater located beneath the well-lit productive euphotic zone via the combined processes of vertical eddy diffusivity, turbulent motions, and upwelling (**Figures 2** and **3**). Other hypothesized pathways of P delivery, including the transport of buoyant P-containing organic matter and P mining by vertically migrating phytoplankton (Karl et al. 1992, Karl & Tien 1997), are less well constrained (**Figure 2**; see also Section 6.3). The delivery of P from intermediate ocean depths (>200 m) is balanced by the

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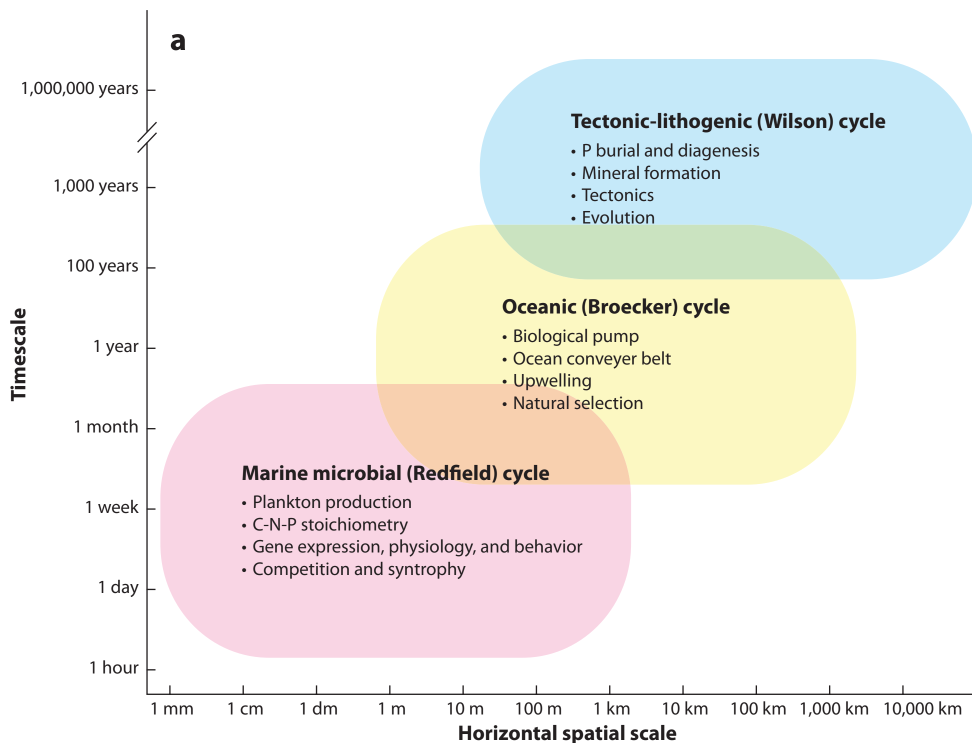

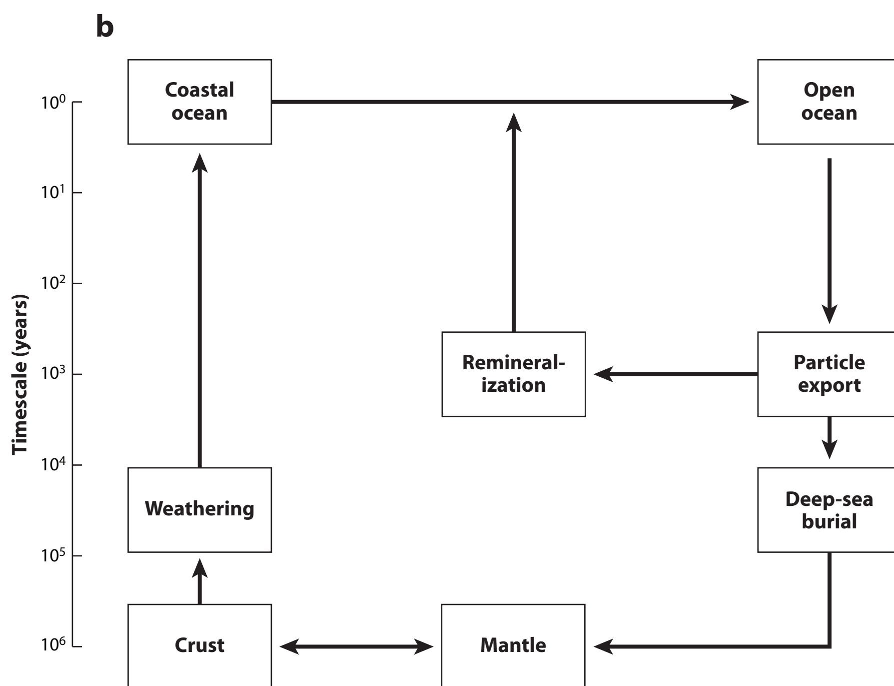

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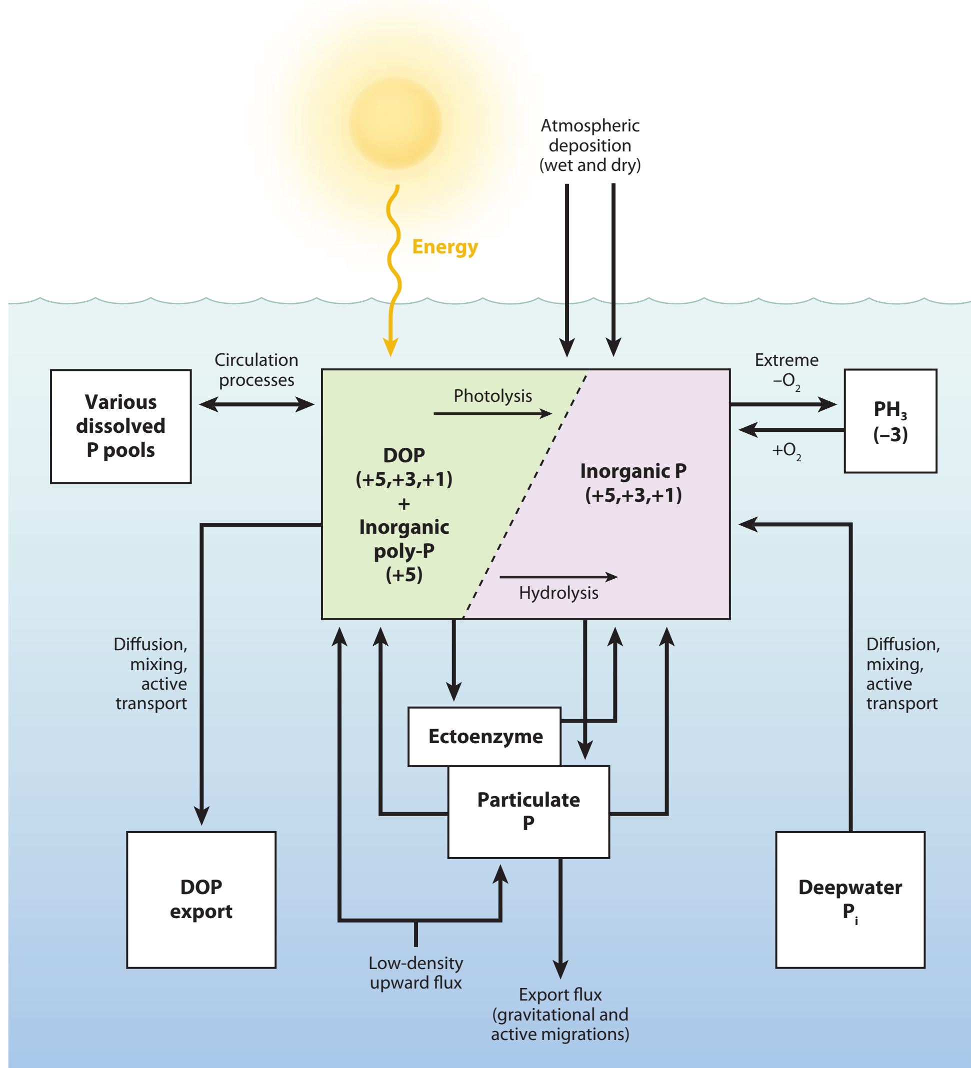

Figure 2

Schematic representation of the inventories (*boxes*) and fluxes (*arrows*) that constitute the open-ocean P cycle. The various probable valence states of P in the particulate and dissolved pools are given in parentheses. Abbreviations: DOP, dissolved organic P; P_i, phosphate; poly-P, polyphosphate.

←

Figure 1

(*a*) Schematic representation of the three major interlinking P cycles, showing approximate time and space scales and selected geophysical, biogeochemical, and biological processes. (*b*) Hypothesized movement of P through the marine P cycle, with approximate timescales for each process. With an oceanic residence time on the order of 10,000–20,000 years, P circulates approximately 10 times throughout the world ocean before it is buried in deep-sea sediments, initiating tectonic reprocessing that requires millions of years to complete.

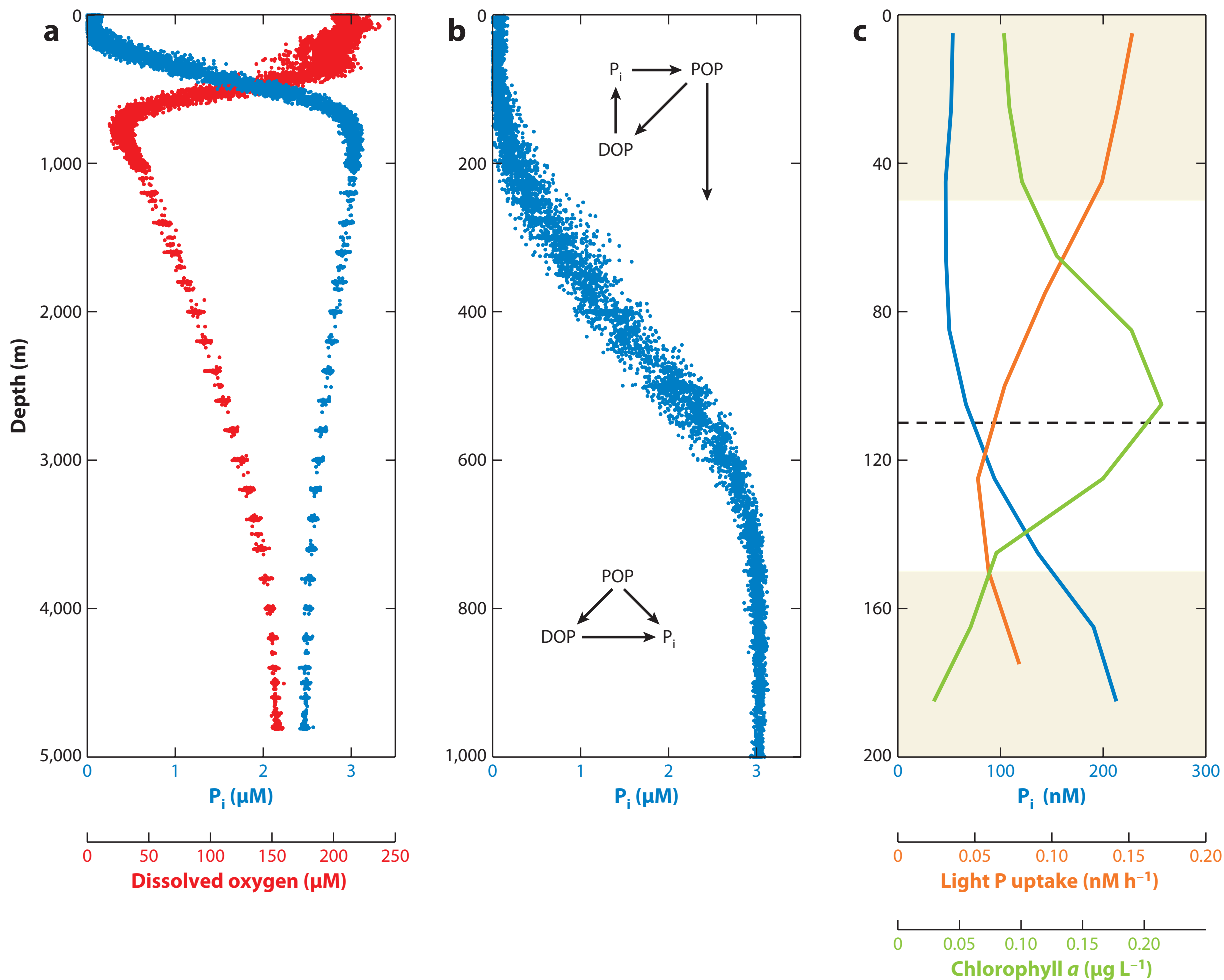

Figure 3

(*a*) Phosphate (P_i) (*blue*) and dissolved oxygen concentrations (*red*) versus depth profiles for the entire water column at station ALOHA in the North Pacific Subtropical Gyre (total water depth = 4,750 m), showing the main feature of opposing trends in concentration as a result of organic matter production by photosynthesis in surface waters and aerobic remineralization of sinking particles at depth. (*b*) P_i profile in the upper 1,000 m of the water column, showing in a schematic view the two main processes of net P_i removal via particulate matter production and gravitational settling (0–150 m) and net P_i production via the remineralization of organic P (>150 m). (*c*) Representative data from station ALOHA showing two distinct regions of the euphotic zone: one region characterized by net uptake of P_i into particulate organic matter (*top*) and another region characterized by net P_i remineralization (*bottom*). The two regions are separated by the deep chlorophyll maximum layer, which is located near the 1% sea-surface light level (*dashed line*). Abbreviations: DOP, dissolved organic P; POP, particulate organic P.

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combined losses resulting from gravitational settling of particulate organic P (POP) (Martin et al. 1987), diurnal migrations of mesozooplankton (Hannides et al. 2009), and P_i adsorption onto mineral particles. As organic particles sink, most of the organophosphorus (OP) is remineralized back to P_i en route to the abyss or following deposition onto the seabed. Consequently, surface-ocean waters are depleted and deep-ocean waters are enriched in P_i (**Figure 3**). The oceanic P cycle operates on intermediate timescales of decades to centuries (**Figure 1**). The very small fraction of POP that escapes initial remineralization plus the sedimentary P_i that is able to concentrate by

subseabed migration can cause authigenic P-containing mineral formation in selected sedimentary habitats. This process, termed phosphogenesis, removes P from the biosphere and connects the oceanic P cycle to the tectonic-lithogenic P cycle, thereby sustaining a long-term global balance.

The delivery of P_i into the euphotic zone supports the solar energy capture by photosynthetic microorganisms that is necessary to power the marine microbial P cycle. The oceanic and marine microbial P cycles are inextricably linked through the bidirectional P flux, which moves upward in the form of regenerated P_i and downward mostly as POP via the biological pump (**Figure 2**). The microbially mediated transformation of P in the surface ocean is the most active portion of the three nested P cycles, with P turnover times on the order of days to months (**Figure 1**).

Similarly to the tectonic-lithogenic and oceanic P cycles, the marine microbial P cycle is considered to be in steady state (P sources equal to P sinks) when measured over a sufficiently long (but poorly constrained) period. However, during the past century, continental mining of phosphate-bearing rock (e.g., calcium phosphate) to extract phosphoric acid for a variety of industrial uses, especially for fertilizer manufacturing, has disrupted the preindustrial linkages among these three nested P cycles. For example, the global riverine P flux to the sea has more than doubled (Caraco 1993), with unknown consequences for marine ecosystems. Furthermore, enhanced stratification in subtropical and tropical marine habitats as a result of greenhouse-gas-induced warming (Polovina et al. 2008) will eventually result in a reduced delivery of P_i to the euphotic zone, leading to P_i deficiency and reduced rates of net primary production and POP export.

3. PHOSPHORUS FORM AND FUNCTION

3.1. Physical and Chemical Properties

As an element, P exhibits allotropy and can exist in three very different physical and chemical states (known as white P, red P, and black P); however, none of these allotropes occur in nature. Instead, P is combined with oxygen, generally as phosphorus pentoxide (P_2O_5) or P_i. In these forms, P is a constituent of nearly 200 different minerals, including fluorapatite and apatite, which are the two most abundant P-containing minerals in the marine environment.

Much more important than allotropy is that P can exist in a range of oxidation states: +5 (P_i), +3 (phosphite; PO_3^{3-}), +1 (hypophosphite; PO_2^{3-}), and −3 (phosphine gas; PH_3). The common assumption that oceanic P occurs exclusively in a fully oxidized state (valence = +5) is no longer tenable (Hanrahan et al. 2005, White & Metcalf 2007). Although not yet well recognized or appreciated, this spectrum of valence states most likely supports a cascade of microbial oxidation-reduction reactions that may have important bioenergetic and ecological consequences analogous to those of the microbial C, N, and S cycles (**Figure 4**; see also Section 4.3).

P has at least 23 isotopes, with masses ranging from 24 to 46; ^{31}P is the only stable form and hence is the most abundant. Of the radioactive isotopes, only ^{32}P and ^{33}P have decay half-lives that are long enough to be useful as tracers for P dynamics in laboratory and field experiments. ^{32}P and ^{33}P are both beta particle (β^-) emitters (1.71 MeV with a 14.3-day half-life and 0.25 MeV with a 25.4-day half-life, respectively) and can be produced naturally or artificially. The natural production pathway involves the interaction of high-energy cosmic rays with argon atoms, causing cosmic ray spallation (Lal et al. 1957). As for most other cosmogenic nuclides, ^{32}P and ^{33}P are produced mainly in the atmosphere (~90%; Lal 2001) and enter the upper ocean via wet deposition. These natural P radionuclides are found in exceedingly small amounts compared with ^{31}P; the combined global inventory is only approximately 1 g P, with oceanic deposition rates of 5.82×10^{-3} and 6.93×10^{-3} atoms cm^{-2} min^{-1} for ^{32}P and ^{33}P, respectively (Lal 2001).

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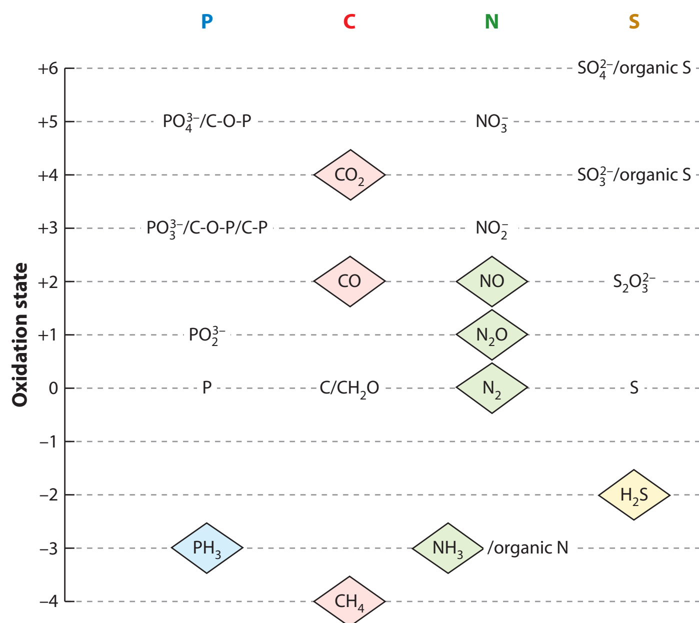

Figure 4

Schematic representation of the variety of possible forms and oxidation states of P in the marine environment compared with those of C, N, and S. Molecules shown in diamonds are gases. Elemental P (oxidation state = 0) and phosphine gas (PH_3) are probably not found in the open ocean.

Despite these low abundances and fluxes, cosmogenic P nuclides are an important tool for studies of the marine microbial P cycle (Lal et al. 1988).

The artificial synthesis of ^{32}P and ^{33}P dates back to the discovery of the neutron (Chadwick 1932) and the subsequent availability of small neutron sources. George de Hevesy—the father of nuclear medicine—was the first to use ^{32}P for metabolic experimentation (Chiewitz & Hevesy 1935). Following the invention of the cyclotron in the early 1930s (patent filed by E.O. Lawrence in 1932 and issued in 1934), large quantities of radioisotopes were produced at the Radiation Laboratory at the University of California, Berkeley, as well as elsewhere, thereby setting the stage for academic research use of ^{32}P (and later ^{33}P) for biological and ecological studies of various aspects of the P cycle.

Finally, the oxygen isotopic abundance of P_i has also been used to track the cycling of P between organic and inorganic pools. Hydrolytic remineralization of P_i from OP compounds incorporates oxygen atoms from ambient water, and repeated cycling leads to P-$\delta^{18}O$ values that are in equilibrium with H_2O-$\delta^{18}O$ both in laboratory cultures of bacteria (Blake et al. 1997) and in natural microbial communities (Paytan et al. 2002). A recent workshop, IsoPhos2012, was convened to discuss P_i-$\delta^{18}O$, including analytical considerations, the need for reference standards, and potential applications in studies of the marine microbial P cycle (Tamburini et al. 2012).

3.2. Characterization, Detection, and Quantitative Analysis of Dissolved and Particulate P Pools

In the marine environment, dissolved P can exist in a variety of different inorganic and organic forms, and most of these are readily bioavailable, albeit at variable rates and assimilation

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efficiencies. The dissolved inorganic P pool is dominated by P_i but may also include contributions from PO_3^{3-}, PO_2^{3-}, pyrophosphate (pyro-P), and inorganic polyphosphate (poly-P). The DOP pool is a diverse mixture of low- and high-molecular-weight (LMW and HMW, respectively) molecules and compounds.

Karl & Björkman (2001) summarized the available methods for studies of the marine microbial P cycle. They defined 50 different analytical procedures to track dissolved and particulate P inventories and fluxes, which I do not repeat here. However, several general comments may be useful. First, analysis of dissolved and particulate P in seawater is neither simple nor straightforward. The broad range of possible inorganic and organic compounds in nature demands that most chemical methods target operationally defined pools, with overlapping reactivities and specificities. For example, both inorganic poly-P and selected dissolved and particulate OP molecules and compounds are highly resistant to most hydrolysis procedures that are designed to measure "total P," which may lead to systematic underestimations in certain marine habitats. Even P_i, the preferred substrate for microbial growth, is difficult to measure with the accuracy that is often required, especially in some oligotrophic habitats, where pool sizes are low (<1 nmol L^{-1}) and turnover times are rapid (<1 h). Furthermore, in most oceanic habitats, the amount of DOP is much larger than the amount of P_i, often by an order of magnitude.

For studies of the marine microbial P cycle, it is important to distinguish between total dissolved P and the pool of BAP. This is a challenge because certain inorganic and OP molecules and compounds (both dissolved and particulate) may have different bioavailability potentials and may be used only under certain conditions of P_i deficiency and by certain microorganisms (Karl 2007a). Finally, POP pools are dominated by nonliving material throughout the world ocean, so the use of POP as an estimate of living (i.e., biomass) P provides only an upper bound. Direct measurements of microbial P are very difficult to achieve. The existence of detectable pools of dissolved ATP and dissolved and nonliving particulate DNA and RNA is enigmatic because of our preconceived notions regarding their obligate associations with living cells. Consequently, DNA sequence analyses of diagnostic P-cycle genes need to either first remove the detrital influence or else measure it directly. This is rarely, if ever, done in ecological studies.

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4. PHOSPHORUS AND THE CENTRAL DOGMA OF BIOLOGY

4.1. Genes, Transcripts, and Proteins

In a now-classic paper titled "On Protein Synthesis," Francis Crick (1958) first hypothesized what has become known as the central dogma of biology—the unidirectional flow of genetic information from DNA to RNA to proteins. He argued that the main function of proteins is to act as enzymes for the catalysis of nearly all cellular reactions, and that the main function of genetic material is to direct protein synthesis. Although there was little direct evidence at that time to support these claims, Crick concluded that "there seems little point in genes doing anything else."

The central dogma has been the guiding light in molecular biology for the past half century, and during the past two decades, remarkable progress has been made in our understanding of the microbial P cycle as a consequence of novel discoveries in the areas of genomics, transcriptomics, and proteomics (Scanlan et al. 2009). Metagenomes provide information about the presence of target genes and therefore metabolic potential. Geographical surveys reveal metabolic patterns that can be used to formulate and test important ecological hypotheses. The Global Ocean Sampling (GOS) expedition, an extensive metagenomic study of marine microorganisms (Rusch et al. 2007), revealed an unprecedented level of genomic diversity in the surface ocean and has served as a sequence library for studies of the microbial P cycle. The metatranscriptome can be used to

inform studies of gene expression and induction intensity and is an important tool for hypothesis testing. Proteomics assesses the culmination of gene expression and transcription and reveals the catalytic patterns and physiological strategies for microbial growth and survival in the sea.

Cellular P metabolism is complex and requires the coordinated activities of numerous proteins and the intracellular presence of a variety of OP compounds, including substrates, cofactors, and regulators. P_i is the preferred P substrate for most microorganisms that have been studied, even though growth can be supported by many other inorganic and OP substrates that may also be present in nature. Pioneering research using *Escherichia coli* as a laboratory model documented an enhanced synthesis of the enzyme alkaline phosphatase (APase, encoded by the *PhoA* gene) upon P_i limitation (Horiuchi et al. 1959). APase is a relatively nonspecific phosphomonoesterase that allows *E. coli* to use a wide range of ester-linked substrates (C-O-P) as alternative sources of P for biosynthesis. Subsequent laboratory efforts revealed that the control of APase synthesis was part of a much more complex P_i starvation induction response that is now known to involve multiple genes and gene clusters (operons) that are all coregulated with *PhoA*. This response process also includes a high-affinity P_i transport system and the enzymatic machinery to transport and assimilate a broad spectrum of LMW P esters (e.g., glycerol-3-P and hexose phosphate), HMW polyanions (e.g., poly-P), and selected organophosphonates (C-P), all of which are regulated at the gene transcription level (**Figure 5**).

The well-orchestrated pattern of gene expression and synthesis of many key P-cycle proteins has been termed the Pho regulon (Torriani-Gorini et al. 1994, Wanner 1996). This gene transcription cascade is initiated by a two-component system that begins with the autophosphorylation of a specific histidine kinase (*PhoR*); this is then followed by the transfer of the P group to the cognate response regulator *PhoB*, which in turn alters the expression of the genes that constitute the Pho regulon (Wanner 1996). The coordinated changes in cell physiology that occur at the onset of P_i limitation prepare the cell for competition and survival under changing environmental conditions. Ironically, both P and (especially) high-energy P are needed to trigger the P starvation response. Also, because the Pho regulon enzymes are induced upon P_i limitation rather than by the presence of hydrolyzable DOP substrates, P_i limitation does not strictly imply P limitation. Despite significant advances in understanding over the past few decades, there are still many Pho-regulated genes for which we have no assigned function, so it is likely that new P-metabolic and regulatory pathways remain to be discovered.

The starvation response in *E. coli* is expressed when the extracellular P_i concentration is approximately 4 μM (Wanner 1996). This value exceeds the highest P_i concentration found anywhere in the open ocean, including P_i-enriched deep-sea habitats, so the response threshold and other features of the Pho regulon in natural assemblages of marine microorganisms may be different from those of laboratory-based models. Furthermore, as mentioned above, it is important to emphasize that the Pho regulon responds to P_i limitation, not to P limitation. Natural assemblages of marine microbes can have low levels of P_i but high levels of bioavailable DOP.

Pioneering research by Scanlan et al. (1993) reported the expression of two novel P_i-binding proteins in *Synechococcus* (strain WH7803) during P_i-deficient growth, and Moore et al. (2005) reported significant clonal and ecotype variability in P_i-acquisition mechanisms for *Prochlorococcus*. More recently, Martiny et al. (2006) examined the presence, arrangement, and regulation of genes involved in the response of *Prochlorococcus* to P_i limitation. They employed a DNA microarray to compare gene expression in a high-light-adapted strain (MED4) with that of a low-light-adapted strain (MIT9313); the two strains varied significantly in their response to P_i limitation. The authors also compared the genomes of 11 *Prochlorococcus* strains that were initially isolated from various geographical regions, and they detected differences that were independent of rRNA-based phylogeny. They hypothesized that this variability may reflect the environmental conditions at the

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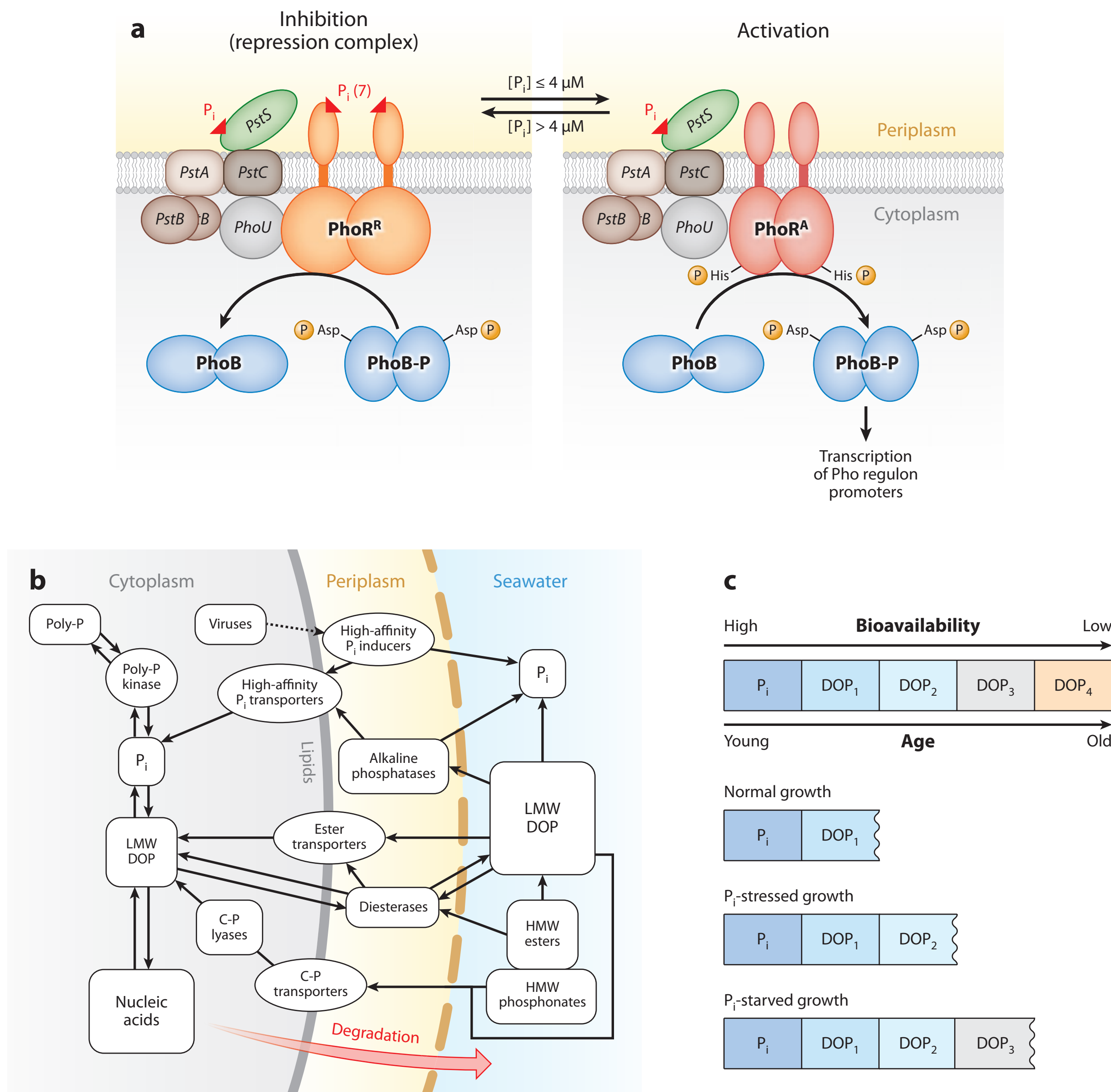

Figure 5

(*a*) Schematic representation of the Pho regulon, initially discovered in *Escherichia coli*, showing selected genes, transporters, regulators, activators, and other controls. The transmembrane signal transduction is initiated by phosphate (P_i) concentration with a threshold for activation of ~4 μM P_i. Adapted from Wanner (1996). (*b*) Schematic representation of the variety of bioavailable P pools in seawater and their probable assimilation pathways. Key substrates include P_i, P esters (C-O-P), and phosphonates (C-P). Abbreviations: DOP, dissolved organic P; HMW, high molecular weight; LMW, low molecular weight; poly-P, polyphosphate. Adapted from Dyhrman et al. (2007). (*c*) Hypothesized sequential utilization of different DOP substrate pools as the microbial communities become increasingly P starved. Adapted from Karl (2007b).

time of isolation, the effect of virus-enhanced lateral gene transfer, or both (Martiny et al. 2006). More recently, Monier et al. (2012) provided field evidence in support of virus–host exchange of P-metabolism genes in a variety of marine eukaryotic phytoplankton. This genetic variation in the metabolic capacity of co-occurring species may lead to P resource partitioning and a reduction in competition for BAP substrates in the sea.

Eukaryotic microorganisms also respond to P_i limitation by regulating the transcription of numerous proteins (Grossman 2000). A comprehensive study by Dyhrman et al. (2012) compared the transcriptome and proteome of the marine diatom *Thalassiosira pseudonana* during P-replete and P-limited growth. A total of 318 transcripts were differentially regulated and 136 proteins were differentially synthesized between the two experimental treatments. As previously observed in bacteria, *T. pseudonana* upregulated genes for P_i acquisition, OP utilization, and cellular P allocation during P-limited growth. The latter process included enhanced poly-P storage and selective substitution of sulfolipids for phospholipids to reduce the P cell quota (Dyhrman et al. 2012). The presence of poly-P in P_i-deficient cultures may have resulted from the induction of the high-affinity P_i-acquisition pathway and short-term luxury uptake of P_i, which has important ecological implications for rapidly changing and pulsed nutrient delivery in marine environments.

The knowledge gained in these laboratory studies, and others like them, can be used for ecological P-cycle hypothesis generation and testing under field conditions. Although the nature of the P_i-stress response in model marine microorganisms varies considerably, many marine microbial genomes and environmental metagenomes contain homologs to genes that are central to the *E. coli* Pho regulon.

4.2. Marine Microbial P-Cycle Components, Contributions, and Controls

It is now well established that the marine microbial P cycle is the most active of the three nested components of the global P cycle (**Figure 1**). Its most basic processes include P uptake, P assimilation into a variety of organic molecules, and organic P remineralization and release of P_i to sustain the cycle (**Figures 2** and **3**). Hidden within these three fundamental processes are numerous regulatory mechanisms and microbial population and community interactions. The survival of microorganisms in low-P_i environments depends on successful P acquisition. High-affinity P_i transporters (low K_m) and a high uptake capacity for P_i (high V_{max}) are competitive advantages for microbes living in environments where P_i delivery is variable in time and space. Furthermore, the ability to obtain P from substrates other than P_i may also confer a competitive advantage but would require a larger genome and more complex metabolic regulation.

4.2.1. P uptake. Marine microorganisms can assimilate at least four different forms of dissolved P: (*a*) inorganic oxyanions of multiple valence states and molecular forms, including P_i, PO_3^{3-}, PO_2^{3-}, pyro-P, and inorganic poly-P; (*b*) monoester- and diester-linked organophosphates (C-O-P); (*c*) organic phosphoanhydrides (C-O-P-O-P); and (*d*) organophosphonates (C-P). The latter three groups include a diverse spectrum of monomeric and polymeric substrates, and they collectively make up the DOP pool. Despite fairly accurate estimation of DOP concentrations in the surface ocean, much less is known about DOP's chemical composition, molecular-weight spectrum, and bioavailability (Karl & Björkman 2002). If DOP is ultimately derived from living organisms, then it should be bioavailable and should not accumulate in the near-surface waters of the global ocean, as is known to be the case (**Figure 2**). Consequently, the elevated DOP:P_i concentration ratios observed in subtropical and tropical habitats worldwide (Karl & Björkman 2002) must be the result of a dynamic process between DOP production and uptake, with the flux balance always in favor of DOP production through the combined processes of exudation,

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grazing, viral lysis, and cell death (**Figure 2**). Field measurements of DOP uptake rates have been made using $^{32}P/^{33}P$-labeled OP substrates. Typically, phosphomonoesters (e.g., glucose-6-P and glycerol-P) or nucleotide triphosphates (e.g., ATP) have been employed in timed incubation experiments. The use of model substrates in field experiments designed to study DOP dynamics is analogous to the use of laboratory-reared microorganisms to understand physiological or genetic responses to controlled growth conditions. Both approaches are important, but neither can capture the diversity and complexity of natural marine habitats and their microbial assemblages. Scaling from, for example, glycerol-3-P or ATP uptake to total DOP is not possible. Because microbes respond to individual molecules and compounds rather than to the bulk DOP pool or P compound classes, additional research needs to be conducted to characterize the DOP pool.

Several approaches have been used to estimate the role of DOP metabolism in the marine microbial P cycle. One involves measurement of DOP production during timed incubations with $^{32}P_i$ and then estimation of pool turnover and hence DOP utilization. Orrett & Karl (1987) used this method to assess the role of DOP in microbial metabolism in the North Pacific Subtropical Gyre (NPSG) by following $^{32}P_i$ incorporation into microbial ATP and RNA pools. The specific radioactivity [nCi ^{32}P (nmol total P)$^{-1}$] of these pools was used to constrain estimates for DOP production, which in this study exceeded the estimated downward vertical flux of DOP in this region by more than two orders of magnitude (Smith et al. 1986). The authors concluded that newly produced DOP must be an important source of P for microbial growth (Orrett & Karl 1987).

Cotner & Wetzel (1992) evaluated DOP utilization in freshwater phytoplankton and bacteria using a kinetic isotope dilution approach that employed tracer levels of $^{32}P_i$ and increasing concentrations of either unlabeled P_i or selected DOP compounds. The isotope dilution of $^{32}P_i$ incorporated into particulate matter by the addition of selected OP compounds relative to the $^{32}P_i$-only control was an index of preferential DOP substrate utilization. Björkman & Karl (1994) used a similar (but not identical) approach to investigate the relative bioavailability of specific DOP substrates in coastal seawaters around Hawaii. A bioavailability factor based on changes in the turnover time of the P_i pool (measured using carrier-free $^{32}P_i$) in the absence or presence of a 1-μM addition of selected organophosphates compared with a positive control containing 1-μM P_i was used as a relative metabolic preference index. Of the compounds tested, nucleotides had the highest bioavailability factors, and monophosphate esters had the lowest (Björkman & Karl 1994). A subsequent study in the oligotrophic NPSG both confirmed the relative preference for nucleotides over monophosphate esters and documented a net P_i regeneration from exogenous nucleotides (Björkman et al. 2000). Because many DOP compounds have low C:P ratios compared with whole-cell C:P stoichiometry (e.g., the molar C:P ratios for glycerol-3-P and glucose-6-P are 3 and 6, respectively, compared with whole-cell C:P ratios of 50–150), DOP metabolism may represent an efficient mechanism for net P_i remineralization. This would be especially important during periods of low growth rate when C demands for maintenance energy generation are high but biosynthetic demands for P are low, as in the mesopelagic and abyssopelagic zones (see Section 5.4).

Another method of quantitatively assessing DOP pool bioavailability involves comparing P_i and total BAP pools by analyzing $^{32}P_i$ labeling of the terminal P group (γ-P) of the total microbial ATP pool (Karl & Bossard 1985). Because the γ-P of ATP turns over rapidly, the specific radioactivity at isotopic equilibrium records the extent of $^{32}P_i$ dilution from all BAP sources, and differences between the theoretical and measured $^{32}P_i/P_i$ values are a direct measure of DOP utilization over the timescale of the incubation period (hours). Björkman & Karl (2003) used this approach to quantify DOP utilization in the oligotrophic NPSG. Their results indicated that the BAP pool was consistently larger than the P_i pool by factors of 1.4–2.8 in the upper water column, indicating that the naturally occurring microbial assemblages obtained a significant portion (often >50%) of their required P from DOP. Because this study analyzed the bulk microbial assemblage and the bulk

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ambient DOP pool, no further characterization of the utilized DOP substrates or identification of the substrate-responsive microbes was possible. However, a subsequent study by Brum (2005) at this same location measured both virus-free dissolved DNA concentrations and production rates, and concluded that the P flux from dissolved DNA pool turnover was sufficient to support the entire DOP demand.

More recently, taxon-specific, quantitative uptake of P_i and specific DOP substrates has been achieved using radioisotopic cell labeling combined with flow cytometric cell sorting and analysis (**Figure 6**). This approach is an improvement over size fractionation, which cannot distinguish heterotrophic bacteria from picocyanobacteria owing to overlapping size spectra, and over previous methods that detected active cells using microautoradiography. Cell sorting also provides the opportunity to test hypotheses regarding relative substrate preference, P resource partitioning, and competition among co-occurring microbial taxa. To date, the most commonly identified taxa in field studies have included *Prochlorococcus*, *Synechococcus*, and "nonpigmented" (assumed to be heterotrophic) bacteria; this last group is sometimes further subdivided by the intensity of DNA staining into low- and high-nucleic-acid subpopulations. Because there is likely to be cell-to-cell variability within each sorted group or taxon, the sorted populations record only the mean condition for the aggregate assemblage, which typically ranges from 10^3 to 10^6 cells per sample depending on the experimental design.

Zubkov et al. (2007) reported that *Prochlorococcus* and low-nucleic-acid bacteria, the latter dominated by SAR11, are the main consumers of $^{33}P_i$ in P-deficient ($P_i < 5$ nmol L^{-1}) habitats of the North Atlantic Subtropical Gyre. They concluded that these two groups are in direct competition for the limiting P_i resource and that larger microbial cells, including picoeukaryotic phytoplankton (also known as plastidic protists), must acquire their P by other means. Subsequently, Hartmann et al. (2011) hypothesized that either DOP utilization or the consumption of particles, including P-rich prokaryotes, is the most likely pathway for P acquisition in plastidic protists.

Michelou et al. (2011) extended these observations in the western Sargasso Sea by comparing per-cell, per-unit-surface-area, and per-unit-cellular-P-quota uptake of both $^{32}P_i$ and $AT^{32}P$. Their results indicated that the average P_i and ATP uptake rates per cell are 50- and 80-fold higher for *Synechococcus* than for *Prochlorococcus* and bacteria, respectively. The same was true when uptake was normalized to cellular P quota (Michelou et al. 2011). However, despite their minimal per-cell (and per-unit-cellular-P-quota) uptake capacity, bacteria as a group dominated (>90%) total P_i and ATP uptake owing to the overwhelming numerical abundance of this microbial group. Although individual *Synechococcus* cells appear to be very competitive for P acquisition even at low substrate concentrations, their population impact on the P cycle was minimal. This important distinction between competition and consumption of a limiting nutrient is the essence of microbial ecology.

Recent taxon-specific P-cycle investigations in the NPSG reported that the dominant photoautotroph, *Prochlorococcus*, competes equally with bacteria for $^{32}P_i$ uptake on a seawater-volume basis (Björkman et al. 2012). However, on a per-cell basis, *Prochlorococcus* was three times more efficient (20 amol cell^{-1} d^{-1}) for P_i assimilation at ambient concentrations (~50 nmol P_i L^{-1}). The kinetic response of both *Prochlorococcus* and bacteria to the addition of exogenous P_i (10–500 nmol L^{-1})

Figure 6

(*a*) Schematic flow diagram depicting sampling, incubation with a ^{32}P radiotracer, flow cytometric taxon sorting, and measurement of group-specific activities. The scattergram is based on chlorophyll-based autofluorescence and forward scatter using a flow cytometer, and subsequent detection of radioactivity in the sorted cells using liquid scintillation counting. Adapted from Lomas et al. (2011). (*b*) Representative taxon-specific P_i uptake of bacteria and *Prochlorococcus* in the light and dark.

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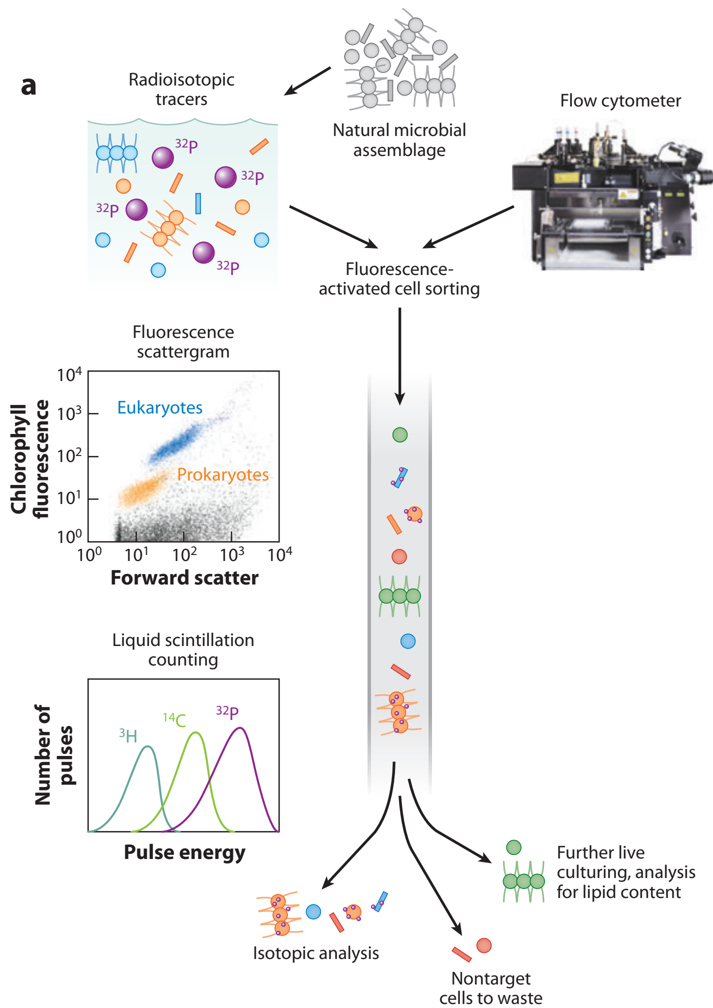

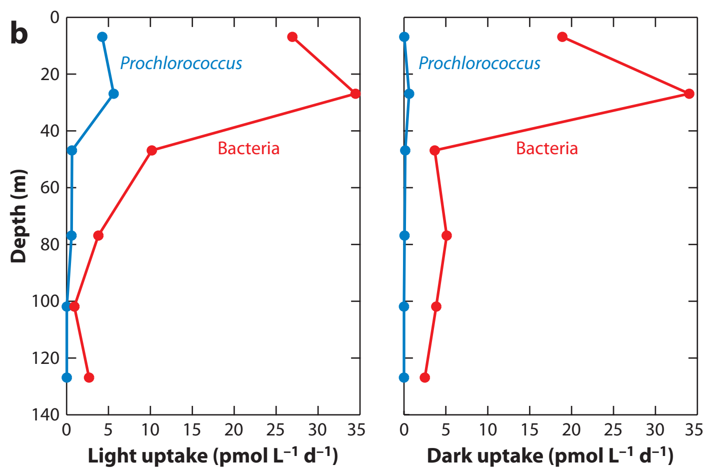

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was small, indicating that internal P_i pools for both groups were nearly saturated at the time of this study (Björkman et al. 2012). Their model DOP substrate, $AT^{32}P$ (labeled in the γ position), displayed more rapid total pool turnover than P_i did and also had a greater kinetic response, with V_{max} values at saturating ATP concentrations (>20 nmol L^{-1}) exceeding those at ambient ATP concentrations by more than 50-fold (Björkman et al. 2012). A follow-on study investigated the light dependence of $^{32}P_i$ and γ-$AT^{32}P$ uptake. Incubation of samples at in situ light levels led to significant increases in $^{32}P_i$ uptake for *Prochlorococcus* compared with uptake after incubation in the dark, with mean light:dark uptake ratios of 2.5:1; there was no significant impact of light on $^{32}P_i$ uptake by nonpigmented bacteria (Duhamel et al. 2012). The uptake of γ-$AT^{32}P$ by *Prochlorococcus* was also higher in the light than in the dark, but again there was no effect on uptake by nonpigmented bacteria (Duhamel et al. 2012).

It remains to be shown whether inorganic and organic P substrates are assimilated simultaneously by the same cell or ecotype or whether these resources are partitioned among separate taxa or subpopulations of the total microbial assemblage. Of importance to the marine microbial P cycle is the relative bioavailability of DOP compounds, which likely leads to preferential uptake of monophosphate esters, nucleotides, vitamins, and alkylphosphonates and the accumulation of polymeric DOP. The turnover of HMW DOP is probably the flux bottleneck in the microbial P cycle, but it may also represent a P buffer or surplus for possible use when more bioavailable supplies are exhausted (Karl 2007a). Ultimately, most (essentially all) DOP is consumed, because deep-sea DOP is <10% of the surface concentration (Thomson-Bulldis & Karl 1998, Karl & Björkman 2002) (see Section 5.4).

In addition to using dissolved P, many microorganisms can use particulate pools, including both P bound in mineral phases and POP. These pools are accessible following ectoenzymatic activity, chemical dissolution, and hydrolysis or by direct particle ingestion (e.g., protistan grazing) in the case of POP. Much less is known about the mechanisms and dynamics of particulate matter diagenesis than about DOP pathways.

4.2.2. P assimilation. Following P_i uptake into the cell or P_i release from transported DOP, P is incorporated into a host of LMW OP compounds. The P cell quota and the subcellular molecular distributions of P vary considerably among taxa and for individual organisms in response to environmental conditions (see Section 5.3). For example, although a majority of microbial P is allocated to nucleic acids, the relative proportion of P in RNA compared with P in DNA is a function of growth rate and genome size. Slow growth and a streamlined genome are both evolutionary strategies for reduced P cell quotas and survival in chronically P_i-deficient marine habitats. Motility (including the effects of gravitational settling, buoyancy regulation, and turbulence) and P_i chemotaxis can increase the flux of P substrates to the cell surface. These and other processes, including P_i-dependent quorum sensing (Van Mooy et al. 2011), serve as important survival mechanisms in P_i-deficient environments, but these processes all require investments in energy.

The subcellular distribution of P is generally determined in laboratory and field experiments by growing cells with radiolabeled P_i and assessing the percentage of ^{32}P (or ^{33}P) in various isolated biochemical pools. In laboratory pure-culture studies, the assumption of equilibrium labeling can be validated. However, in field studies of mixed populations of microorganisms in various and unknown stages of growth, there is no guarantee that all cellular P pools achieve radioisotopic equilibrium during the labeling period.

Cuhel & Waterbury (1984) conducted a comprehensive laboratory study of the incorporation of P into different subcellular fractions of *Synechococcus* strain WH7803. The culture was grown on a P-replete medium (initial concentration 80 μM P_i) at maximum growth rate (doubling time

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approximately 15 h), under continuous light; $^{32}P_i$ was used for uniform labeling over several generations. Total P was 6.6 fg cell^{-1} (molar C:P = 123:1), with 51.4% in the LMW fraction, 43.8% in RNA, and 3.2% in DNA. The virtual absence of phospholipids during P-replete growth was noteworthy. Van Mooy & Devol (2008) employed ^{33}P labeling in a field study conducted in the relatively P-deficient surface waters of the NPSG and found that approximately 45% of the total uptake was in RNA, 20% was in phospholipids, and the remainder was in DNA or grouped under "other." The proportion of P in RNA increased as total $^{33}P_i$ uptake increased, over the range 4–18 nmol P_i L^{-1} d^{-1}.

Bertilsson et al. (2003) documented the elemental and P cell quota flexibility of marine picocyanobacteria using cultures of *Prochlorococcus* (strain MED4) and *Synechococcus* (strains WH8012 and WH8103), the two most abundant phototrophs in P-stressed marine habitats. For P-replete growth, the P cell quotas (in femtograms per cell) were 1.0 (MED4), 1.8 (WH8012), and 3.3 (WH8103), compared with 0.3 (MED4), 0.5 (WH8012), and 0.8 (WH8103) for P-deficient growth. The C:P and N:P molar ratios both increased significantly during P stress (C:P = 464–779:1 and N:P = 59–109:1, respectively) and indicated an efficient P-sparing metabolic strategy. Although the authors did not examine the subcellular allocations of P in this study, they did indicate that the P-deficient MED4 culture allocated >50% of the total P cell quota to a single copy of its 1.66-Mb genome, leaving little in reserve for RNA, phospholipids, and LMW OP.

Building on the Bertilsson et al. (2003) study, Van Mooy et al. (2006) discovered that substitution of S for P in phospholipids can dramatically reduce the P cell quota during P-deficient growth. Using axenic laboratory cultures of the MED4 strain of *Prochlorococcus*, they found that <1% of the total $^{33}P_i$ incorporated into the cell was allocated to lipids. Furthermore, they showed that the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) and the glycolipids mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively) constituted 94% ± 5% of total lipid mass during P-deficient growth. A subsequent study confirmed that S-for-P substitution is a general metabolic strategy for both cyanobacteria and eukaryotic phytoplankton to reduce the P cell quota during P_i-deficient growth (Van Mooy et al. 2009). In short-term laboratory experiments where P_i-replete cells of the diatom *T. pseudonana* were transferred to a P_i-depleted medium or vice versa, the phospholipid content adjusted rapidly (in 24–48 h) for exponentially growing cells, indicating that the S-for-P strategy is a dynamic process that tracks environmental P_i availability (Martin et al. 2011). In a series of field experiments, Van Mooy et al. (2009) demonstrated that total microbial community phospholipid synthesis (expressed as percentage of total P uptake) is positively correlated with ambient P_i concentration, ranging from <1% in the Sargasso Sea (P_i < 10 nM) to ~20% in the South Pacific (P_i > 150 nM).

Inorganic poly-P can represent a significant proportion of the P quota under certain conditions, especially when P_i is present in excess of that needed to support growth. These conditions can be met either in P_i-replete habitats or in chronically P_i-deficient environments following a stochastic pulse of P_i. In theory, any marine habitat that is not P_i limited is P replete and could support poly-P formation. However, in addition to the presence of P_i, cells need energy to transport P_i and polymerize it into poly-P. Indeed, one of the major roles of poly-P may be to serve as an energy reserve, because the reversible polymerization-depolymerization catalyzed by the enzyme poly-P kinase involves ATP [e.g., $(\text{poly-P})_n + \text{ADP} \leftrightarrow (\text{poly-P})_{n-1} + \text{ATP}$]. The presence or absence of poly-P in environmental samples, therefore, may be an indication of the availability of both P_i and excess energy. In most P_i-replete open-ocean environments, this may be restricted to light-saturated, phototrophic microorganisms. Orchard et al. (2010) recently reported the presence of poly-P in field-collected samples of *Trichodesmium* in the oligotrophic Sargasso Sea. ^{31}P-NMR measurements indicated that poly-P constituted up to 25% of the P cell quota, despite physiological

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indicators of P_i stress that included elevated particulate matter C:P and N:P ratios, high APase activity, and low ambient P_i concentrations (Orchard et al. 2010). Analysis of the GOS database for the presence of homologs of three key genes involved in poly-P metabolism revealed that the greatest abundance occurred in low-P_i habitats (Temperton et al. 2011), a result that is consistent with Orchard et al.'s (2010) study. More recently, Martin & Van Mooy (2013) found high poly-P concentrations (~40 nmol P L^{-1}) in particulate matter sampled along a coastal-to-open-ocean transect in the Northeast Pacific and higher ratios of poly-P to chlorophyll offshore, although P_i was >200 nmol L^{-1} along the entire transect. Given the broad range of important physiological functions (Kornberg et al. 1999), the expression of poly-P kinases and intracellular storage of poly-P may be important survival mechanisms in both P-deficient and P-replete marine habitats.

Dyhrman et al. (2009) recently reported that C-P-bonded compounds are 10% (range 8–17%) of the total cellular P in laboratory cultures of *Trichodesmium erythraeum* strains IMS101 and ST6-5, but not in the related species *T. tenue* or *T. thiebautii*. Unfortunately, the ^{31}P-NMR method employed in this study is unable to determine the biochemical structure or cellular function of phosphonates in *Trichodesmium*. A more recent study by Metcalf et al. (2012) detected methylphosphonate (MPn) in association with membrane polysaccharides, but the exact function is still unknown (see Section 5.5).

Gundersen et al. (2002) were the first to use transmission electron microscopy and X-ray microanalysis to measure the major elemental composition of individual microbial cells in the sea. In principle, this method detects all forms of cellular P (including both dynamic and storage pools) as well as the chemically inert organophosphonates; however, P attribution to specific biochemical classes is not possible. Results from the Sargasso Sea demonstrated that total C, N, and P quotas of bacterial cells with volumes ranging from 0.01 to 2.0 μm^3 were best described as a power function of biovolume with scaling factors of <1.0. This result indicates that larger bacteria have less C, N, and P per unit cell volume than smaller bacteria. For P, the number of femtograms per cell was equal to $3.89\,(\pm 0.15) \times V^{0.937(\pm 0.058)}$ ($r^2 = 0.873$, $P < 0.0001$, $n = 84$); the "average" bacterium, with a biovolume of 0.048 μm^3, had a mean P cell quota of 0.226 fg (Gundersen et al. 2002).

Twining et al. (2010) recently compared P, S, and a suite of trace metal cell quotas using synchrotron X-ray fluorescence analysis of individual *Synechococcus* cells. The *Synechococcus* cells ($n = 10$–35) were sampled from three physically and biogeochemically distinct mesoscale eddies within the oligotrophic waters of the Sargasso Sea. Significant differences were observed for P cell quotas, with cells from the deep chlorophyll maximum layer of the mode-water eddy containing nearly 2.5-fold more P (geometric mean P = 50.8 ± 10.2 amol $cell^{-1}$) than cells from either cyclonic or anticyclonic eddies. This result is consistent with the prediction of nutrient entrainment into mode-water eddies and rapid uptake by microbial cells. Repeat (Lagrangian point-of-reference) sampling of the cyclonic eddy on three occasions over a five-day period revealed short-term variability of up to 50% in cellular P quotas, emphasizing that P assimilation by microorganisms is temporally dynamic.

More recently, taxon-specific C, N, and P measurements have been compared for naturally occurring *Prochlorococcus*, *Synechococcus*, and pico- and nanoeukaryote populations sampled from an oligotrophic and a more nutrient-replete habitat (Martiny et al. 2013). The results showed large stoichiometric differences among taxa, with the highest C:P (>300:1) and N:P (~50:1) ratios for flow-cytometrically sorted *Prochlorococcus* cells sampled from the oligotrophic Sargasso Sea (Martiny et al. 2013).

4.2.3. P remineralization. Most of the P_i and DOP assimilated by microorganisms in the surface ocean is locally remineralized back to P_i, which is then available for another round of uptake to support recycled primary production and solar energy capture. In open-ocean ecosystems,

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approximately 90% of gross primary production is supported by local recycling of P; the remaining 10%, or less, is supported by the delivery of new P (usually in the form of P_i) from allochthonous sources. It is therefore imperative to distinguish gross (or total) P uptake and assimilation from net processes. On relatively long timescales (decades to centuries), the system is assumed to be in P steady state (P delivery = P export; **Figure 2**); however, the steady-state assumption is probably not appropriate for shorter timescales (days to years). If the supply of P_i temporarily exceeds the local rate of export, then organic P pools expand until a new balance between P supply and P export is achieved, provided all other production-limiting nutrients are available. The initial pulse of new P_i can sustain several cycles of P_i uptake and remineralization before the system resets to its initial condition. However, if the supply of new P_i is reduced, either abruptly or gradually over a longer period, then P stress and eventually P limitation could lead to fundamental changes in ecosystem structure and function (see Section 6).

The net production of POP (and to a lesser extent DOP) in the euphotic zone supports a continuous downward flux of P that sustains the approximately 1–3 mmol m^{-3} differential in P_i concentration from the surface to the abyss (see **Figure 3** and Section 5.1). This downward vertical flux of OP is balanced by the delivery of regenerated P back to the euphotic zone to maintain ecosystem function. As particles sink, there is a continuous loss of C, N, and P through the combined effects of particle disaggregation, dissolution/hydrolysis, ingestion by zooplankton and other animals, and remineralization by particle-attached microorganisms. The DOP and suspended (nonsinking) POP derived from these processes are then available for remineralization by free-living microbial populations in the mesopelagic and deep-sea environments. Whether the sinking particle-associated or free-living microbes are the most important component for P_i remineralization is neither known nor easily determined (**Figures 2** and **3**).

Pioneering field research conducted by Knauer et al. (1979) documented that the C:N and C:P ratios of sinking particles increase with depth in subtropical oceanic ecosystems. The so-called solubilization length scales are important for understanding the mechanisms involved in the oceanic P cycle and for assessing the capacity for long-term C sequestration (Christian et al. 1997). If the C:N and C:P ratios were identical in the upward inorganic flux and the downward particulate organic flux, there would be no possibility for net C sequestration. Consequently, it is essential to understand the controls on particle export and subsequent remineralization processes.

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4.3. Organophosphonates (C-P), Phosphite Metabolism, and the Marine P Redox Cycle

The chemical synthesis of organic derivatives of phosphonic acid (also known as phosphorous acid) dates back to the end of the nineteenth century, with large-scale production possible after the discovery of the Michaelis-Arbuzov reaction in 1898 (reviewed in Freedman & Doak 1957). Synthetic C-P compounds are currently used in a variety of industrial and commercial applications. Furthermore, it has been suggested that various forms of C-P may have served as prebiotic P carriers (De Graaf et al. 1997); the presence of alkylphosphonates in the Murchison meteorite supports this hypothesis (Cooper et al. 1992). Although organophosphonates appear to be ancient molecules, they were discovered in living organisms only 50 years ago. An unknown, ninhydrin-positive spot on a paper chromatogram from an acid hydrolysate of the sheep rumen protozoan *Tetrahymena pyriformis* was later purified and identified as 2-aminoethane phosphonic acid (2-AEP, also known as ciliatine) (Horiguchi & Kandatsu 1959). The authors concluded their discovery paper with the statement "It will be interesting to investigate whether this compound is widely distributed in nature."

Research conducted over the intervening decades has documented the widespread occurrence of a broad range of C-P compounds in diverse taxa, including marine bacteria, protists, and invertebrates (Hilderbrand & Henderson 1983). Some lower marine invertebrates are particularly rich in organophosphonates; e.g., the sea anemone *Anthopleura elegantissima* can have 30–50% of its total P in the form of 2-AEP (Quin 1965). Despite Hilderbrand's (1983) authoritative treatise *The Role of Phosphonates in Living Systems*, the physiological and ecological roles of marine organophosphonates remain largely unknown.

Most synthetic and biogenic C-P compounds are resistant to chemical (acid and base) hydrolysis, thermal decomposition, photolysis, and the action of phosphatases, making these compounds markedly distinct from C-O-P forms (Ternan et al. 1998, Nowack 2003). The C-P bond strength (62 kcal mol^{-1}) is similar to C-O-P molecules, but the activation energy is much higher (Black et al. 1991). For example, 2-AEP is stable in 8-*M* HCl at 150°C for 48 h and in 5-*M* NaOH at 120°C for 8 h (Ternan et al. 1998). Kittredge et al. (1962) hypothesized that the chemical stability of the C-P bond may lead to a buildup of C-P compounds in the marine DOP pool and could even represent a dead end in the marine P cycle. However, they also suggested that "a more interesting alternative" would be if the C-P compounds were actively degraded by marine microorganisms. At that time, there was no evidence to support either prediction.

Kolowith et al. (2001) used tangential flow ultrafiltration to isolate HMW (1–100-nm) DOM (U-DOM) and employed ^{31}P-NMR to characterize 16 samples from the North and South Pacific Ocean, the North Atlantic Ocean, and the North Sea. The U-DOM, which represented 20–40% of the total DOM, contained approximately 75% C-O-P and 25% C-P regardless of geographical location. No further molecular characterization of the C-P compounds was or has since been made, but it is likely that the U-DOM includes a diverse spectrum of C-P derivatives from lipids, carbohydrates, and proteins in both native and partially degraded forms. A more recent study using electrodialysis and reverse osmosis to concentrate LMW and HMW DOP pools and then ^{31}P-NMR to characterize them discovered that the LMW pool was enriched in C-O-P and depleted in C-P compounds relative to the HMW pool (Young & Ingall 2010). By contrast, they detected only C-O-P in the corresponding POP samples (Kolowith et al. 2001). Assuming that DOP is derived from POP, these data on the relative proportions of C-O-P and C-P support the hypothesis of an enrichment of organophosphonates in U-DOM.

The discovery of 2-AEP and related C-P compounds stimulated interest in the C-P biosynthesis and biodegradation pathways; the spot on a chromatogram founded a new subdiscipline of P biochemistry (Kittredge & Roberts 1969) and microbial ecology. Currently, much more is known about C-P biodegradation than about C-P biosynthesis. The first step in C-P bond synthesis is thought to be the reversible, intramolecular rearrangement of phosphoenolpyruvate (PEP) to phosphonopyruvate catalyzed by the enzyme PEP phosphomutase (Seidel et al. 1988). Because the reaction equilibrium favors PEP, a coupled and irreversible enzymatic reaction that is catalyzed by phosphonopyruvate decarboxylase yields phosphonoacetaldehyde and CO_2 (White & Metcalf 2007). Beyond these initial steps, the biosynthetic pathways are poorly known. However, a novel C-P biosynthetic pathway leading to the formation of MPn was recently reported in *Nitrosopumilus maritimus*, an isolate of the group I marine Thaumarchaeota phylum (Metcalf et al. 2012). This was an unexpected discovery because MPn was not thought to be a natural product. To link this pathway back to the marine environment, the authors screened the GOS microbial metagenomic database (Rusch et al. 2007) for the presence of homologs to their newly discovered MPn synthase. They identified several genes, including one from a *Pelagibacter* scaffold, even though that gene is absent from the genomes of isolated *Pelagibacter* strains.

Interest in C-P compound degradation has been keen ever since Zeleznick et al. (1963) showed that *E. coli* could grow on MPn as the sole source of P. Several independent C-P degradation

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pathways have been described (Kononova & Nesmeyanova 2002, Quinn et al. 2007), but the two most well studied enzymes are phosphonatase and C-P lyase. Homologous genes for these degradation pathways are distributed in distantly related bacteria, thus providing strong evidence for lateral gene transfer during evolution (Huang et al. 2005). The C-P substrate specificity of phosphonatase is limited (e.g., 2-AEP) compared with the relatively broad specificity observed for C-P lyase (Wanner 1994). In this regard, C-P lyase may be the organophosphonate equivalent of APase because both enzymes have broad substrate specificity, are P_i starvation inducible, and are under Pho regulon control.

Laboratory studies of the two most abundant marine picocyanobacteria (*Prochlorococcus* and *Synechococcus*) and the marine N_2-fixing cyanobacterium *Trichodesmium* have documented the presence of genes responsible for the transport and degradation of organophosphonates (Palenik et al. 2003, Dyhrman et al. 2006, Ilikchyan et al. 2009). Genes encoding these C-P degradation pathways have also been reported in isolated marine heterotrophs and in marine metagenomic sequence libraries (Quinn et al. 2007). Martinez et al. (2010) recently screened marine bacterioplankton fosmid libraries for the ability to complement a strain of *E. coli* that was unable to use 2-AEP as a sole source of P. Using this functional screening approach, they identified a novel 2-AEP degradation pathway and then analyzed the marine metagenomic database to confirm that the genes in this new pathway are both abundant and widespread in the marine environment (Martinez et al. 2010).

The ability to degrade C-P compounds varies across the microbial world. Some bacterial strains contain more than one C-P degradation pathway, some contain degradation pathways that are C-P substrate rather than P_i starvation inducible, and some incorporate C as well as P. White et al. (2010b) compared P cell quotas for *Trichodesmium* strain IMS101 growing on P_i, two model C-O-P substrates (AMP and glucose-6-P), and two model C-P substrates (MPn and 2-AEP). Despite comparable rates of C and N_2 fixation on all P substrates tested, both net P assimilation rates and P cell quotas were significantly lower, and the corresponding C:P molar ratio significantly higher, for growth on the organophosphonates. The authors hypothesized that luxury P uptake is not possible during growth on C-P substrates, which has important implications for the elemental stoichiometry and P cell quotas of marine microbes growing on C-P compounds in their natural habitats. No bioenergetic budget was reported in this or any other study of C-P metabolism, so whether the free energy of C-P hydrolysis is conserved during the oxidation of reduced P is unknown.

Despite the enormous progress that has been made in C-P gene expression and biochemistry over the past few decades (see Section 4.1), our current understanding of the ecological significance of C-P metabolism is in its infancy. Although P acquisition under conditions of P_i limitation is most likely the selective force to retain the ability to degrade C-P compounds, the biosynthetic function is less well understood. The redox change of P_i in C-P biosynthesis requires a significant investment of energy in the form of ATP (Kim et al. 1998), so the presence of reduced C-P compounds in organisms from energy-limited marine ecosystems suggests that they may have a fundamental role leading to a competitive advantage in nature. Many biogenic C-P compounds are structural analogs of naturally occurring phosphate esters and may confer stability (e.g., membrane-associated phosphonolipids; Kennedy & Thompson 1970), thereby reducing the requirement for maintenance energy expenditure. Because most marine microbes are slow growing and have low growth efficiencies, a reduction of maintenance energy costs would confer a selective ecological advantage. Some C-P compounds are antibiotics or antimetabolites, so their synthesis could protect selected marine microbes from viral lysis, protistan grazing, or biofouling. Finally, the recent discovery of MPn as a marine microbial natural product with an estimated 10^{26} individual cells participating in global ocean MPn biosynthesis (Metcalf et al. 2012), coupled with efficient MPn degradation (Karl et al. 2008a), leads to the inevitable conclusion that P redox chemistry must be a common feature

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of the marine microbial P cycle (**Figure 2**). Future research goals are to identify the environmental controls on C-P compound cycling, the microorganisms involved, and the bioenergetics of P oxidation and reduction pathways, especially in energy-limited marine environments.

In addition to a marine P redox cycle sustained by the coupled synthesis and degradation of C-P-bonded OP compounds, there may be a microbially mediated redox cycling of inorganic P compounds (**Figure 2**). The microbiological oxidation of PO_3^{3-} (valence = +3) to P_i (valence = +5) was first documented in soils more than 80 years ago (Rudakow 1929, cited in Heinen & Lauwers 1974), and subsequent laboratory studies by Casida (1960) and Malacinski & Konetzka (1966) confirmed bacterial growth on PO_3^{3-} as the sole source of P. Heinen & Lauwers (1974) also isolated a substrate-specific PO_2^{3-} (valence = +1) oxidase from *Bacillus caldolyticus* and showed that the electron flow during oxidation was partially coupled to NADH production. Tsubota (1959) studied bacterial P_i reduction in paddy fields and demonstrated the formation of both PO_3^{3-} and PO_2^{3-} under anaerobic conditions. Collectively, these studies document the exciting potential for a ubiquitous P redox cycle (i.e., $P^{+5} \rightarrow P^{+3} \rightarrow P^{+1} \rightarrow P^{+3} \rightarrow P^{+5}$) in nature (**Figure 4**).

There is growing evidence for an active, microbially mediated P redox cycle in the sea. Recent progress on the P redox cycle has involved laboratory-based genetic and physiological investigations of isolated bacteria as well as the screening of assembled metagenomic and genomic databases; field-based ecological studies are nonexistent. C-P lyase can also oxidize PO_3^{3-} to P_i (Metcalf & Wanner 1991), and PO_2^{3-} oxidation to P_i proceeds via a PO_3^{3-} intermediate (Metcalf & Wolfe 1998). Costas et al. (2001) characterized a novel P-oxidizing enzyme from *Pseudomonas stutzeri* strain WM88, phosphite dehydrogenase, and showed that it stoichiometrically couples NADH formation to P_i production, thereby demonstrating partial conservation of the energy released during the oxidation (Costas et al. 2001). Subsequent genetic analyses identified a second operon specific to PO_2^{3-} oxidation, and both systems are regulated by the Pho regulon (White & Metcalf 2004) (see also Section 4.1). Consequently, in addition to serving as a P source for cellular biosynthesis, the oxidation of reduced inorganic P may serve as an energy source. Indeed, Schink & Friedrich (2000) showed chemolithoautotrophic bacterial growth by dissimilatory PO_3^{3-} oxidation using sulfate as the electron acceptor [e.g., $4HPO_3^{2-} + SO_4^{2-} + H^+ \rightarrow 4HPO_4^{2-} + HS^-$; $\Delta G^{\circ\prime} = -364$ kJ (mol sulfate)$^{-1}$, where $\Delta G^{\circ\prime}$ is the Gibbs standard free energy change]. The authors speculated that PO_3^{3-} might be derived via the degradation of organophosphonates. This was the first and, to date, only report of reduced P oxidation as an obligate type of bacterial metabolism. Recently, Yang & Metcalf (2004) reported that *E. coli* APase, one of the most well studied enzymes, also catalyzes the oxidation of PO_3^{3-} to yield P_i and H_2; the end-product H_2 is a source of energy for many microorganisms. This novel catalytic function—i.e., PO_3^{3-}-dependent, H_2-evolving hydrogenase activity—is unprecedented in both P and H biochemistry (Yang & Metcalf 2004).

The potential significance of PO_3^{3-} oxidation in the marine microbial P cycle is unknown, but two recent reports of PO_3^{3-} utilization by *Prochlorococcus* have begun to explore a possible ecological role. Martinez et al. (2012) investigated reduced P utilization in several *Prochlorococcus* strains. Although strain MIT9301 was unable to grow on 2-AEP as a sole source of P, it was able to use PO_3^{3-}. The oxidation of PO_3^{3-} was mediated by an NAD-dependent PO_3^{3-} dehydrogenase, and although no energy budget was attempted, at least a portion of the available energy may have been conserved via NADH production. In contrast to strain MIT9301, the two other *Prochlorococcus* strains tested (MED4 and MIT9313) were unable to grow on PO_3^{3-} as the sole source of P (Martinez et al. 2012). To link their laboratory-based results back to the field, they analyzed the occurrence and abundance of the PO_3^{3-}-utilization genes in several marine microbial metagenomic and metatranscriptomic sequence libraries. They found that in the P_i-depleted region of the Sargasso Sea, an estimated 2–9% and 22–59% of the naturally occurring *Prochlorococcus* cells at

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50 and 100 m, respectively, may be able to oxidize PO_3^{3-} (Martinez et al. 2012). In a separate study, Feingersch et al. (2011) investigated the characteristics of an organophosphonate binding protein (encoded by *PhnD*) that had been reported in globally abundant marine microorganisms, including *Prochlorococcus*. The authors expressed two different *Prochlorococcus PhnD* genes in *E. coli* and measured the binding specificities of several substrates (five organophosphonates, PO_3^{3-}, and P_i) using isothermal calorimetry (Feingersch et al. 2011). One protein (encoded by *PhnD2*) bound strongly with MPn, ethylphosphonate, and PO_3^{3-}, whereas the other (encoded by *PhnD1*) bound strongly to PO_3^{3-}, with only weak affinities to MPn and P_i. Both the *PhnD1* and *PhnD2* genes are present in metatranscriptomic data sets from the Sargasso Sea, suggesting that PO_3^{3-} transport and oxidation may be an important source of P in selected oligotrophic marine habitats.

Phosphite is a potent fungicide that is especially active against the oomycetes (Griffith et al. 1989). The biochemical mode of action appears to be at the site of adenine nucleotide synthesis, and exposure leads to decreased intracellular concentrations of NAD and ATP (Griffith et al. 1990). Therefore, in addition to the possibility of PO_3^{3-} production as an intermediate in OP degradation or PO_2^{3-} oxidation, it may also be actively produced as an antibiotic by some marine organisms. Presently, there are no data on the concentrations or fluxes of any inorganic or organic reduced P compound in the sea, so there is a vital need for method development. Although total dissolved and particulate C-P pools have been measured using ^{31}P-NMR, we currently lack information about molecular composition and C-P bioavailability for microbial assimilation. Field research on the microbial P redox cycle is just getting started.

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5. PHOSPHORUS AND THE CENTRAL DOGMA OF MICROBIAL OCEANOGRAPHY

5.1. Global P Distributions and Controls

Throughout the global ocean, there are systematic variations of P_i both between major ocean basins and with ocean depth (**Figure 7**). These regional distributions are a manifestation of the large-scale global ocean circulation superimposed on the biological processes of P uptake, particulate matter export, and remineralization, which are collectively termed the biological pump. Globally, surface mixed-layer P_i concentrations vary by more than 1,000-fold, from <1 nmol L^{-1} in the oligotrophic North Atlantic (Cavender-Bares et al. 2001) to >1 μmol L^{-1} in the Southern Ocean (**Figure 7**). The lowest P_i concentrations are below the theoretical uptake capacity for many marine phytoplankton (Wu et al. 2000), so these regions must be P_i stressed. However, despite the vanishingly low P_i (<1 nmol L^{-1}), these habitats have significant DOP concentrations (~50–100 nmol P L^{-1}) and contain diverse pico- and nanophytoplankton assemblages that support primary production, so there appears to be ample P for microbial growth (Cavender-Bares et al. 2001). Finally, an evaluation of the P status of natural microbial assemblages must also consider the difference between long-term chronic P_i stress and short-term acute P_i limitation. Chronic (i.e., lasting for much longer than the lifetime of a cell) P_i limitation probably leads to changes in relative gene frequencies of the natural assemblage and selection for the species or ecotypes best suited for survival (Coleman & Chisholm 2010).

5.2. Ecological Stoichiometry and N Versus P Limitation

All microorganisms contain a nearly identical suite of biomolecules with common structural and metabolic functions. This biochemical uniformity establishes the bulk elemental composition of

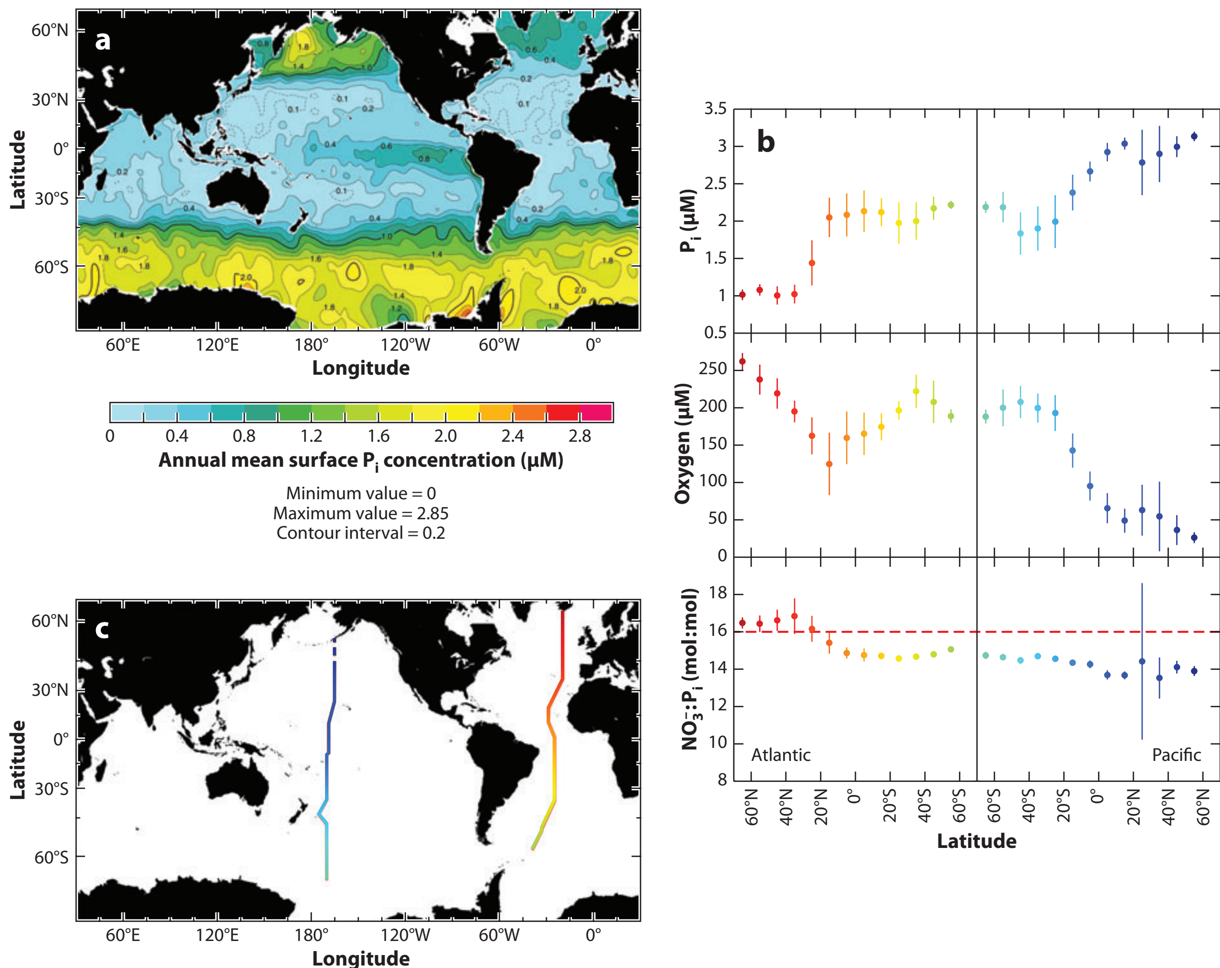

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Figure 7

(*a*) Global distribution of surface phosphate (P$_i$) concentrations. The highest concentrations (∼2 μM; *yellow*, *orange*, and *red*) are in the Southern Ocean, and the lowest (<0.1 μM; *light blue*) are in the subtropical ocean gyres. Adapted from the NOAA Oceanographic Data Center's World Ocean Database (**http://www.nodc.noaa.gov/OC5/WOD/pr_wod.html**). (*b*,*c*) P$_i$ concentrations, oxygen concentrations, and nitrate: P$_i$ (NO_3^-:P$_i$) ratios (panel *b*) along a transect moving down the Atlantic and up the Pacific (panel *c*) for water depths of 500–1,500 m in 10°-latitude bins, presented as mean values ±1 SD. The red dashed line in the NO_3^-:P$_i$ plot is the Redfield ratio of 16:1. Data from the World Ocean Circulation Experiment Hydrographic Program Office (**http://woce.nodc.noaa.gov/woce_v3/wocedata_1/whp**) for surveys A16C, -N, and -S (Atlantic) and P15 (Pacific).

life (Sterner & Elser 2002). Redfield (1934) noted that the dissolved NO_3^-:P$_i$ molar ratio in deep seawater was constrained within relatively narrow limits despite large changes in the absolute nutrient concentrations. Furthermore, and more important, the dissolved NO_3^-:P$_i$ ratio was similar to the N:P ratio observed in phytoplankton. Redfield (1934) hypothesized that there must be an intimate coupling between the chemical composition of plankton and that of seawater. He further reasoned that this well-constrained stoichiometric relationship was controlled by the metabolic activities of N_2-fixing cyanobacteria, which were ultimately limited by P$_i$ (Redfield 1934, 1958). This elemental stoichiometry of marine microbes, the so-called Redfield ratio of 106C:16N:1P

by atoms, has achieved canonical status in aquatic sciences over the past several decades and has become a unifying foundation of ocean biogeochemistry (Redfield et al. 1963, Lenton & Watson 2000).

The concept of a well-constrained N:P ratio in microorganisms is a fundamental consequence of the biochemical composition of cells (Arrigo 2005). Each of the most abundant biopolymers (e.g., proteins, nucleic acids, polysaccharides, and lipids) has a different elemental composition, so the bulk C:N:P ratio is expected to vary as a function of metabolic activity and growth rate. A comprehensive compilation of the elemental composition of marine phytoplankton based on laboratory culture experiments revealed stoichiometric plasticity, with molar N:P ratios ranging from <5:1 for P-replete growth to >100:1 for P-deficient growth (Geider & La Roche 2002). The critical molar N:P ratio that marked the transition between N limitation and P limitation of phytoplankton growth was in the range of 20–50:1, well above the Redfield N:P ratio of 16:1 (Geider & La Roche 2002).

Klausmeier et al. (2004) developed a physiological N:P optimization model for phytoplankton growth under various P resource conditions. Their model predicts a variable optimum N:P ratio ranging from 8.2:1 to 45:1, depending on environmental conditions. They hypothesized that the Redfield N:P ratio of 16:1, rather than being a fixed value, is the average N:P for phytoplankton growth in nature. A recent theoretical assessment of the balance between protein synthesis and ribosome (rRNA) production during nutrient-replete microbial growth predicted a protein:rRNA ratio of 3 ± 0.7:1, which corresponds to an N:P ratio of 16 ± 3:1 (Loladze & Elser 2011). Nutrient limitation by N or P constrains protein or rRNA synthesis, respectively, leading to N:P ratios that are higher or lower than the optimal (Redfield) ratio. Consequently, the elemental stoichiometry measured in a given marine ecosystem appears to have a fundamental basis in microbial P metabolism.

There is a long-standing and lively debate in the oceanographic literature regarding the nature of the so-called growth-limiting nutrient. Unfortunately, the views on nutrient control of primary production have become siloed; geochemists mostly favor P, whereas biologists favor fixed N. Codispoti (1989) summarized the key scientific issues, specifically the balance between rates of N_2 fixation and denitrification along with the bioavailability of P and iron (Fe). In low-nutrient marine habitats, extended periods of fixed N limitation should select for N_2-fixing microorganisms and force the ecosystem toward P limitation; P would then be the ultimate production rate-limiting macronutrient (see Section 5.3).

Christian (2005) compared the results of a physical-biogeochemical-ecosystem model that assumed either constant or variable C-N-P elemental stoichiometry for phytoplankton in the oligotrophic NPSG. His model predicted that the ecosystem should be strongly P_i limited if C-N-P is allowed to vary owing to the growth of N_2-fixing microorganisms; this result is supported by field observations (Karl et al. 1997). N_2 fixation in the NPSG is controlled by the availability of P_i and Fe, and ultimately by the ability of diazotrophs to compete for these common limiting resources. The author concluded that this modulation in ecosystem elemental stoichiometry is important for the C cycle, specifically the maintenance of a biological CO_2 sink. A more recent study has reported systematic latitudinal trends in the elemental stoichiometry of marine particulate matter, with the highest molar C:P (195:1) and molar N:P (28:1) ratios occurring in nutrient-depleted low-latitude gyres, which may be a manifestation of N_2-fixation-dependent P_i limitation (Martiny et al. 2013).

A large part of this debate about N versus P limitation centers on the distinction between the proximate limiting nutrient, as defined by Liebig's law of the minimum, and the ultimate limiting nutrient that controls ocean production and metabolism over much longer timescales (Tyrrell 1999). Diagnosis of proximate nutrient limitation of phytoplankton and bacterial production has been performed in many field studies through short-term nutrient bioassay experiments (e.g.,

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Rivkin & Anderson 1997, Davey et al. 2008, Moore et al. 2008). Although the results vary considerably with region, depth, time of the year, and other environmental variables, the greatest metabolic/growth response typically results from the addition of both N+P (or N+P+Fe) for phototrophs and organic C+N+P for heterotrophs, indicating natural conditions of multiple-nutrient stress or nutrient colimitation. If fixed N and Fe are both available, then P supply is the critical factor. If, however, Fe delivery limits local N_2 fixation, as it would in regions far removed from continental dust sources, then N limits ecosystem production (see Section 5.3). The entire process is self-regulating in that the net rate of N_2 fixation is upregulated by a deficit of fixed N (in the presence of adequate supplies of P and Fe) and is downregulated by excess fixed N. This complex interplay of essential nutrient sources and sinks can lead to seasonal (or longer) oscillations in the limiting nutrient, in microbial community structure and function, and in P-cycle dynamics (Karl 2002). Consequently, the integrated net effect, articulated by the Redfield ratio hypothesis, is a result of ecological processes acting on multiple time and space scales. Ultimately, a Redfield ocean is one where N and P are colimiting, and all indications are—at least to a first-order approximation—that this is a reasonable description of most marine habitats. However, even small deviations from Redfield stoichiometry can disrupt the mean flows of matter and energy, leading to complex, non-steady-state ecosystem dynamics that are often challenging to sample and interpret (see Section 5.3).

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5.3. Coupled and Uncoupled Bioelemental Cycles in the Sea

The marine microbial P cycle is inextricably linked to all other biophilic elements. These essential bioelements are mostly assimilated, transported, and remineralized by a common set of physical, chemical, and biological processes, and they therefore have distribution patterns similar to those of P_i (Deutsch & Weber 2012). However, under certain conditions, the normally coupled fluxes of P, N, and Fe are decoupled. For example, Fe delivery to the surface waters by eddy diffusion and upwelling is supplemented by atmospheric deposition (both dry and wet) of dust particles that contain bioavailable Fe (**Figure 8**). This process varies in both time and space, and is most important downwind of continental deserts (Parekh et al. 2005, Moore et al. 2006). Furthermore, some dust sources (e.g., the Saharan desert) contain P_i as well as Fe and therefore lead to severe fixed N deficiency. Likewise, the normal supply of fixed N, as NO_3^-, can be supplemented via the metabolic activities of N_2-fixing microorganisms (**Figure 8**); the P cycle has no analogous source pathway. Diazotrophy [$N_2 \rightarrow$ ammonia (NH_3)] is a highly specialized and energy-demanding process that is restricted to a small number of microbial taxa. Although initial investigations focused on *Trichodesmium* as the dominant diazotroph in the sea (Carpenter & Romans 1991), we now recognize a much broader diversity of diazotrophs, including unicellular cyanobacteria and N_2-fixing symbionts of eukaryotic phytoplankton (Karl et al. 2002, Zehr & Kudela 2011, Zehr 2013). Although some heterotrophic marine bacteria can also fix N_2 (Halm et al. 2012), the open ocean does not have large amounts of organic matter, so solar energy is generally required to power N_2 fixation (Karl et al. 2008b, Luo et al. 2013). In addition to needing energy (usually sunlight), the enzyme nitrogenase requires Fe, so N_2 fixation is ultimately linked to the atmospheric Fe deposition pathway. Sustained net growth of diazotrophs also requires a supply of P_i, so ecosystems that depend on diazotrophy as a substantial source of fixed N are likely to be P_i stressed. The nutrient control of N_2 fixation is site specific (Monteiro et al. 2011) owing in large part to the geographical variability of atmospheric dust deposition and oxygen minimum zones (Deutsch et al. 2007, Luo et al. 2013). Consequently, controls on N_2-fixation rates, N:P mass ratio regulation, and C export and sequestration may have ecosystem response times on the order of decades or longer (Moore et al. 2006). Finally, because N_2 fixation may ultimately be controlled by Fe

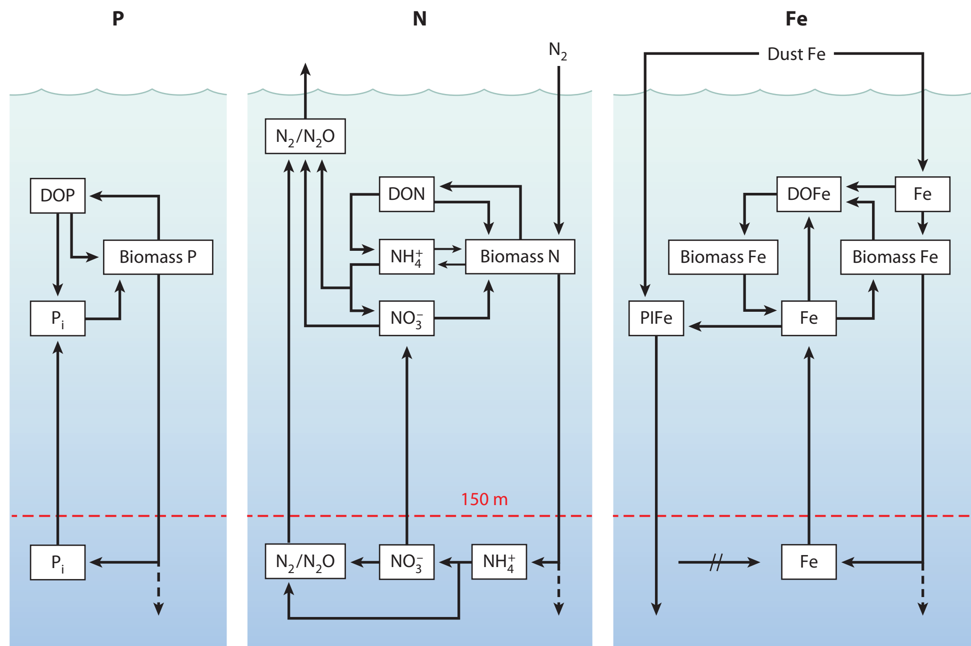

Figure 8

Schematic representation of the probable delivery and cycling pathways for P, N, and Fe to the open sea. All three elements are delivered to the surface ocean via advective processes from the deep-sea nutrient reservoir, but N has an additional source via N_2 fixation, and Fe delivery is enhanced by dry and wet atmospheric deposition. These pathways lead to a decoupling of the major bioelement cycles in the sea, and ultimately to P control of microbial processes. The dashed red line at 150 m depicts the approximate boundary between the euphotic zone (above) and the mesopelagic zone (below). Abbreviations: DOFe, dissolved organic Fe; DON, dissolved organic N; DOP, dissolved organic P; P_i, phosphate; PIFe, particulate inorganic Fe. Adapted from Karl (2002).

availability, and because dust delivery to the ocean is climate sensitive, there may be inextricably linked feedback mechanisms that regulate N_2 fixation, atmospheric CO_2 concentrations, and dust deposition over relatively long periods (Michaels et al. 2001).

5.4. The Enigma of Alkaline Phosphatase Activity in the Sea

Studies of the marine microbial P cycle have made extensive use of enzymatic biomarkers to assess the P status of natural microbial assemblages. Because P_i starvation in bacteria is known to result in a significant increase in APase, and because of the availability of several simple assays for APase in environmental samples, there is a relatively large amount of data on APase in marine ecosystems (see the reviews Karl & Björkman 2002 and Hoppe 2003). The presence of APase in a given field sample is often interpreted as an indicator of P_i deficiency, but the extant literature is confusing and at times contradictory. Indeed, there are alternative pathways leading to APase expression and, therefore, novel ecological interpretations for the presence of APase in the sea (Karl & Björkman 2002).

All living organisms in the sea, from bacteria to marine mammals, have APase, so it is important to identify the source or sources of APase in a given habitat, but this is not always possible. Because standard volumetric APase activities (e.g., in nanomoles per liter per hour) vary with

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biomass, APase activity must be normalized on a per-cell, per-unit-biomass, or per-unit-P-cell-quota (or equivalent) basis for comparative analyses to be meaningful. This is often a challenge for mixed assemblages of microorganisms of various sizes. Furthermore, the common use of synthetic substrates added at saturating concentrations yields only maximum potential hydrolysis rates; extrapolation to in situ rates is not possible. Finally, at least three distinct APase gene families have been described (*PhoA*, *PhoD*, and *PhoX*), each with its own unique substrate specificity, metal cofactor(s), cellular localization, and kinetics. Even though *PhoA* and *PhoX* are thought to have similar functions, both genes are expressed in *Trichodesmium* strain IMS101 during P starvation (Orchard et al. 2009); the specific functions may therefore be complementary, rather than redundant, for survival in oligotrophic seawaters.

A recent improvement in APase detection methodology employs an insoluble fluorogenic substrate-based reaction termed enzyme-labeled fluorescence, which can be used to detect and localize APase activity in single cells using epifluorescence microscopy or flow cytometry (González-Gil et al. 1998). In a recent study of the Gulf of Aqaba, Mackey et al. (2007) observed that large eukaryotic phytoplankton were enzyme-labeled-fluorescence (APase) positive but picophytoplankton were not, despite low P_i concentrations (<50 nM) and low molar $NO_3^-:P_i$ ratios (<5:1). Consequently, different subpopulations of the microbial community express P_i-limitation proteins under different conditions, so molecular or biogeochemical diagnoses may need to be applied in a species- or ecotype-specific manner (Mackey et al. 2007).

Koike & Nagata (1997) detected APase activities in deep waters (1,000–4,000 m) of the North Pacific that were nearly equivalent to those of surface samples on a per-unit-volume basis, despite a >100-fold decrease in bacterial biomass for abyssal waters. If APase activity per cell reflects P_i deficiency, then deep-sea microorganisms would exhibit little or no APase activity, because the deep sea is P_i replete (>2 μM P_i at depths of >1,000 m) when compared with surface waters. Equally intriguing was the 1,000-fold increase in the ratio of APase to β-glucosidase, a key bacterial enzyme responsible for polysaccharide hydrolysis (**Figure 9**). The authors hypothesized that this enigmatic enrichment of APase activity in the deep sea may be the result of fragmentation of sinking particles, and that the origin of the deep-sea APase was likely to have been the P_i-limited surface ocean (Koike & Nagata 1997).

Elevated APase activities at abyssal depths have now been reported for the Indian Ocean (Hoppe & Ullrich 1999) and the North Atlantic (Baltar et al. 2009). Hoppe & Ullrich (1999) observed per-bacterial-cell APase values that were nearly 40 times greater at 800 m than at the ocean's surface, despite a decrease in the activities of other hydrolytic enzymes (**Figure 9**). However, unlike Koike & Nagata (1997), who concluded that the deep-sea APase must have originated elsewhere, Hoppe & Ullrich (1999) hypothesized that abyssal APase enrichment is a manifestation of enhanced C acquisition involving the assimilation of bioavailable DOP compounds. The elevated APase would locally regenerate P_i but (more important) would also capture the C skeleton that could provide energy for cell maintenance, precursors for net biosynthesis, or both. Previous research by Wilkins (1972) documented the induction of APase by nucleotide base starvation, and others have shown that the synthesis of some P_i-starvation genes, including the one encoding APase, can be triggered by both C and N starvation (Wanner & McSharry 1982). An independent analysis of the oligotrophic North Pacific metagenome revealed APase genes (especially *PhoD* and *PhoX*) in deep water (500–4,000 m), with abundances that were approximately equal to that of the ubiquitous single-copy *RecA* gene (Luo et al. 2011).

This wealth of mostly new information on the distribution and activity of APase in deep-ocean waters calls into question previous interpretations of APase being an indicator of P_i deficiency only. There are now at least two fundamentally different ecological processes that can lead to elevated cellular APase, and it is premature to conclude that one applies only to surface water and the other

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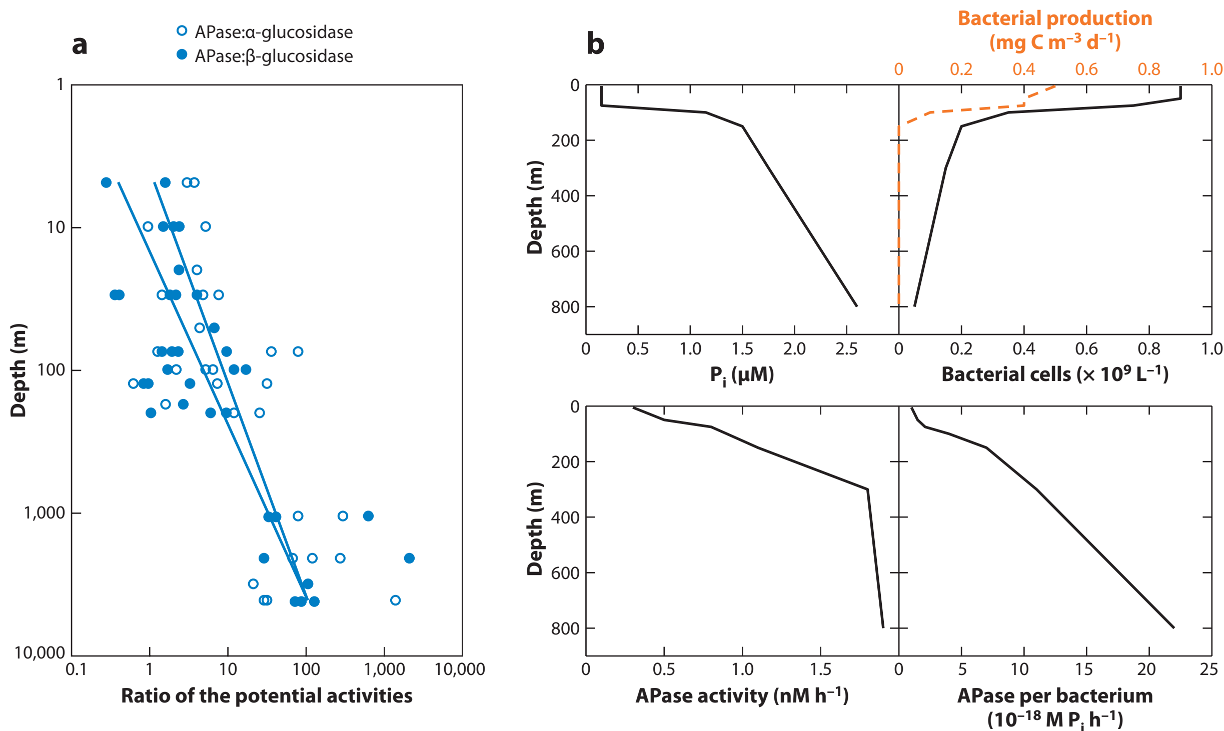

Figure 9

Key observations in the P-enriched deep sea that have informed our understanding of the role of alkaline phosphatase (APase) in the sea. (*a*) APase:α-glucosidase and APase:β-glucosidase activity ratios as functions of water depth in the central North Pacific, showing a dramatic >1,000-fold increase in the deep sea. Adapted from Koike & Nagata (1997). (*b*) Phosphate (P_i) concentration, bacterial cell abundance and production, volumetric APase activity, and bacterial-cell-specific APase as functions of water depth for samples collected in the Indian Ocean (0°, 65°E), showing enhanced APase and especially bacterial-cell-specific APase in the deep sea. Adapted from Hoppe & Ullrich (1999).

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applies only to deep water. Clearly, most surface-ocean bacteria are also C, N, and energy limited, so there is no justification for assuming that surface-ocean APase should track only P_i limitation.

Both APase encoded gene expression and enzyme activities can increase by up to 1,000-fold during P_i limitation compared with preinduced levels. Furthermore, O'Brien & Herschlag (2001) reported that APase supports a $>10^{17}$-fold hydrolysis rate enhancement for selected phosphomonoesters over the uncatalyzed rate. It is unclear why a cell would require such a large excess of catalytic activity (e.g., in the abyssal ocean) if the kinetic characteristics of APase (e.g., K_m and V_{max}) are similar to those of other hydrolytic enzymes. It is conceivable, even probable, that the high levels of APase induced in selected environmental samples may be a response to a secondary or tertiary role for the enzyme rather than to its assumed primary catalytic function. For example, after the phosphomonoester pool has been exhausted, the much less efficient diesterase activity (estimated to be 10^6 times less efficient than the monoesterase activity; O'Brien & Herschlag 2001) may be key for releasing P_i from the relatively large pool of dissolved nucleic acids present in the sea. Yang & Metcalf (2004) recently reported that bacterial APase (*PhoA* type) can oxidize PO_3^{3-} and produce H_2 at a reaction rate that is approximately 10^3 times slower than that of phosphomonoester hydrolysis. O'Brien & Herschlag (2001) hypothesized that the promiscuous substrate

specificity exhibited by APase may have facilitated evolution of new enzymes via the processes of gene duplication and natural selection. Although there are few deepwater DOP concentration data, the pool sizes, especially for APase substrates, are probably very small or nonexistent (total DOP < 25 nmol L^{-1}; Thomson-Bulldis & Karl 1998, Karl & Björkman 2002). Consequently, the metabolic strategy of DOP capture for C and energy may require a large excess of catalytic potential, as appears to be the case for APase expression in nature. A resolution of this deep-sea APase enigma would be a welcome advance in our understanding of the marine microbial P cycle.

5.5. P-Cycle Connections to Reduced-Gas Dynamics

Many reduced biogenic gases, including methane (CH_4), hydrogen (H_2), and nitrous oxide (N_2O), are present in oligotrophic ocean ecosystems at concentrations well in excess of their equilibria with the atmosphere. Consequently, there must be one or more local production sources and a continuous seawater-to-air gas flux. The microbial P cycle has recently been implicated, either directly or indirectly, in the production of all three biogenic gases (**Figure 10**).

During N_2 fixation, which is ultimately controlled by P availability, NH_3 and H_2 are produced in equimolar stoichiometry (Simpson & Burris 1984). Much but not all of the NH_3 is incorporated into organic matter, and much but not all of the H_2 (and potential energy) is captured by uptake hydrogenases in the cell (Wilson et al. 2010). The remainder of the NH_3 and H_2 is excreted from the cell (Mulholland et al. 2004, Wilson et al. 2010). Both NH_3 and H_2 represent potential energy sources for the growth of selected microorganisms, so the P-dependent metabolic processes that produce them are also potential conduits for solar energy flux. For example, NH_3 oxidation supports the growth of chemolithoautotrophic bacteria and archaea, and H_2 oxidation by the Knallgas reaction ($H_2 + O_2 \rightarrow H_2O$ + energy) is coupled to ATP formation in many microorganisms. Microbial oxidation of NH_3 (nitrification) also leads to N_2O production, the primary source of N_2O in the surface ocean (Dore et al. 1998) (**Figure 10**). Another potential source of H_2 in surface waters may be the oxidation of PO_3^{3-} via bacterial APase activity (Yang & Metcalf 2004) (**Figure 10**). Therefore, it is conceivable that a $P_i \leftrightarrow PO_3^{3-}$ redox cycle may also serve as a mechanism for solar energy capture, storage, and transduction (see Section 4.3). Currently, there are no estimates of PO_3^{3-} concentrations or fluxes in the marine environment that are required to test this hypothesis.

The presence of excess CH_4 in oxygenated ocean habitats has been termed "the marine methane enigma" because CH_4 was thought to be produced only in strictly anoxic environments (Kiene 1991). Karl et al. (2008a) described a novel pathway for the aerobic production of CH_4 in the sea via bacterial metabolism of MPn. Beversdorf et al. (2010) subsequently demonstrated the aerobic production of CH_4 by *Trichodesmium* growing on MPn as a sole source of P. More recently, Metcalf et al. (2012) reported a novel MPn biosynthetic pathway and the presence of an MPn synthase–encoding gene in the GOS metagenomic sequence database. Based on its distribution and frequency of occurrence, the authors concluded that the Karl et al. (2008a) aerobic CH_4 hypothesis is likely to be an integral component of the marine microbial P cycle (**Figure 10**). Once formed, CH_4 can be used as an energy source for selected microorganisms, and this therefore represents a potential mechanism for solar energy capture, storage, and transduction.

6. STATION ALOHA: AN ACCESSIBLE OPEN-OCEAN SENTINEL FOR RESEARCH ON THE MARINE MICROBIAL PHOSPHORUS CYCLE

6.1. Background and Motivation

The subtropical gyres of the world ocean are extensive, coherent regions that occupy approximately 40% of the surface of the Earth. With a surface area of nearly 2×10^7 km^2, the NPSG

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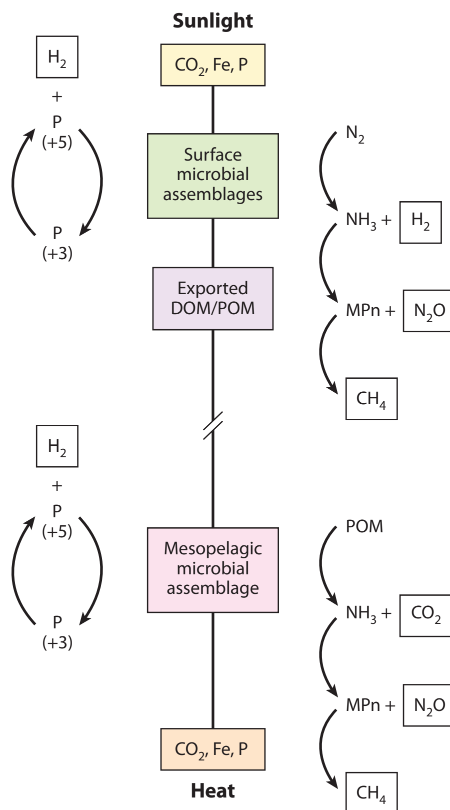

Figure 10

Schematic representation of hypothesized pathways of matter and energy flow leading to the production of selected greenhouse gases in low-nutrient ocean ecosystems. Energy derived from sunlight supports microbial P reduction, including both the phosphite (P+3)–phosphate (P+5) cycle (*left*) and N_2 fixation (*top right*). N_2 fixation leads to a cascade of metabolic reactions, including the simultaneous production of NH_3 and H_2, the subsequent oxidation of NH_3 by chemolithoautotrophic bacteria and archaea, and production of N_2O and synthesis of methylphosphonate (MPn), the latter supporting aerobic methane (CH_4) production upon subsequent decomposition. In the mesopelagic zone, remineralization of exported dissolved and particulate organic matter (DOM and POM, respectively) leads to NH_3 production, initiating a second, energy-limited biogenic gas cascade. Ultimately, the energy required to sustain the mesopelagic microbial processes is derived mostly from solar energy, which is eventually dissipated as heat.

is one of the Earth's largest contiguous biomes. Pioneering P-cycle research in the NPSG conducted by Perry (1972, 1976) suggested that P_i might control microbial growth and productivity in the surface waters. This conclusion was based on two independent lines of evidence: (*a*) higher-than-anticipated C:P ratios in POM, and (*b*) high biomass-normalized rates of APase activity. Subsequent P-cycle research in the NPSG conducted during the Vertical Transport and Exchange (VERTEX) program in August 1983 investigated DOP cycling (Orrett & Karl 1987) as well as POP export and remineralization (Martin et al. 1987). However, an experimental assessment of the P_i-control hypothesis was not conducted until the establishment in October 1988 of station ALOHA (A Long-Term Oligotrophic Habitat Assessment; 22°45′N, 158°W), the open-ocean benchmark site of the Hawaii Ocean Time-Series (HOT) research program (Karl & Lukas 1996).

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Based on our understanding of the microbial P cycle at the start of the HOT program, we made several ecological predictions about P pools and their dynamics. First, we recognized that the concentration of P_i at any sampling opportunity would be the net result of many complex interactions, including multiple P_i sources and sinks. However, we predicted that coupled microbial P_i uptake and particle export from the euphotic zone would serve to scavenge P_i to relatively low concentrations (<50 nmol L^{-1}) and regulate the pool to a narrowly defined range. Because earlier investigations of the NPSG often reported undetectable P_i concentrations (less than ~30 nmol L^{-1}) using existing methods, we devised a novel high-sensitivity method for use in the HOT program (Karl & Tien 1992). Furthermore, we hypothesized that if turbulent diffusion and upwelling were the primary sources for both P_i and NO_3^-, then these two major nutrients should covary in surface waters such that the NO_3^-:P_i ratio would be close to the delivery ratio, i.e., the Redfield ratio. Any surface-ocean changes in P_i concentration should display a stoichiometric covariance in NO_3^-. Finally, we hypothesized that the C:P and N:P stoichiometry of both suspended and sinking particulate matter would also be similar to that of the source waters, and that P export would be constrained by the P_i delivery flux over an appropriately long observation period (weeks to a month), thereby approaching a steady-state system. None of these first-order predictions have been supported by the ALOHA observations. Our preconceived notion of a relatively homogeneous, climax-like successional community has given way to a new NPSG P-cycle paradigm.

6.2. The Station ALOHA P-Cycle Portfolio

On approximately monthly intervals since October 1988, systematic measurements of a suite of core parameters, including selected P pool inventories and fluxes, have been made at station ALOHA. These observations, supplemented by hypothesis-testing experimentation, constitute the most comprehensive microbial P-cycle database for any oceanic ecosystem (**Tables 1–3**). Biogeochemical assessments at or near station ALOHA range in scope from a complete 24-year (and counting) record of monthly P_i, ATP, and POP concentrations, as well as POP export from the euphotic zone, to a spectrum of more specialized measurements that were conducted for brief portions of the observation period but rarely at the same time (**Table 1**). Examples include the partial chemical characterization of the DOP pool (September 1991–March 1992), P-flux estimates based on cosmogenic ^{32}P:^{33}P activity ratio (February 1999–April 2000), estimates of $^{32}P_i$ uptake and the BAP pool (October 2000–November 2001), and APase activity and kinetic characterization (February 2008–November 2009). Other key measurements were conducted only once, in some cases at a single depth (e.g., a global analysis of microbial community gene expression at 75 m on March 9, 2006, at 0330 local time; Frias-Lopez et al. 2008). Unfortunately, because the P cycle is not in a long-term steady state at station ALOHA (Karl 1999, 2007a; Karl et al. 2001a), we are unable to integrate these independent P-cycle observations, so they should be viewed as single frames of the ongoing ALOHA motion picture.

In addition to field measurements and experiments, P-cycle data have been used in a number of conceptual and numerical modeling studies (**Table 2**). These range in scope from C-N-P remineralization ratios (Li et al. 2000) and the impacts of N_2 fixation (Christian 2005) to *Trichodesmium* vertical migration and P_i mining (White et al. 2006) and potential consequences of artificial upwelling (Karl & Letelier 2008). Furthermore, the ALOHA metagenomes and metatranscriptomes have served as a vital resource for a broad range of scientific investigations (**Table 2**).

6.3. Dissolved P Pools, Dynamics, and Stoichiometry

Long-term measurements of dissolved P pools at station ALOHA have revealed unexpected temporal variability, ranging from monthly to decadal scales. For example, the P_i concentrations

Table 1 A chronology of selected marine microbial P-cycle-related observations and experiments conducted at or near station ALOHA in the North Pacific Subtropical Gyre (1988–present)

Sampling period	Event(s)	Primary reference(s)
October 1988–present (>250 cruises)	Establishment of station ALOHA at 22°45′N, 158°W; monthly measurements of P_i (0–4,600 m), DOP (0–1,000 m), POP (0–1,000 m), P-ATP (0–1,000 m), and P export from the euphotic zone	Karl et al. 1993, 1996, 2001b; Hebel & Karl 2001; **http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html**
August 1989 (1 cruise)	Measurement of dissolved and particulate matter inventories and dynamics (including P_i, DOP, POP, and ATP) during a large bloom of *Trichodesmium*	Karl et al. 1992
October 1988–November 1994 (59 cruises)	Development and field testing of the MAGIC method for P_i analysis	Karl & Tien 1992, 1997
October 1988–November 1994 (59 cruises)	Estimation of C, N, and P export and solubilization time and length scales	Christian et al. 1997
October 1988–February 2001 (123 cruises)	Measurement of water column profiles of DOP (0–1,000 m)	Karl et al. 2001b; **http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html**
October 1989–July 1997 (17 cruises)	Comparison of P_i estimation using standard, MAGIC, and modified MAGIC methods; determination of abyssal ocean DOP	Thomson-Bulldis & Karl 1998
1989–1999 (104 cruises)	Assessment of multiyear variability in DOC, DON, and DOP inventories	Church et al. 2002
May 1990 and October 1991	Measurement of C:N:P elemental stoichiometry of isolated *Trichodesmium* colonies	Letelier & Karl 1996
September 1991–March 1992 (6 cruises)	Partial characterization of DOP using controlled UV light–induced photodecomposition	Karl & Yanagi 1997
January–May 1997 (HALE ALOHA)	Observation of rapid coupling between nutrient (NO_3^- and P_i) upwelling and microbial/biogeochemical processes	Letelier et al. 2000
June 1992–October 2004	Measurement of deep-sea (2,800- and 4,000-m) POP export and remineralization rates using moored sediment traps	Karl et al. 2012
September 1993 (1 cruise)	Measurement of C:N:P stoichiometry of positively and negatively buoyant *Trichodesmium* colonies; measurement of dark P_i uptake of sinking colonies to test the P-mining hypothesis	Letelier & Karl 1998
January 1994–December 2005 (125 cruises)	Assessment of zooplankton migration as a vehicle for P export	Hannides et al. 2009
July 1996–August 1997 (5 cruises)	Measurement of light and dark P_i uptake and regeneration rates, DOP production, BAP estimation, and selected DOP substrate bioavailability	Björkman et al. 2000
June 1998–February 1999 (6 cruises)	Measurement of dissolved and particulate ATP and GTP concentrations and dissolved ATP metabolism	Björkman & Karl 2005
February 1999–April 2000 (10 cruises)	Determination of cosmogenic ^{32}P:^{33}P activity ratio; estimation of P_i and DOP residence times	Benitez-Nelson & Karl 2002

(Continued)

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Table 1 (Continued)

Sampling period	Event(s)	Primary reference(s)
October 1999 (1 cruise)	Application of tangential flow ultrafiltration/^{31}P-NMR for characterization of DOP and POP	Sannigrahi et al. 2006
November 1999 (1 cruise)	Measurement of C-N-P remineralization of particulate and dissolved organic matter	Kaiser & Benner 2012
January 2000–December 2001 (21 cruises)	Comparison of P dynamics and controls on N_2 fixation for samples and experiments at station ALOHA to those in the Southeast Pacific BIOSOPE cruise and Southwest Pacific DIAPALIS cruise	Moutin et al. 2008
February–July 2000 (3 cruises)	Discovery of novel unicellular, diazotrophic cyanobacteria at station ALOHA	Zehr et al. 2001
March 2000 (1 cruise)	Determination of P_i-δ^{18}O (0–3,000 m)	Colman et al. 2005
March 2000–November 2001 (15 cruises)	Measurements of light and dark $^{32}P_i$ uptake (0–175 m)	Duhamel et al. 2012
July 2000 and August 2001 (2 cruises)	Assessment of P_i control of N_2 fixation	Zehr et al. 2007
October 2000–November 2001 (8 cruises)	Measurement of $^{32}P_i$ and total P uptake (0–175 m); estimation of BAP; determination of POP turnover time	Björkman & Karl 2003
June 2002–November 2003 (4 cruises)	Assessment of concentration-dependent P_i uptake using ^{32}P radiolabeling and size fractionation	Björkman et al. 2012
October 2002 (1 cruise)	Construction and analysis of microbial community DNA (fosmid) libraries for seven samples from the surface (10 m) to the abyss (4,000 m)	DeLong et al. 2006
December 2002 (1 cruise)	Measurement of the concentration, production, and turnover of dissolved DNA	Brum 2005
December 2002 (1 cruise)	Measurement of production and turnover of "virus-free" dissolved DNA concentrations	Brum 2005
July 2003 (1 cruise)	Measurement of total microbial RNA synthesis rates using $^{32}P_i$ estimation; *Prochlorococcus* RNA synthesis using the RIBOTRACE method; determination of nutrient controls of RNA synthesis	Van Mooy & Devol 2008
July 2003 (1 cruise)	Measurement of S substitution for P in microbial membrane lipids and consequences of NH_4^+ amendments	Van Mooy et al. 2009
June–July 2004 (VERTIGO cruise)	Comparison of C-N-P export using conventional surface tethered and neutrally buoyant sediment traps	Lamborg et al. 2008
July and November 2004 (2 cruises)	Assessment of the metabolic response of microbial assemblages to deepwater nutrient (P_i) enrichment	McAndrew et al. 2007
July 2004–May 2007 (10 cruises)	Assessment of the temporal variability in phytoplankton response to deepwater nutrient (P_i) additions	Mahaffey et al. 2012
November 2004–March 2005 (3 cruises)	Assessment of P_i and Fe control and size distribution of N_2 fixation	Grabowski et al. 2008
November 2004–September 2007 (31 cruises)	Measurement of N_2 fixation (0–125 m) using $^{15}N_2$	Church et al. 2009

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Table 1 ***(Continued)***

Sampling period	Event(s)	Primary reference(s)
June 2005–September 2006 (4 cruises) and August 2007 (BloomER)	Discovery of aerobic production of methane from microbial methylphosphonate decomposition and survey of C-P lyase genes	Karl et al. 2008a
July 2005 (1 cruise)	Measurement of nutrient inventories and dynamics (including P_i) and controls on diazotroph diversity, abundances, and N_2-fixation rates within a large anticyclonic eddy	Fong et al. 2008
March 2006 (1 cruise)	Analysis of DNA metagenomic and RNA transcriptomic sequences from a 75-m depth sample	Frias-Lopez et al. 2008
October 2006 (1 cruise)	Comparison of the relative frequency of occurrence for selected P-cycle genes in samples collected at the Bermuda and Hawaii time-series sites (0–100 m)	Coleman & Chisholm 2010
February 2008–November 2009 (6 cruises)	Depth profiles (0–120 m) of APase activity and kinetic characterization using MUF-P substrate; measurement of P_i uptake and turnover	Duhamel et al. 2011
May–June 2008	Implementation of Project OPPEX—a field test of the Karl-Letelier P-dependent, two-stage phytoplankton bloom hypothesis	White et al. 2010a
July 2008 (POOB cruise)	Assessment of nutrient (N and P) regulation of APase activity	Duhamel et al. 2010
July–August 2008 (OPEREX cruise)	Evaluation of P_i and DOP (MPn) control of inorganic C and N_2 fixation	Watkins-Brandt et al. 2011
July–August 2008 (OPEREX cruise)	Assessment of the impacts of mesoscale eddies on P_i inventories and related microbial and biogeochemical parameters	Guidi et al. 2012
October 2008 (1 cruise)	Measurement of size-fractionated and flow-cytometric-cell-sorted uptake of $^{32}P_i$ and ^{32}P-ATP (0–175 m)	Duhamel et al. 2012
September 2009 (1 cruise)	Construction of 16S ribosomal DNA and RNA tag sequence (V6 to V8 region) libraries at 25 and 1,000 m	Hunt et al. 2012
July 2010 (1 cruise)	Assessment of the role of bacterial quorum sensing for P acquisition in *Trichodesmium* consortia	Van Mooy et al. 2011
July–August 2010 (2 cruises)	Evaluation of taxon-specific $^{32}P_i$ and ^{32}P-ATP uptake and kinetic characterization using flow cytometry	Björkman et al. 2012
March 2011–January 2012	Discovery of a novel symbiotic, unicellular, diazotrophic cyanobacterium	Thompson et al. 2012

Abbreviations: APase, alkaline phosphatase; BAP, bioavailable P; DOC, dissolved organic C; DON, dissolved organic N; DOP, dissolved organic P; MPn, methylphosphonate; MUF-P, methylumbelliferyl phosphate; P_i, phosphate; POP, particulate organic P.

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in the near-surface mixed layer (25-m depth) exhibit stochastic variation of more than 30-fold (from <5 nmol L^{-1} to >150 nmol L^{-1}) over the 24-year observation period (**Figure 11**). This high-frequency (<monthly) variability is not observed deeper in the euphotic zone or in the upper mesopelagic zone; it is exclusively a surface phenomenon. Furthermore, this degree of variability is not observed for either the larger DOP pool (Karl et al. 2001b) or the smaller POP pool (Hebel & Karl 2001).

Table 2 Conceptual and numerical models and observation-based analyses of selected P-cycle processes at station ALOHA

Reference(s)	Hypothesis, event, or primary objective
Karl et al. 1992	Development of a *Trichodesmium* P-transport model for sustaining near-surface plankton blooms
Baines et al. 1994	Development of conceptual, comparative models for relationships between primary production and export between lakes and oceans
Karl et al. 1995, 1997, 2001a; Karl 1999, 2002; Cullen et al. 2002; Bidigare et al. 2009; Church et al. 2009	Development of a conceptual framework for climate forcing of plankton community structure and function, including P dynamics, N_2 fixation, N:P stoichiometry, and related processes
Karl & Tien 1997	Development of a conceptual model for the importation of P into the euphotic zone by passive and active upward particle flux pathways
Li et al. 2000	Development and application of a two-end-member mixing model to constrain the elemental stoichiometry and oxygen consumption of organic matter remineralization
Fennel et al. 2002	Development of a deterministic model for N_2 fixation using N versus P limitation, and simulation of station ALOHA N_2-fixing blooms
Sakamoto et al. 2004	Development of a conceptual framework for the influence of Rossby-wave-induced nutrient dynamics for the period January 1997–January 2000 using HALE ALOHA mooring observations and TOPEX/Poseidon satellite sea-surface height anomalies
Christian 2005	Analysis of fixed and variable three-element (C-N-P) ecosystem models coupled to mixed-layer and CO_2 flux models
Rothstein et al. 2006 (PARADIGM program)	Discussion of marine ecosystem models from conceptual to statistical to numerical simulations, including application of a data assimilation model for N_2 fixation
White et al. 2006	Development of a numerical model for *Trichodesmium* vertical migration
Karl 2007b	Development of a conceptual model for sequential utilization of various DOP compound classes by selective alternative P-capture mechanisms during P limitation
White et al. 2007, Dore et al. 2008	Development of a conceptual framework for data-based analysis of environmental controls of diazotroph blooms
Fennel 2008, Karl & Letelier 2008, Letelier et al. 2008	Development of a conceptual model for P_i control of N_2 fixation and C sequestration following enhanced upwelling in low-nutrient, low-chlorophyll ecosystems, and response to criticism by Fennel
Konstantinidis & DeLong 2008	Analysis of a fosmid library to explore patterns of genetic recombination and clonal divergence in deep-sea microbial assemblages
Monteiro & Follows 2009	Development of an N_2-fixation-dependent biogeochemical oscillator model
Martinez et al. 2010	Discovery of a novel C-P utilization pathway based on an analysis of fosmid metagenomic libraries
Monteiro et al. 2010	Projection of the global distribution of marine diazotrophs based, in part, on cell and *nifH*/gene abundance observations
Luo et al. 2011	Assessment of the depth distribution (0–4,000 m) of alkaline phosphatase and phosphonate utilization genes from an analysis of the metagenomic library
Karl et al. 2012	Development of a conceptual model for photoperiodism as a key environmental cue for C-N-P export processes
Luo et al. 2012	Assembly of a global database on marine N_2 fixation and estimation of annual rate
McSorley et al. 2012	Discovery of a novel oxidative enzymatic pathway for cleavage of C-P bonds using a metagenomic library
Martinez et al. 2012	Discovery of genes for phosphite utilization by *Prochlorococcus marinus* using fosmid and metagenomic libraries

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Table 2 *(Continued)*

Reference(s)	Hypothesis, event, or primary objective
Flombaum et al. 2013	Assessment of environmental controls (including P_i) on the global distribution of picophytoplankton
Luo et al. 2013	Assessment of environmental controls (including P_i) on global marine N_2 fixation

Abbreviations: DOP, dissolved organic P; P_i, phosphate.

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The P_i-concentration-versus-depth profiles in the upper water column (0–100 m) conform to one of two general patterns (**Figure 12**): (*a*) those that exhibit a uniform concentration, with gradients of $\leq$0.05 μmol P_i m^{-4} (termed type I profiles), or (*b*) those that exhibit distinct near-surface (0–30 m) P_i maxima (termed type II profiles). Type I profiles can be subdivided into low (type I-L) or high (type I-H) categories based on whether the mean P_i concentration is $\leq$60 or >60 nmol L^{-1} (Karl & Tien 1997). The type II profiles were unexpected and are still not completely understood. They may be unstable or transient features, but they are not rare. During the period 1988–2012, 36% of the profiles (86 out of 236 cruises) exhibited near-surface P_i enrichments. Type II profiles may result from differential rates of uptake and remineralization of P_i, from local atmospheric delivery of P_i to the surface ocean, or from the upward flux of P by either active (e.g., phytoplankton vertical migration) or passive (e.g., upward flux of buoyant P-containing organic particles) processes (Karl & Tien 1997). The delivery of P, especially via atmospheric deposition or the upward flux of low-density P-enriched organic matter, would have important implications for the estimation of the net export of P (and C) from the euphotic zone and for controls on net primary production and N_2 fixation. Regardless of the P source or mechanism of near-surface ocean enrichment, both the mean euphotic-zone P_i concentration and the P_i profile characteristics are highly variable on monthly timescales.

One approach for investigating the possible source of P_i for type II profiles is to make simultaneous measurements of arsenate (AsO_4^{3-}) in the surface waters. Because AsO_4^{3-} is derived from atmospheric deposition, the co-occurrence of a type II profile of AsO_4^{3-} might suggest an atmospheric source for P_i. However, type II AsO_4^{3-} concentration profiles have not been observed at station ALOHA (Karl & Tien 1997).

Another approach might be to make simultaneous measurements of the distribution and dynamics of cadmium (Cd). Marine plankton concentrate Cd in close proportion to total P even in the open sea, far removed from continental sources (Martin & Broenkow 1975). Cd-concentration-versus-depth profiles reveal nutrient-like behavior—namely, depletion at the surface and enrichment with global, linear covariance with P_i at depth—suggesting control by the biological pump (Boyle et al. 1976). This predictable stoichiometric relationship between Cd and P has proven to be useful in paleoceanographic P_i concentration reconstructions (Boyle 1988); however, a recent study has shown that Fe availability may alter the Cd:P ratio in natural assemblages of phytoplankton (Cullen et al. 2003). Nevertheless, measurements of Cd:P might be useful for studies of the contemporary marine microbial P cycle. If the "normal" delivery of P_i from deep water is inextricably linked to Cd, then anomalous or unusual P_i delivery pathways (such as that proposed here for P_i at station ALOHA) might be detected by changes either in the dissolved Cd:P of surface water or in the Cd:P of suspended or exported particulate matter. This approach might be useful for constraining or eliminating from further consideration one or more of the hypothetical pathways leading to type II P_i profiles at station ALOHA.

Another P-cycle enigma is the fact that the temporal dynamics of surface-ocean P_i concentration are fundamentally distinct from those of NO_3^- concentration, which we initially hypothesized

Table 3 Selected pools and fluxes of the station ALOHA P cycle

Parameter	Depth range or horizon (m)	Mean values, integrated inventories, or ranges[a]
Major dissolved P pools		
P_i	0–100	2–16 mmol P m^{-2}
	300–500	278 ± 2.4 mmol P m^{-2}
	500–1,000	1,456 ± 2.2 mmol P m^{-2}
DOP (total)	0–100	15–30 mmol P m^{-2}
	300–500	18.2 ± 0.5 mmol P m^{-2}
	500–1,000	23.2 ± 0.9 mmol P m^{-2}
DOP (UV labile)	0–100	14.9 mmol P m^{-2}
DOP (UV stable)	0–100	7.7 mmol P m^{-2}
DOP (DNA)	0–100	118 mg DNA m^{-2}
DOP (ATP)	0–100	4–15 μmol ATP m^{-2}
DOP (bioavailable)	0–100	7–15% of total DOP
Major particulate P pools		
PP (total)	0–100	1.0–1.6 mmol P m^{-2}
	300–500	0.47 ± 0.02 mmol P m^{-2}
	500–1,000	0.96 ± 0.05 mmol P m^{-2}
PP (biomass)[b]	0–100	50–75% of total PP
	300–500	20% of total PP
	500–1,000	<15% of total PP
PP (ATP)	0–100	2–4 mg ATP m^{-2}
	300–500	0.47 ± 0.02 mg ATP m^{-2}
	500–1,000	0.66 ± 0.03 mg ATP m^{-2}
PP (DNA)	0–100	350–400 mg DNA m^{-2}
Major P fluxes		
P_i uptake	0–100	0.08–0.4 mmol P m^{-2} d^{-1}
P_i turnover	0–100	Days to weeks
Total P uptake	0–100	~2 × P_i uptake
APase (V_{max})	0–100	0.1–0.3 mmol P m^{-2} d^{-1}
P_i import (eddy diffusivity)[c]	150	5.1 mmol P m^{-2} d^{-1}
PP export (passive)	150	10.6 ± 0.3 μmol P m^{-2} d^{-1}
	300	5.6 ± 0.5 μmol P m^{-2} d^{-1}
	500	4.0 ± 0.4 μmol P m^{-2} d^{-1}
	4,000	0.64 ± 0.03 μmol P m^{-2} d^{-1}
PP export (migrant)	150	79% of PP export
P export (total)[d]	150	6.9 mmol P m^{-2} $year^{-1}$
P residence time	0–150	~5 years
Related fluxes		
C fixation	0–100	40–50 mmol C m^{-2} d^{-1}
N_2 fixation	0–100	0.04–0.2 mmol N m^{-2} d^{-1}
PC export	150	2,372 ± 56 μmol C m^{-2} d^{-1}
	300	1,282 ± 83 μmol C m^{-2} d^{-1}
	500	916 ± 53 μmol C m^{-2} d^{-1}
	4,000	291 ± 9.5 μmol C m^{-2} d^{-1}

(*Continued*)

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Table 3 (*Continued*)

Parameter	Depth range or horizon (m)	Mean values, integrated inventories, or ranges[a]
PN export	150	301 ± 7.1 μmol N m^{-2} d^{-1}
	300	127 ± 9.0 μmol N m^{-2} d^{-1}
	500	78 ± 5.0 μmol N m^{-2} d^{-1}
	4,000	12.1 ± 0.7 μmol N m^{-2} d^{-1}

Abbreviations: DOP, dissolved organic P; P_i, phosphate; PC, particulate C; PN, particulate N; PP, particulate P.

[a]From various sources, including Karl et al. 1996, 2001a,b; Karl & Tien 1997; Karl & Yanagi 1997; Björkman et al. 2000; Hebel & Karl 2001; Björkman & Karl 2003, 2005; Brum 2005; Karl 2007a; Church et al. 2009; Duhamel et al. 2011, 2012. Values are shown as the mean ± SE (n = 52 to 225) or as a range when the concentrations are more variable over time (e.g., in the near-surface ocean). Most of the station ALOHA data are publicly available at **http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html**.

[b]Assumes ATP × 250 = biomass C, and a biomass C:P molar ratio of 100.

[c]Calculated using the mean measured $d[P_i]/dz$ gradient across the phosphocline (5.37 μmol P m^{-4}; Karl et al. 2001b) and an eddy diffusivity coefficient K_v of 3×10^{-5} m^2 s^{-1}; flux = $K_v(d[P_i]/dz)$.

[d]Sum of passive particle and active migrant fluxes.

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should be tightly coupled. Specifically, NO_3^- profiles never exhibit a type II structure, and, moreover, stochastic winter pulses of NO_3^- into the upper 0–100-m portion of the water column, presumably due to deep mixing events, do not show corresponding P_i enrichments (Karl et al. 2001b) (**Figure 13**). These differences may be due to differential net supply or removal processes, or both, but a detailed dynamical and ecological understanding is lacking.

Major differences have also been observed between the molar ratios of the inorganic (NO_3^-:P_i) and total (inorganic plus organic) N:P pools, with the greatest difference in the upper 200 m of the water column (Karl et al. 2001b). Whereas the inorganic N:P is much less than the Redfield ratio of 16:1, the organic N:P is typically greater than the Redfield ratio (**Figure 13**). Unfortunately, without additional information on dissolved organic matter bioavailability, it is impossible to predict whether N or P is the proximate limiting nutrient. At station ALOHA, the NO_3^-:P_i ratio of deep water (>400 m) is approximately 14:1, with relative N depletion (compared with the Redfield ratio) most likely to be a result of long-term NO_3^- loss via denitrification (Karl et al. 2001b) (**Figure 7**). Periodically, but without warning, the near-surface total dissolved N:P ratio reverts to a near-Redfield value, where it remains for at most a few months before once again accumulating N relative to P (**Figure 13**). These events are probably caused by large, rapid mixing events, though we have little direct evidence for either the mechanism or ecological consequences.

Karl & Letelier (2008) hypothesized that the delivery of ALOHA deep water containing both NO_3^- and P_i, but with an NO_3^-:P_i ratio that is suboptimal for nutrient-replete growth, would elicit a two-stage bloom. The first microbial responders will rapidly assimilate the available nutrients in Redfield stoichiometry (N:P = 16:1), leaving a small residual pool of P_i after all of the NO_3^- has been assimilated. Indeed, this process may contribute to the unexpected stochastic variability observed in the near-surface P_i pool. If bioavailable Fe is present in sufficient concentrations, as it appears to be at station ALOHA (Boyle et al. 2005), then this NO_3^--deficient, P_i- and Fe-replete habitat would select for the proliferation of N_2-fixing microorganisms. Through metabolism and growth, the diazotrophs would scavenge the residual P_i, add new fixed N to the system, and lead to a net sequestration of CO_2 as the excess P_i-supported biomass is eventually exported to the deep sea (Karl & Letelier 2008). This model explains some, but not all, of the P_i pool dynamics observed at station ALOHA during the past 24 years.

Finally, in addition to these high-frequency (approximately monthly) variations in P_i, we have observed much longer (years to a decade), unexpected changes in euphotic-zone P_i inventories

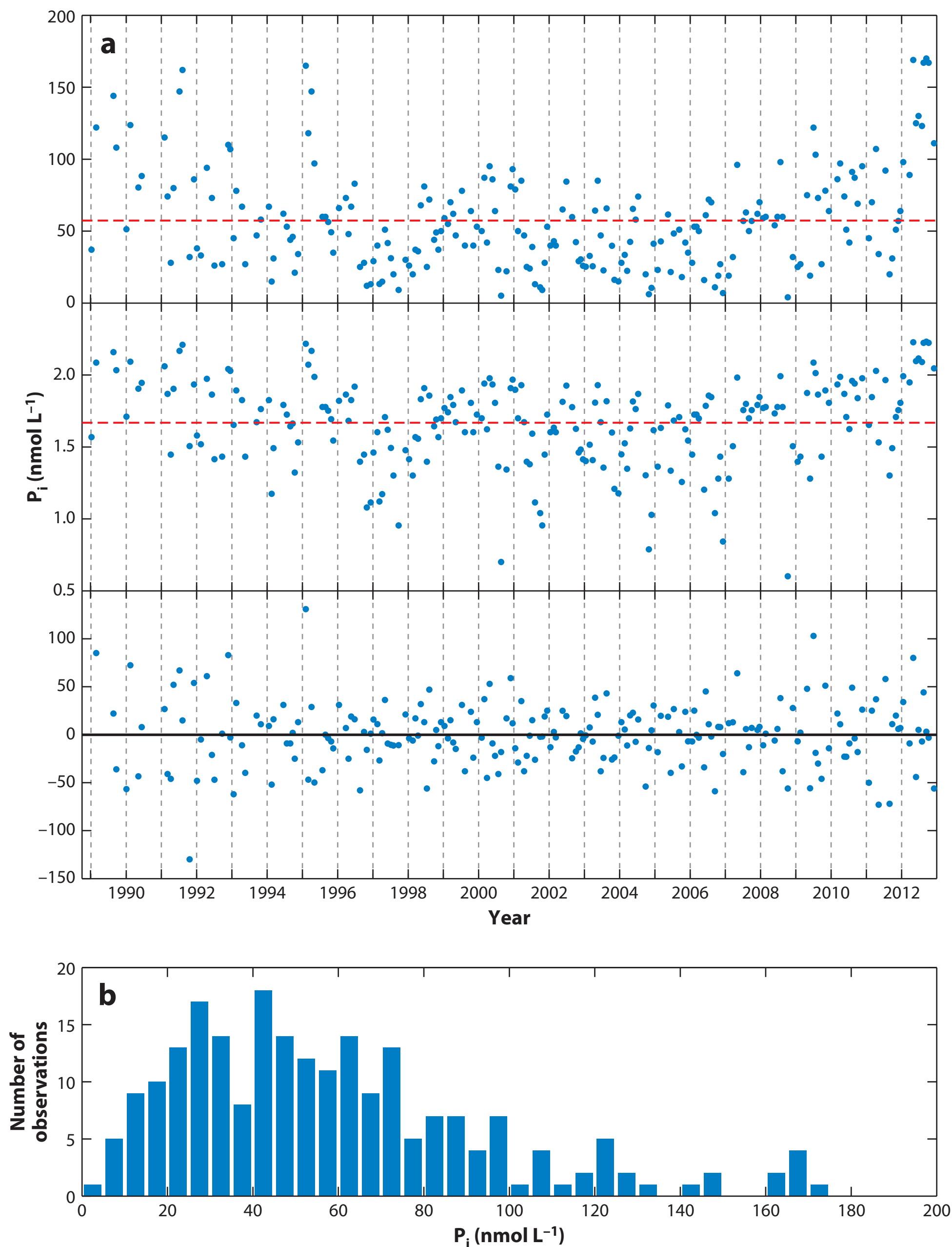

Figure 11

Time-series observations of phosphate (P_i) concentration at 25 m at station ALOHA for the period 1988–2012. (*a*) Linear (*top*) and $\log_{10}$ (*middle*) P_i concentrations, along with the cruise-to-cruise (effectively month-to-month) differences in concentration (*bottom*). The $\log_{10}$ graph emphasizes the large range observed during the past 24 years. The dashed red lines show the mean value of 57.3 nmol L^{-1} for the entire data set. (*b*) A frequency histogram of P_i concentrations, binned into 5 nmol L^{-1} increments.

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(**Figure 14**). At the start of the HOT program in 1988, the 0–100-m inventory of P_i was approximately 10 mmol m^{-2}. During the first 6 years of sampling, this inventory systematically decreased by 50%, to approximately 5 mmol m^{-2}, a net loss of ∼0.8 mmol y^{-1}. Karl & Tien (1997) hypothesized that N_2 fixation was responsible for this net loss of P_i. However, shortly after that paper was written, there was a large but short-lived (<6 months) stochastic increase in the P_i inventory, effectively restoring it to 1988 concentrations. Over the next 3 years (1995–1997), there was again removal of P_i, but it occurred at a much more rapid net rate (∼2.3 mmol y^{-1}) and led to an even lower inventory (3 mmol m^{-2}). Not long after these trends were described (Karl et al. 2001b), there was a less intense but extended period during which the 0–100-m P_i pool was partially restored, to ∼6–7 mmol m^{-2}. From 1998 to 2009, the P_i inventory remained relatively low (<5 mmol m^{-2}), after which there was another period of restoration that raised the inventory back to 1988 levels or higher (**Figure 14**).

As mentioned above, these P_i pool dynamics are independent of NO_3^- inventories, which have remained low and relatively constant over the 24-year observation period, with the exception of stochastic winter pulses that do not alter the P_i pool (Karl et al. 2001b). We believe that these unexpected subdecadal dynamics in P_i inventories are the result of complex climate-ocean interactions that affect both physical and biogeochemical dynamics (Karl 1999, Karl et al. 2001a). These temporal changes in P_i availability have numerous and far-reaching ecological consequences, including for the rate of solar energy capture, net ecosystem metabolism, and CO_2 sequestration. We are just beginning to understand the mechanisms that link these processes to the microbial P cycle.

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6.4. Suspended and Sinking Particulate P Pools, Dynamics, and Stoichiometry

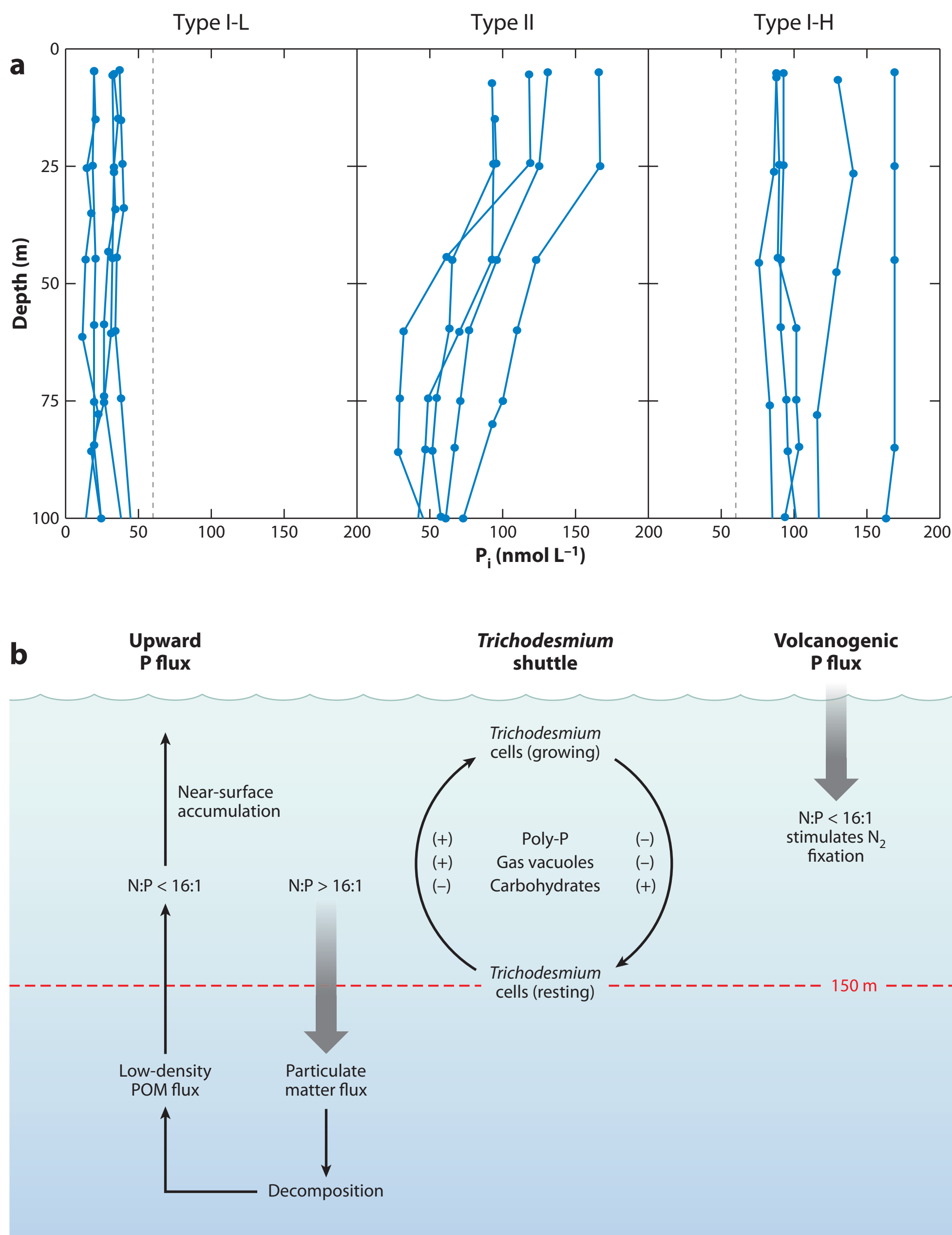
At station ALOHA, we observe seasonal and interannual variations in POP, especially in the upper 0–75 m of the water column (Hebel & Karl 2001). The C:N:P elemental stoichiometry of the suspended particulate matter pool varies both seasonally and with depth for a given season (Hebel & Karl 2001); both the C:P and N:P ratios increase substantially and predictably throughout the summer. Superimposed on these seasonal dynamics has been an approximately 50% decrease in the multidecade POP inventories of the upper water column (**Figure 15**). Moreover, the decreasing POP pool is characterized by C:P and N:P ratios of the residual suspended particulate matter becoming increasingly P poor. At the start of the observation period, the molar C:N:P ratio (131C:18N:1P) was close to the canonical Redfield ratio of 106C:16N:1P, but by 2011 the P was significantly depleted relative to C and N (186C:28N:1P) (**Figure 15**).

Finally, the export rate of P from the euphotic zone, as measured using free-drifting sediment traps, has also systematically decreased over the observation period, whereas the export rates of C and N have not (**Figure 16**). Consequently, the C:N:P stoichiometry of the sinking particles is also depleted in P relative to both C and N compared with the Redfield ratio, with N:P ratios approaching 50:1 (**Figure 16**). These temporal dynamics in POP inventories and export are even more enigmatic considering that the rate of primary production (as measured using the in situ ^{14}C technique) has increased by nearly 50% since 1989, rising from rates of approximately 400 mg C m^{-2} d^{-1} to rates that exceed 600 mg C m^{-2} d^{-1} (Saba et al. 2010; HOT data available at **http://hahana.soest.hawaii.edu**). These observations are all consistent with the hypothesized selection for N_2-fixing microorganisms and with a switch from an N-controlled biome to one controlled by P, or perhaps by P and Fe (Karl 1999, 2002; Karl et al. 2001b; Cullen et al. 2002).

6.5. Building the Long-Term Picture

The fundamental role of nutrient dynamics in biogeochemical processes and ecosystem modeling demands that we have a comprehensive, mechanistic understanding of inventories and fluxes

(**Table 3**). Karl et al. (2001a) hypothesized that the environmental conditions necessary to promote selection for N_2-fixing microorganisms (e.g., water column stratification, nutrient resupply rates, and N:P delivery ratio) have changed since the late 1970s, resulting in a state favorable to N_2 fixation that has persisted to the present. If the biomass of N_2-fixing microbes and the rates of N_2 fixation in the NPSG are increasing over time owing to climate-driven changes in the environment, then the biome is being forced into severe P limitation. Ecological consequences might include changes in the standing stocks and turnover rates of dissolved and particulate P and altered C-N-P



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composition of new biomass production, which could in turn select for microorganisms that do not require as much P for growth, or for slower-growing microbes with lower P requirements resulting from reduced rRNA content or streamlined genomes. The potential ecological consequences of the hypothesized N_2-fixation-forced P_i limitation are profound. It is conceivable that the NPSG has alternative or multiple stable states that are controlled largely by the net N:P nutrient delivery ratio (Karl 2002), and that once a new state is established, it is resilient to change until some new environmental threshold is achieved (Ives & Carpenter 2007).

Corno et al. (2007) have suggested that the P-cycle changes observed at station ALOHA may be linked to the magnitude, duration, and synchrony of two dominant NPSG climate indices—the El Niño–Southern Oscillation and Pacific Decadal Oscillation. Because these oscillations are independent and have different frequencies, phase shifts, and temporal alignment, it may be difficult to predict the ecological and biogeochemical consequences with any certainty. Indeed, Bidigare et al. (2009) used both ALOHA observations and model simulations to reveal a cascade of interacting physical, chemical, and biological shifts that could result from rapid climate changes. The conceptual model of temporal alternation between N and P control of production and export processes (Karl 1999) reconciles the paradigms that were developed in the 1960s to explain the fixed Redfield ratio and N-controlled ecosystem processes of that era with the new P-cycle paradigms presented here. The extent to which greenhouse-gas-induced warming and other changes to the surface ocean will affect the ability to switch between these two ecosystem states is unknown, but it seems almost certain that the dimensions of the subtropical gyres will expand and that the oceans as a whole will then become more stratified and more nutrient depleted.

The emergent P-cycle data set from station ALOHA is unique, robust, and rich with previously undocumented phenomena and ecological insights. Although the ongoing ocean time-series study at station ALOHA has certainly not resolved all of these important matters, it does provide an unprecedented data set to begin the next phase of hypothesis testing with numerous research opportunities. For nearly 25 years, station ALOHA has served as a benchmark site for investigating

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Figure 12

(*a*) Variability in near-surface (0–100 m) phosphate (P_i) concentration profiles at station ALOHA observed during the period 1988–2012. These individual profiles were selected as examples of what have been termed type I profiles (those exhibiting a uniform concentration, with gradients of ≤0.05 μmol P_i m^{-4}) and type II profiles (those exhibiting distinct near-surface P_i maxima). Type I is subdivided into low (type I-L) and high (type I-H) categories based on whether the mean P_i concentration is ≤60 or >60 nmol L^{-1}. (*b*) Conceptual views of three nontraditional delivery pathways that might contribute to near-surface P_i enrichments: the upward P-flux model, the *Trichodesmium* shuttle model, and the volcanogenic P-flux model. (*Left*) In the upward P-flux model, sinking particulate organic matter (POM) with N:P greater than the Redfield ratio (16:1) is selectively decomposed to yield a lipid-rich, P-rich, N-poor organic fraction (low-density POM) that eventually rises. Near the sea surface, this material is further degraded by microorganisms or photolytic processes to produce P_i. (*Center*) The *Trichodesmium* shuttle model is based on the ability of this organism to migrate and store P_i at depth as polyphosphate (poly-P). Either an external stimulus (e.g., decreased turbulence) or an internal stimulus (e.g., exhaustion of stored carbohydrate or accumulation of P) triggers the *Trichodesmium* population to form gas vacuoles that allow the cells to change their positions in the water column and (most important) to increase the mean irradiance flux. Near the surface, these cells are selected for because of their ability to fix N_2 and maintain balanced growth at the expense of the stored poly-P, resulting in a surface bloom. When intracellular P is depleted, the cells continue to reduce CO_2, which is stored in the cell as carbohydrates. This ballast causes them to sink and start the cycle all over again. (*Right*) In the volcanogenic P-flux model, P derived from volcanic eruptions (which are low in N) uncouples N and P and stimulates N_2 fixation. Hawaiian basalts are P rich and N poor, and for the past 25 years there has been a nearly continuous eruption of the Kilauea volcano on the island of Hawaii. The dashed red line at 150 m depicts the approximate boundary between the euphotic zone (above) and the mesopelagic zone (below).

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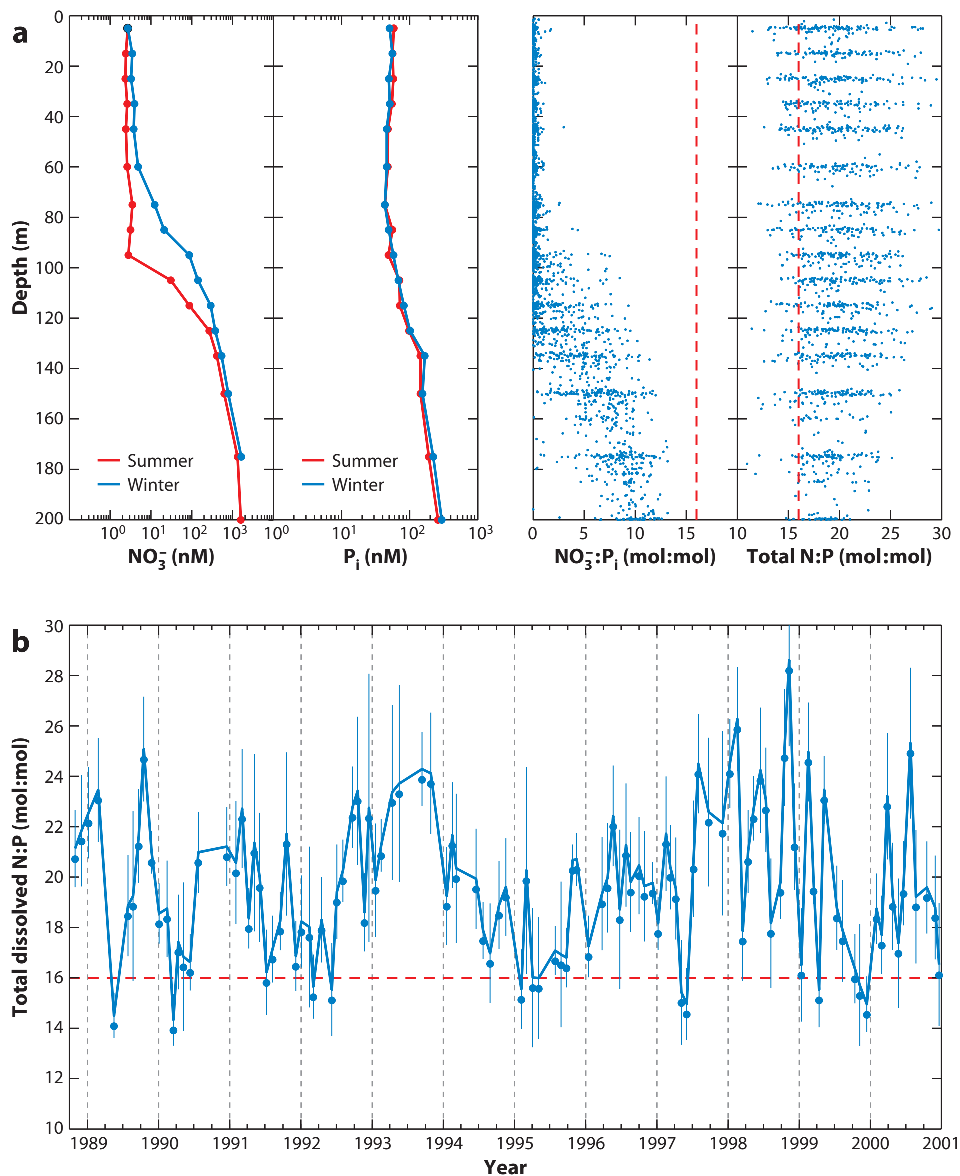

Figure 13

(*a*) Nitrate (NO_3^-) and phosphate (P_i) concentrations versus water depth for samples collected at station ALOHA during the period 1988–2000. The left subpanel shows summer (June–August; *red*) and winter (December–February; *blue*) mean values; the right subpanel shows molar ratios for inorganic N:P only ($NO_3^-:P_i$) and total N:P (inorganic plus organic) for the complete data set relative to the Redfield ratio of 16:1 (*red dashed lines*). (*b*) Time series of total dissolved N:P showing an aperiodic return to the Redfield ratio of 16:1 (*red dashed line*).

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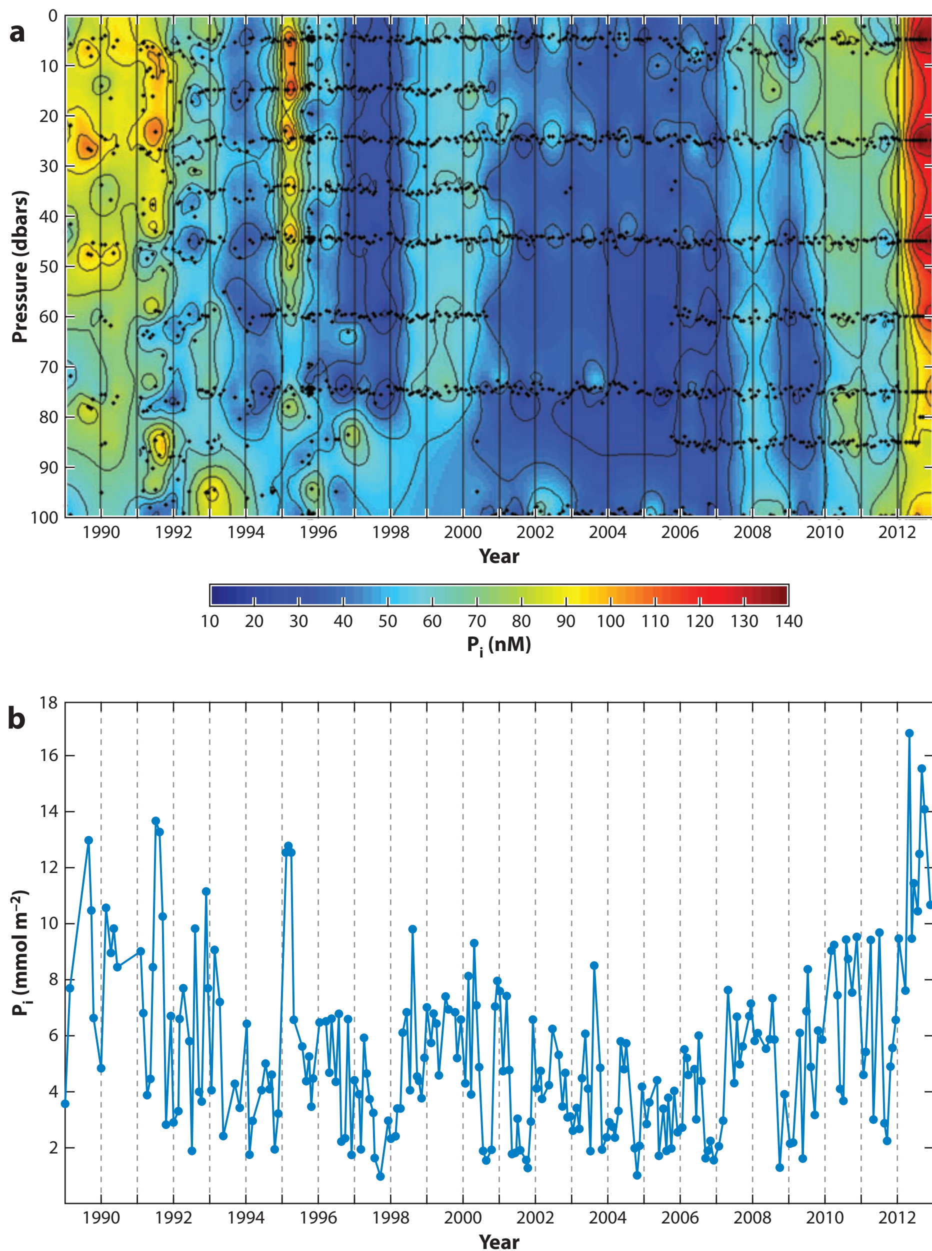

Figure 14

(*a*) Upper-ocean (0–100 m) phosphate (P_i) concentration contour plot showing large interannual variability in P_i inventory during the period 1988–2012. (*b*) P_i inventory (0–100 m integral) during the same period.

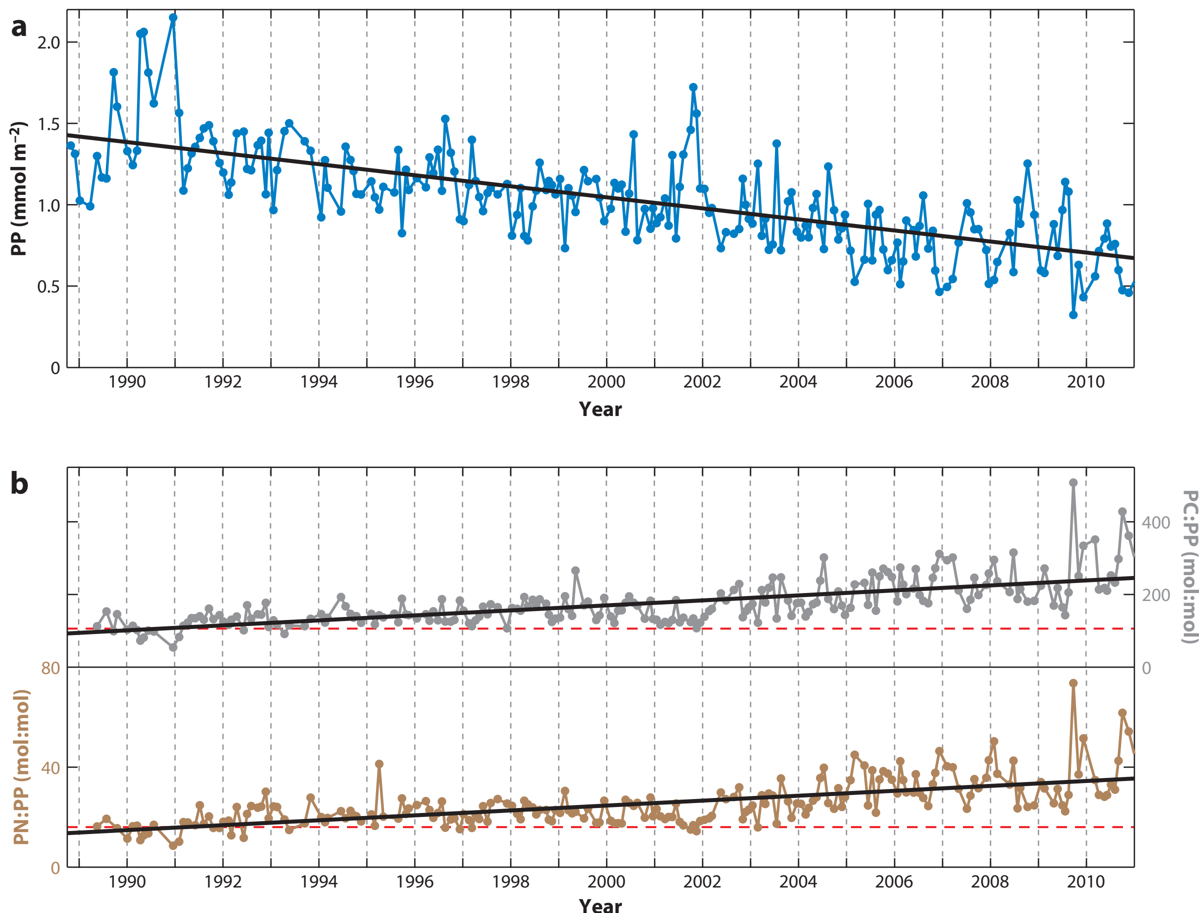

Figure 15

(*a*) Time-series inventory of particulate P (PP; 0–75-m depth-integrated values) at station ALOHA for the period 1988–2010, showing a systematic decline of approximately 50% during this period. (*b*) Time series of particulate matter stoichiometry over the same period, showing a systematic depletion in PP relative to both particulate C (PC) and particulate N (PN) compared with the Redfield reference ratios of 106C:1P and 16N:1P (*red dashed lines*).

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variability in the coupled physical, chemical, and microbiological processes, with an emphasis on coupled C-N-P cycles, including the impacts of environmental variability and climate change. What is now needed is a series of expeditions focused specifically on the marine microbial P cycle, where a complete set of measurements and experiments (see **Table 1**) can be performed simultaneously. The Center for Microbial Oceanography: Research and Education (C-MORE)–sponsored field program Hawaii Ocean Experiment–Phosphorus Rally (HOE-PhoR), planned to coincide with the silver anniversary of the HOT program in 2013, will build on current knowledge to conduct the most comprehensive study of the microbial P cycle ever attempted.

7. CONCLUSIONS AND A PROSPECTUS FOR THE FUTURE

Cellular P metabolism is complex, and P biogeochemistry and microbial oceanography are even more so. Tom Brock, a pioneer in aquatic microbial ecology, once proclaimed that ecology is

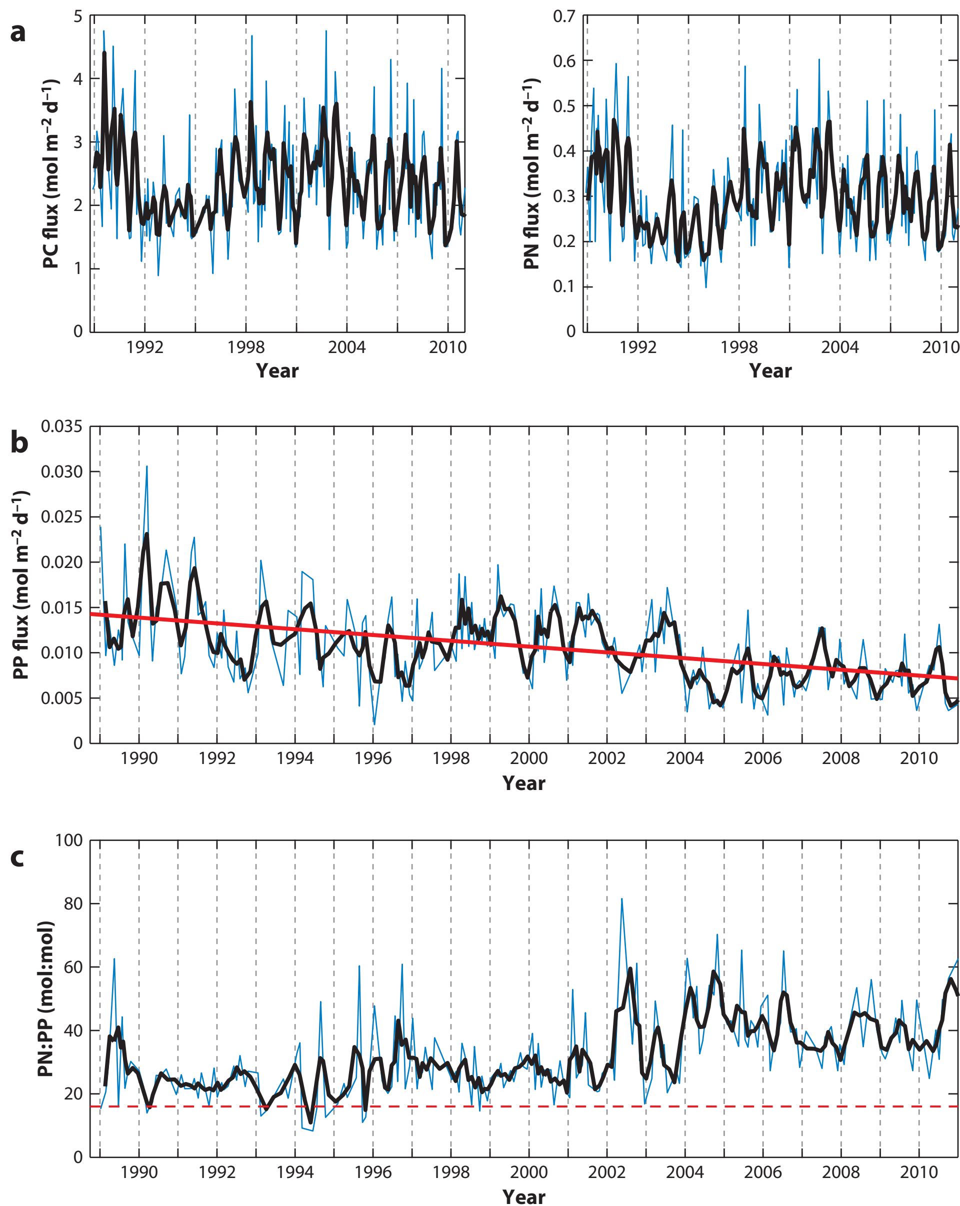

Figure 16

Particulate matter export from the euphotic zone (150 m) at station ALOHA measured using a drifting, surface-tethered sediment trap array. (*a*) Particulate C (PC) and particulate N (PN) fluxes. (*b*) Particulate phosphorus (PP) flux. (*c*) PN:PP molar ratio of the collected particles relative to the Redfield reference ratio of 16:1 (*red dashed line*).

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"physiology under the worst possible conditions" (Brock 1966), and this is still an accurate assessment. Given the vast expanse of the oceanic habitat, it is difficult to imagine how human activity could ever alter the natural P cycle, but it has. Indeed, human activities have altered all three P cycles: tectonic-lithogenic, oceanic, and marine microbial. Through accelerated mining of phosphate-bearing rock in the past few decades, we have more than doubled the flux of P to the sea (Seitzinger et al. 2010). This has resulted in eutrophication of the coastal ocean and possibly selection for N_2-fixing cyanobacteria, just as the overuse of P-based detergents altered many lake ecosystems in the 1960s. The full ecological consequences of the acceleration of the tectonic-lithogenic P cycle are unknown.

Human activities have also begun to alter the large-scale oceanic P cycle through planetary-scale greenhouse-gas-induced warming. The future ocean on a warmer planet will probably be more stratified, with reduced mixed-layer P_i concentrations and lower gross, net, and export productivity. Given their low P cell quotas, high surface-to-volume ratios, and streamlined genomes, marine microorganisms like SAR11 and *Prochlorococcus* appear to have a selective ecological advantage.

Planned future uses of the ocean for energy and food production could have additional unintended consequences. For example, the use of cold, deep seawater for air conditioning or for the generation of electricity via ocean thermal energy conversion will transfer large volumes of P-enriched seawater to surface coastal habitats, leading to enhanced productivity (possibly of harmful algae) and to the formation of oxygen deficits (dead zones) following the decomposition of the sedimented organic matter. Recovery of P prior to waste effluent discharge in these novel uses of the sea may alleviate the undesirable environmental impacts and at the same time capture P for reuse in agriculture and other industrial applications. At the current demand, the limited, nonrenewable supply of phosphate-bearing rock will be exhausted within this century, with dire consequences for human nutrition and food security (Elser & Bennett 2011). Clearly, the human population has exceeded its carrying capacity, and once the supply of phosphate-bearing rock is depleted, the ability to support our planet's burgeoning human population will be diminished. In the end, as Louis Pasteur proclaimed many years ago, "the microbes will have the final word." We urgently need to improve our understanding of the marine microbial P cycle. It should be a thrilling next decade of discovery.

SUMMARY POINTS

1. The marine microbial P cycle is an essential component of the much larger global P cycle that is critical for oceanic productivity, C sequestration, and planetary habitability.
2. Marine P pools include both inorganic and organic forms that are interconnected through the metabolic activities of microorganisms.
3. Recent investigations have discovered an active P redox cycle with variable P valence states (e.g., +5, +3, and +1), analogous to the marine N and S cycles. Whether microbial oxidation of reduced P yields metabolically useful energy, as hypothesized in this review, is unknown.
4. The marine microbial P cycle is inextricably linked to other bioelement cycles, specifically the C, N, and Fe cycles, which are coupled via the process of N_2 fixation.
5. Ongoing time-series investigations at the deepwater station ALOHA have revealed a complex and dynamic P cycle, with previously undescribed phenomena and unsolved mysteries.

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6. The marine microbial P cycle holds enormous potential as a focal point for future research.

NOTE ADDED IN PROOF

The C-MORE program's HOE-PhoR expeditions were successfully completed in 2013 (leg I, May 22–June 5; leg II, September 16–28). More information and cruise data are available at **http://hahana.soest.hawaii.edu/hoephor/hoephor.html**.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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I thank many P-cycle colleagues for their invaluable insights and contributions, some of which are presented in this review. Science is arguably a team sport, so a special thanks goes out to the HOT and C-MORE scientists and technical and support staff who made the field observations at station ALOHA possible. I would especially like to thank Karin Björkman and Ricardo Letelier for their enduring collaborations and for providing constructive comments on an earlier draft of this review. Finally, I am grateful to the *Annual Review of Marine Science* Editorial Committee for the invitation and the Annual Reviews production team for their excellent work. Funding for my P-cycle research has been provided by the National Science Foundation and the Gordon and Betty Moore Foundation.

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LITERATURE CITED

Abell J, Emerson S, Renaud P. 2000. Distributions of TOP, TON and TOC in the North Pacific subtropical gyre: implications for nutrient supply in the surface ocean and remineralization in the upper thermocline. *J. Mar. Res.* 58:203–22

Alberts B. 2011. Editor's note. *Science* 332:1149

Arrigo KR. 2005. Marine microorganisms and global nutrient cycles. *Nature* 437:349–55

Atkins WRG, Harvey HW. 1925. The variation with depth of certain salts utilized in plant growth in the sea. *Nature* 116:784–85

Baines SB, Pace ML, Karl DM. 1994. Why does the relationship between sinking flux and planktonic primary production differ between lakes and oceans? *Limnol. Oceanogr.* 39:213–26

Baltar F, Aristegui J, Sintes E, van Aken HM, Gasol JM, Herndl GJ. 2009. Prokaryotic extracellular enzymatic activity in relation to biomass production and respiration in the meso- and bathypelagic waters of the (sub)tropical Atlantic. *Environ. Microbiol.* 11:1998–2014

Benitez-Nelson CR. 2000. The biogeochemical cycling of phosphorus in marine systems. *Earth-Sci. Rev.* 51:109–35

Benitez-Nelson CR, Karl DM. 2002. Phosphorus cycling in the North Pacific Subtropical Gyre using cosmogenic ^{32}P and ^{33}P. *Limnol. Oceanogr.* 47:762–70

Bertilsson S, Berglund O, Karl DM, Chisholm SW. 2003. Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea. *Limnol. Oceanogr.* 48:1721–31

Beversdorf LJ, White AE, Björkman KM, Letelier RM, Karl DM. 2010. Phosphonate metabolism of *Trichodesmium* IMS101 and the production of greenhouse gases. *Limnol. Oceanogr.* 55:1768–78

Bidigare RR, Chai F, Landry MR, Lukas R, Hannides CCS, et al. 2009. Subtropical ocean ecosystem structure changes forced by North Pacific climate variations. *J. Plankton Res.* 31:1131–39

Björkman KM, Duhamel S, Karl DM. 2012. Microbial group specific uptake kinetics of inorganic phosphate and adenosine-5′-triphosphate (ATP) in the North Pacific Subtropical Gyre. *Front. Microbiol.* 3:189

Björkman KM, Karl DM. 1994. Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters. *Mar. Ecol. Prog. Ser.* 111:265–73

Björkman KM, Karl DM. 2003. Bioavailability of dissolved organic phosphorus in the euphotic zone at Station ALOHA, North Pacific Subtropical Gyre. *Limnol. Oceanogr.* 48:1049–57

Björkman KM, Karl DM. 2005. Presence of dissolved nucleotides in the North Pacific Subtropical Gyre and their role in cycling of dissolved organic phosphorus. *Aquat. Microb. Ecol.* 39:193–203

Björkman K, Thomson-Bulldis AL, Karl DM. 2000. Phosphorus dynamics in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.* 22:185–98

Black S, Morel B, Zapf P. 1991. Verification of the chemical convention. *Nature* 351:515–16

Blake RE, O'Neil JR, Garcia GA. 1997. Oxygen isotope systematic of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds. *Geochim. Cosmochim. Acta* 61:4411–22

Boyle EA. 1988. Cadmium: chemical tracer of deepwater paleoceanography. *Paleoceanography* 3:471–89

Boyle EA, Bergquist BA, Kayser RA, Mahowald N. 2005. Iron, manganese, and lead at Hawaii Ocean Time-series station ALOHA: temporal variability and an intermediate water hydrothermal plume. *Geochim. Cosmochim. Acta* 69:933–52

Boyle EA, Sclater FR, Edmond JM. 1976. On the marine geochemistry of cadmium. *Nature* 263:42–44

Brock TD. 1966. *Principles of Microbial Ecology*. Englewood Cliffs, NJ: Prentice Hall

Brum JR. 2005. Concentration, production and turnover of viruses and dissolved DNA pools at Stn ALOHA, North Pacific Subtropical Gyre. *Aquat. Microb. Ecol.* 41:103–13

Canfield DE, Kristensen E, Thamdrup B. 2005. The phosphorus cycle. *Adv. Mar. Biol.* 48:419–40

Caraco NF. 1993. Disturbance of the phosphorus cycle: a case of indirect effects of human activity. *Trends Ecol. Evol.* 8:51–54

Carpenter EJ, Romans K. 1991. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science* 254:1356–58

Casida LE Jr. 1960. Microbial oxidation and utilization of orthophosphite during growth. *J. Bacteriol.* 80:237–341

Cavender-Bares KK, Karl DM, Chisholm SW. 2001. Nutrient gradients in the western North Atlantic Ocean: relationship to microbial community structure, and comparison to patterns in the Pacific Ocean. *Deep-Sea Res. I* 48:2373–95

Chadwick J. 1932. Possible existence of a neutron. *Nature* 129:312

Chiewitz O, Hevesy G. 1935. Radioactive indicators in the study of phosphorus metabolism in rats. *Nature* 136:754–55

Christian JR. 2005. Biogeochemical cycling in the oligotrophic ocean: Redfield and non-Redfield models. *Limnol. Oceanogr.* 50:646–57

Christian JR, Lewis MR, Karl DM. 1997. Vertical fluxes of carbon, nitrogen and phosphorus in the North Pacific subtropical gyre near Hawaii. *J. Geophys. Res.* 102:15667–77

Church MJ. 2008. Resource control of bacterial dynamics in the sea. See Kirchman 2008, pp. 335–82

Church MJ, Ducklow HW, Karl DM. 2002. Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre. *Limnol. Oceanogr.* 47:1–10

Church MJ, Mahaffey C, Letelier RM, Lukas R, Zehr JP, Karl DM. 2009. Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific Subtropical Gyre. *Glob. Biogeochem. Cycles* 23:GB2020

Codispoti LA. 1989. Phosphorus versus nitrogen limitation of new and export production. In *Productivity of the Ocean: Present and Past*, ed. WH Berger, VS Smetacek, G Wefer, pp. 377–94. New York: Wiley & Sons

Coleman ML, Chisholm SW. 2010. Ecosystem-specific selection pressures revealed through comparative population genomics. *Proc. Natl. Acad. Sci. USA* 107:18634–39

Colman AS, Blake RE, Karl DM, Fogel ML, Turekian KK. 2005. Marine phosphate oxygen isotopes and organic matter remineralization in the oceans. *Proc. Natl. Acad. Sci. USA* 102:13023–28

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Cooper GW, Onwo WM, Cronin JR. 1992. Alkyl phosphonic acids and sulfonic acids in the Murchison meteorite. *Geochim. Cosmochim. Acta* 56:4109–15

Corno G, Karl DM, Church MJ, Letelier RM, Lukas R, et al. 2007. Impact of climate forcing on ecosystem processes in the North Pacific Subtropical Gyre. *J. Geophys. Res.* 112:C04021

Costas AMG, White AK, Metcalf WW. 2001. Purification and characterization of a novel phosphorus-oxidizing enzyme from *Pseudomonas stutzeri* WM88. *J. Biol. Chem.* 276:17429–36

Cotner JB Jr,Wetzel RG. 1992. Uptake of dissolved inorganic and organic phosphorus compounds by phytoplankton and bacterioplankton. *Limnol. Oceanogr.* 37:232–43

Crick FH. 1958. On protein synthesis. *Symp. Soc. Exp. Biol.* 12:139–63

Cuhel RL, Waterbury JB. 1984. Biochemical composition and short term nutrient incorporation patterns in a unicellular marine cyanobacterium *Synechococcus* (WH7803). *Limnol. Oceanogr.* 29:370–74

Cullen JJ, Franks PJS, Karl DM, Longhurst A. 2002. Physical influences on marine ecosystem dynamics. In *The Sea*, Vol. 12, *Biological-Physical Interactions in the Sea*, ed. AR Robinson, JJ McCarthy, BJ Rothschild, pp. 297–336. New York: Wiley & Sons

Cullen JT, Chase Z, Coale KH, Fitzwater SE, Sherrell RM. 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. *Limnol. Oceanogr.* 48:1079–87

Davey M, Tarran GA, Mills MM, Ridame C, Geider RJ, La Roche J. 2008. Nutrient limitation of picophytoplankton photosynthesis and growth in the tropical North Atlantic. *Limnol. Oceanogr.* 53:1722–33

De Duve C. 1991. *Blueprint for a Cell: The Nature and Origin of Life*. Burlington, NC: Neil Patterson

De Graaf RM, Visscher J, Schwartz AW. 1997. Reactive phosphonic acids as prebiotic carriers of phosphorus. *J. Mol. Evol.* 44:237–41

DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, et al. 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311:496–503

Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP. 2007. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* 445:163–67

Deutsch C, Weber T. 2012. Nutrient ratios as a tracer and driver of ocean biogeochemistry. *Annu. Rev. Mar. Sci.* 4:113–41

Dore JE, Letelier RM, Church MJ, Lukas R, Karl DM. 2008. Summer phytoplankton blooms in the oligotrophic North Pacific Subtropical Gyre: historical perspective and recent observations. *Prog. Oceanogr.* 76:2–38

Dore JE, Popp BN, Karl DM, Sansone FJ. 1998. A large source of atmospheric nitrous oxide from subtropical North Pacific surface waters. *Nature* 396:63–66

Duhamel S, Björkman KM, Karl DM. 2012. Light dependence of phosphorus uptake by microorganisms in the subtropical North and South Pacific Ocean. *Aquat. Microb. Ecol.* 67:225–38

Duhamel S, Björkman KM, Van Wambeke F, Moutin T, Karl DM. 2011. Characterization of alkaline phosphatase activity in the North and South Pacific Subtropical Gyres: implication for phosphorus cycling. *Limnol. Oceanogr.* 56:1244–54

Duhamel S, Dyhrman ST, Karl DM. 2010. Alkaline phosphatase activity and regulation in the North Pacific Subtropical Gyre. *Limnol. Oceanogr.* 55:1414–25

Dyhrman ST, Ammerman JW, Van Mooy BAS. 2007. Microbes and the marine phosphorus cycle. *Oceanography* 20(2):110–16

Dyhrman ST, Benitez-Nelson CR, Orchard ED, Haley ST, Pellechia PJ. 2009. A microbial source of phosphonates in oligotrophic marine systems. *Nat. Geosci.* 2:696–99

Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, et al. 2006. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. *Nature* 439:68–71

Dyhrman ST, Jenkins BD, Rynearson TA, Saito MA, Mercier ML, et al. 2012. The transcriptome and proteome of the diatom *Thalassiosira pseudonana* reveal a diverse phosphorus stress response. *PLoS ONE* 7:e33768

Elser JJ, Bennett E. 2011. A broken biogeochemical cycle. *Nature* 478:29–31

Feingersch R, Philosof A, Mejuch T, Glaser F, Alalouf O, et al. 2011. Potential for phosphite and phosphonate utilization by *Prochlorococcus*. *ISME J.* 6:827–34

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Fennel K. 2008. Widespread implementation of controlled upwelling in the North Pacific Subtropical Gyre would counteract diazotrophic N_2 fixation. *Mar. Ecol. Prog. Ser.* 371:301–3

Fennel K, Spitz YH, Letelier RM, Abbott MR, Karl DM. 2002. A deterministic model for N_2 fixation at stn. ALOHA in the subtropical North Pacific Ocean. *Deep-Sea Res. II* 49:149–74

Flombaum P, Gallegos JL, Gordillo RA, Rincón J, Zabala LL, et al. 2013. Present and future global distributions of the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc. Natl. Acad. Sci. USA* 110:9824–29

Föllmi KB. 1996. The phosphorus cycle, phosphogenesis and marine phosphate-rich deposits. *Earth-Sci. Rev.* 40:55–124

Fong AA, Karl DM, Lukas R, Letelier RM, Zehr JP, Church MJ. 2008. Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean. *ISME J.* 2:663–76

Freedman LD, Doak GO. 1957. The preparation and properties of phosphonic acids. *Chem. Rev.* 57:479–523

Frias-Lopez J, Shi Y, Tyson GW, Coleman ML, Schuster SC, et al. 2008. Microbial community gene expression in ocean surface waters. *Proc. Natl. Acad. Sci. USA* 105:3805–10

Froelich PN, Bender ML, Luedtke NA, Heath GR, DeVries T. 1982. The marine phosphorus cycle. *Am. J. Sci.* 282:474–511

Geider RJ, La Roche J. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37:1–17

González-Gil S, Keafer B, Jovine RVM, Aguilera A, Lu S, Anderson DM. 1998. Detection and quantification of alkaline phosphatase in single cells of phosphorus-limited marine phytoplankton. *Mar. Ecol. Prog. Ser.* 164:21–35

Grabowski MNW, Church MJ, Karl DM. 2008. Nitrogen fixation rates and controls at Stn ALOHA. *Aquat. Microb. Ecol.* 52:175–83

Griffith JM, Smillie RH, Grant BR. 1990. Alterations in nucleotide and pyrophosphate levels in *Phytophthora palmivora* following exposure to the antifungal agent potassium phosphonate (phosphite). *J. Gen. Microbiol.* 136:1285–91

Griffith JM, Smillie RH, Niere JO, Grant BR. 1989. Effect of phosphate on the toxicity of phosphite in *Phytophthora palmivora*. *Arch. Microbiol.* 152:425–29

Grossman A. 2000. Acclimation of *Chlamydomonas reinhardtii* to its nutrient environment. *Protist* 151:201–24

Guidi L, Calil PHR, Duhamel S, Björkman KM, Doney SC, et al. 2012. Does eddy-eddy interaction control surface phytoplankton distribution and carbon export in the North Pacific Subtropical Gyre? *J. Geophys. Res.* 117:G02024

Gundersen K, Heldal M, Norland S, Purdie DA, Knap AH. 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-series Study (BATS) site. *Limnol. Oceanogr.* 47:1525–30

Halm H, Lam P, Ferdelman TG, Lavik G, Dittmar T, et al. 2012. Heterotrophic organisms dominate nitrogen fixation in the South Pacific Gyre. *ISME J.* 6:1238–49

Hannides CCS, Landry MR, Benitez-Nelson CR, Styles RM, Montoya JP, Karl DM. 2009. Export stoichiometry and migrant-mediated flux of phosphorus in the North Pacific Subtropical Gyre. *Deep-Sea Res. I* 56:73–88

Hanrahan G, Salmassi TM, Khachikian CS, Foster KL. 2005. Reduced inorganic phosphorus in the natural environment: significance, speciation and determination. *Talanta* 66:435–44

Hartmann M, Grob C, Scanlan DJ, Martin AP, Burkill PH, Zubkov MV. 2011. Comparison of phosphate uptake rates by the smallest plastidic and aplastidic protists in the North Atlantic subtropical gyre. *FEMS Microbiol. Ecol.* 78:327–35

Hebel DV, Karl DM. 2001. Seasonal, interannual and decadal variations in particulate matter concentrations and composition in the subtropical North Pacific Ocean. *Deep-Sea Res. II* 48:1669–96

Heinen W, Lauwers AM. 1974. Hypophosphite oxidase from *Bacillus caldolyticus*. *Arch. Microbiol.* 95:267–74

Hilderbrand RL. 1983. The effects of phosphonates on living systems. In *The Role of Phosphonates in Living Systems*, ed. RL Hildebrand, pp. 139–70. Boca Raton, FL: CRC

Hilderbrand RL, Henderson TG. 1983. Phosphonic acids in nature. In *The Role of Phosphonates in Living Systems*, ed. RL Hildebrand, pp. 5–30. Boca Raton, FL: CRC

Hoppe H-G. 2003. Phosphatase activity in the sea. *Hydrobiologia* 493:187–200

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Hoppe H-G, Ullrich S. 1999. Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. *Aquat. Microb. Ecol.* 19:139–48

Horiguchi M, Kandatsu M. 1959. Isolation of 2-aminoethane phosphonic acid from rumen protozoa. *Nature* 184:901–2

Horiuchi T, Horiuchi S, Mizuno D. 1959. A possible negative feedback phenomenon controlling formation of alkaline phosphomonoesterase in *Escherichia coli*. *Nature* 183:1529–30

Huang J, Su Z, Xu Y. 2005. The evolution of microbial phosphonate degradative pathways. *J. Mol. Evol.* 61:682–90

Hunt DE, Lin Y, Church MJ, Karl DM, Tringe SG, et al. 2012. Relationship between abundance and specific activity of bacterioplankton in open ocean surface waters. *Appl. Environ. Microbiol.* 79:177–84

Ilikchyan IN, McKay RML, Zehr JP, Dyhrman ST, Bullerjahn GS. 2009. Detection and expression of the phosphonate transporter gene *phnD* in marine and freshwater picocyanobacteria. *Environ. Microbiol.* 11:1314–24

Ives AR, Carpenter SR. 2007. Stability and diversity of ecosystems. *Science* 317:58–62

Kaiser K, Benner R. 2012. Organic matter transformations in the upper mesopelagic zone of the North Pacific: chemical composition and linkages to microbial community structure. *J. Geophys. Res.* 117:C01023

Karl DM. 1999. A sea of change: biogeochemical variability in the North Pacific subtropical gyre. *Ecosystems* 2:181–214

Karl DM. 2000. Phosphorus, the staff of life. *Nature* 406:31–32

Karl DM. 2002. Nutrient dynamics in the deep blue sea. *Trends Microbiol.* 10:410–18

Karl DM. 2007a. The marine phosphorus cycle. In *Manual of Environmental Microbiology*, ed. CJ Hurst, RL Crawford, JL Garland, DA Lipson, AL Mills, LD Stetzenbach, pp. 523–39. Washington, DC: Am. Soc. Microbiol. 3rd ed.

Karl DM. 2007b. Microbial oceanography: paradigms, processes and promise. *Nat. Rev. Microbiol.* 5:759–69

Karl DM, Beversdorf L, Björkman KM, Church MJ, Martinez A, DeLong EF. 2008a. Aerobic production of methane in the sea. *Nat. Geosci.* 1:473–78

Karl DM, Bidigare RR, Church MJ, Dore JE, Letelier RM, et al. 2008b. The nitrogen cycle in the North Pacific trades biome: an evolving paradigm. In *Nitrogen in the Marine Environment*, ed. DG Capone, DA Bronk, MR Mulholland, EJ Carpenter, pp. 705–69. San Diego: Academic

Karl DM, Bidigare RR, Letelier RM. 2001a. Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: the domain shift hypothesis. *Deep-Sea Res. II* 48:1449–70

Karl DM, Björkman KM. 2001. Phosphorus cycle in seawater: dissolved and particulate pool inventories and selected phosphorus fluxes. In *Marine Microbiology*, ed. JH Paul, pp. 239–70. Methods Microbiol. Vol. 30. San Diego: Academic

Karl DM, Björkman KM. 2002. Dynamics of DOP. In *Biogeochemistry of Marine Dissolved Organic Matter*, ed. DA Hansell, CA Carlson, pp. 249–366. San Diego: Academic

Karl DM, Björkman DM, Dore JE, Fujieki L, Hebel DV, et al. 2001b. Ecological nitrogen-to-phosphorus stoichiometry at Station ALOHA. *Deep-Sea Res. II* 48:1529–66

Karl DM, Bossard P. 1985. Measurement of microbial nucleic acid synthesis and specific growth rate by $^{32}PO_4$ and [^{3}H]adenine: field comparison. *Appl. Environ. Microbiol.* 50:706–9

Karl DM, Christian JR, Dore JE, Hebel DV, Letelier RM, et al. 1996. Seasonal and interannual variability in primary production and particle flux at Station ALOHA. *Deep-Sea Res. II* 43:539–68

Karl DM, Church MJ, Dore JE, Letelier RM, Mahaffey C. 2012. Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation. *Proc. Natl. Acad. Sci. USA* 109:1842–49

Karl DM, Letelier RM. 2008. Nitrogen fixation-enhanced carbon sequestration in low nitrate, low chlorophyll seascapes. *Mar. Ecol. Prog. Ser.* 364:257–68

Karl DM, Letelier R, Hebel DV, Bird DF, Winn CD. 1992. *Trichodesmium* blooms and new nitrogen in the North Pacific gyre. In *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*, ed. EJ Carpenter, DG Capone, JG Rueter, pp. 219–37. Dordrecht: Kluwer

Karl DM, Letelier R, Hebel D, Tupas L, Dore J, et al. 1995. Ecosystem changes in the North Pacific subtropical gyre attributed to the 1991–92 El Niño. *Nature* 373:230–34

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Karl DM, Letelier R, Tupas L, Dore J, Christian J, Hebel D. 1997. The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean. *Nature* 388:533–38

Karl DM, Lukas R. 1996. The Hawaii Ocean Time-series (HOT) program: background, rationale and field implementation. *Deep-Sea Res. II* 43:129–56

Karl DM, Michaels A, Bergman B, Capone D, Carpenter E, et al. 2002. Dinitrogen fixation in the world's oceans. *Biogeochemistry* 57/58:47–98

Karl DM, Tien G. 1992. MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. *Limnol. Oceanogr.* 37:105–16

Karl DM, Tien G. 1997. Temporal variability in dissolved phosphorus concentrations in the subtropical North Pacific Ocean. *Mar. Chem.* 56:77–96

Karl DM, Tien G, Dore J, Winn CD. 1993. Total dissolved nitrogen and phosphorus concentrations at US-JGOFS Station ALOHA: Redfield reconciliation. *Mar. Chem.* 41:203–8

Karl DM, Yanagi K. 1997. Partial characterization of the dissolved organic phosphorus pool in the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.* 42:1398–405

Kennedy KE, Thompson GA Jr. 1970. Phosphonolipids: localization in surface membranes of *Tetrahymena*. *Science* 168:989–91

Kiene RP. 1991. Production and consumption of methane in aquatic systems. In *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*, ed. JE Rogers, WB Whitman, pp. 111–46. Washington, DC: Am. Soc. Microbiol.

Kim A, Kim J, Martin BM, Dunaway-Mariano D. 1998. Isolation and characterization of the carbon-phosphorus bond-forming enzyme phosphoenolpyruvate mutase from the mollusk *Mytilus edulis*. *J. Biol. Chem.* 273:4443–48

Kirchman DL, ed. 2008. *Microbial Ecology of the Oceans*. Hoboken, NJ: Wiley & Sons. 2nd ed.

Kittredge JS, Roberts E. 1969. A carbon-phosphorus bond in nature. *Science* 164:37–42

Kittredge JS, Roberts E, Simonsen DG. 1962. The occurrence of free 2-aminoethylphosphonic acid in the sea anemone, *Anthopleura elegantissima*. *Biochemistry* 1:624–28

Klausmeier CA, Litchman E, Daufresne T, Levin SA. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* 429:171–74

Knauer GA, Martin JH, Bruland KW. 1979. Fluxes of particulate carbon, nitrogen, and phosphorus in the upper water column of the northeast Pacific. *Deep-Sea Res. A* 26:97–108

Koike I, Nagata T. 1997. High potential activity of extracellular alkaline phosphatase in deep waters of the central Pacific. *Deep-Sea Res. II* 44:2283–94

Kolowith LC, Ingall ED, Benner R. 2001. Composition and cycling of marine organic phosphorus. *Limnol. Oceanogr.* 46:309–20

Kononova SV, Nesmeyanova MA. 2002. Phosphonates and their degradation by microorganisms. *Biochemistry* 67:184–95

Konstantinidis KT, DeLong EF. 2008. Genomic patterns of recombination, clonal divergence and environment in marine microbial populations. *ISME J.* 2:1052–65

Kornberg A, Rao NN, Ault-Riché D. 1999. Inorganic polyphosphate: a molecule of many functions. *Annu. Rev. Biochem.* 68:89–125

Lal D. 2001. Cosmogenic isotopes. In *Encyclopedia of Ocean Sciences*, ed. J Steele, S Thorpe, K Turekian, pp. 550–60. San Diego: Academic

Lal D, Chung Y, Platt T, Lee T. 1988. Twin cosmogenic radiotracer studies of phosphorus recycling and chemical fluxes in the upper ocean. *Limnol. Oceanogr.* 33:1559–67

Lal D, Narasappaya N, Zutshi PK. 1957. Phosphorus isotopes ^{32}P and ^{33}P in rain water. *Nucl. Phys.* 3:69–75

Lamborg CH, Buesseler KO, Valdes J, Bertrand CH, Bidigare R, et al. 2008. The flux of bio- and lithogenic material associated with sinking particles in the mesopelagic "twilight zone" of the northwest and North Central Pacific Ocean. *Deep-Sea Res. II* 55:1540–63

Lavoisier A. 1790. *Elements of Chemistry, in a New Systematic Order, Containing All the Modern Discoveries*. Trans. R Kerr. Edinburgh: William Creech. **http://ebooks.adelaide.edu.au/l/lavoisier/antoine_laurent/elements**

Lenton TM, Watson AJ. 2000. Redfield revisited. 1. Regulation of nitrate, phosphate, and oxygen in the ocean. *Glob. Biogeochem. Cycles* 14:225–48

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Letelier RM, Karl DM. 1996. Role of *Trichodesmium* spp. in the productivity of the subtropical North Pacific Ocean. *Mar. Ecol. Prog. Ser.* 133:263–73
Letelier RM, Karl DM. 1998. *Trichodesmium* spp. physiology and nutrient fluxes in the North Pacific subtropical gyre. *Aquat. Microb. Ecol.* 15:265–76
Letelier RM, Karl DM, Abbott MR, Flament P, Freilich M, et al. 2000. Role of late winter mesoscale events in the biogeochemical variability of the upper water column of the North Pacific Subtropical Gyre. *J. Geophys. Res.* 105:28723–29
Letelier RM, Strutton PG, Karl DM. 2008. Physical and ecological uncertainties in the widespread implementation of controlled upwelling in the North Pacific Subtropical Gyre. *Mar. Ecol. Prog. Ser.* 371:305–8
Li Y-H, Karl DM, Winn CD, Mackenzie FT, Gans K. 2000. Remineralization ratios in the subtropical North Pacific gyre. *Aquat. Geochem.* 6:65–86
Loladze I, Elser JJ. 2011. The origins of the Redfield nitrogen-to-phosphorus ratio are in a homoeostatic protein-to-rRNA ratio. *Ecol. Lett.* 14:244–50
Lomas MW, Bronk DA, van den Engh G. 2011. Use of flow cytometry to measure biogeochemical rates and processes in the ocean. *Annu. Rev. Mar. Sci.* 3:537–66
Luo H, Zhang H, Long RA, Benner R. 2011. Depth distributions of alkaline phosphatase and phosphonate utilization genes in the North Pacific Subtropical Gyre. *Aquat. Microb. Ecol.* 62:61–69
Luo Y-W, Doney SC, Anderson LA, Benavides M, Berman-Frank I, et al. 2012. Database of diazotrophs in global ocean: abundances, biomass and nitrogen fixation rates. *Earth Syst. Sci. Data* 4:47–73
Luo Y-W, Lima ID, Karl DM, Doney SC. 2013. Data-based assessment of environmental controls on global oceanic nitrogen fixation. *Biogeosciences* 10:7367–412
Mackey KRM, Labiosa RG, Calhoun M, Post AF, Paytan A. 2007. Phosphorus availability, phytoplankton community dynamics, and taxon-specific phosphorus status in the Gulf of Aqaba, Red Sea. *Limnol. Oceanogr.* 52:873–85
Mahaffey C, Björkman KM, Karl DM. 2012. Phytoplankton response to deep seawater nutrient addition in the North Pacific Subtropical Gyre. *Mar. Ecol. Prog. Ser.* 460:13–34
Mahaffey C, Williams RG, Wolff GA, Anderson WT. 2004. Physical supply of nitrogen to phytoplankton in the Atlantic Ocean. *Glob. Biogeochem. Cycles* 18:GB1034
Malacinski G, Konetzka WA. 1966. Bacterial oxidation of orthophosphite. *J. Bacteriol.* 91:578–82
Martin JH, Broenkow WW. 1975. Cadmium in plankton: elevated concentrations off Baja California. *Science* 190:884–85
Martin JH, Knauer GA, Karl DM, Broenkow WW. 1987. VERTEX: carbon cycling in the Northeast Pacific. *Deep-Sea Res. A* 34:267–85
Martin P, Van Mooy BAS. 2013. Fluorometric quantification of polyphosphate in environmental plankton samples: extraction protocols, matrix effects, and nucleic acid interference. *Appl. Environ. Microbiol.* 79:273–81
Martin P, Van Mooy BAS, Heithoff A, Dyhrman ST. 2011. Phosphorus supply drives rapid turnover of membrane phospholipids in the diatom *Thalassiosira pseudonana*. *ISME J.* 5:1057–60
Martinez A, Osburne MS, Sharma AK, DeLong EF, Chisholm SW. 2012. Phosphite utilization by the marine picocyanobacterium *Prochlorococcus* MIT9301. *Environ. Microbiol.* 14:1363–77
Martinez A, Tyson GW, DeLong EF. 2010. Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. *Environ. Microbiol.* 12:222–38
Martiny AC, Coleman ML, Chisholm SW. 2006. Phosphate acquisition genes in *Prochlorococcus* ecotypes: evidence for genome-wide adaptation. *Proc. Natl. Acad. Sci. USA* 103:12552–57
Martiny AC, Pham CTA, Primeau FW, Vrugt JA, Moore K, et al. 2013. Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nat. Geosci.* 6:279–83
Matthews DJ. 1916. On the amount of phosphoric acid in the sea-water off Plymouth Sound. *J. Mar. Biol. Assoc. UK* 11:122–30
Matthews DJ. 1917. On the amount of phosphoric acid in the sea-water off Plymouth Sound. II. *J. Mar. Biol. Assoc. UK* 11:251–57
McAndrew PM, Björkman KM, Church MJ, Morris PJ, Jachowski N, et al. 2007. Metabolic response of oligotrophic plankton communities to deep water nutrient enrichment. *Mar. Ecol. Prog. Ser.* 332:63–75

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
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McSorley FR, Wyatt P, Martinez A, DeLong EF, Hove-Jensen B, Zechel DL. 2012. PhnY and PhnZ comprise a new oxidative pathway for enzymatic cleavage of a carbon-phosphorus bond. *J. Am. Chem. Soc.* 134:8364–67

Metcalf WW, Griffin BM, Cocchillo RM, Gao J, Janga SC, et al. 2012. Synthesis of methylphosphonic acid by marine microbes: a source of methane in the aerobic ocean. *Science* 337:1104–7

Metcalf WW, Wanner BL. 1991. Involvement of the *Escherichia coli phn* (*psiD*) gene cluster in assimilation of phosphorus in the form of phosphonates, phosphite, P_i esters, and P_i. *J. Bacteriol.* 173:587–600

Metcalf WW, Wolfe RS. 1998. Molecular genetic analysis of phosphite and hypophosphite oxidation by *Pseudomonas stutzeri* WM88. *J. Bacteriol.* 180:5547–58

Meybeck M. 1982. Carbon, nitrogen and phosphorus transport by world rivers. *Am. J. Sci.* 282:401–50

Michaels AF, Karl DM, Capone DG. 2001. Element stoichiometry, new production and nitrogen fixation. *Oceanography* 14(4):68–77

Michelou VK, Lomas MW, Kirchman DL. 2011. Phosphate and adenosine-5′-triphosphate uptake by cyanobacteria and heterotrophic bacteria in the Sargasso Sea. *Limnol. Oceanogr.* 56:323–32

Monier A, Welsh RM, Gentemann C, Weinstock G, Sodergren E, et al. 2012. Phosphate transporters in marine phytoplankton and their viruses: cross-domain commonalities in viral-host gene exchanges. *Environ. Microbiol.* 14:162–76

Monteiro FM, Dutkiewicz S, Follows MJ. 2011. Biogeographical controls on the marine nitrogen fixers. *Glob. Biogeochem. Cycles* 25:GB2003

Monteiro FM, Follows MJ. 2009. On the interannual variability of nitrogen fixation in the subtropical gyres. *J. Mar. Res.* 67:71–88

Monteiro FM, Follows MJ, Dutkiewicz S. 2010. Distribution of diverse nitrogen fixers in the global ocean. *Glob. Biogeochem. Cycles* 24:GB3017

Moore CM, Mills MM, Langlois R, Milne A, Achterberg EP, et al. 2008. Relative influence of nitrogen and phosphorus availability on phytoplankton physiology and productivity in the oligotrophic sub-tropical North Atlantic Ocean. *Limnol. Oceanogr.* 53:291–305

Moore JK, Doney SC, Lindsay K, Mahowald N, Michaels AF. 2006. Nitrogen fixation amplifies the ocean biogeochemical response to decadal timescale variations in mineral dust deposition. *Tellus* 58B:560–72

Moore LR, Ostrowski M, Scanlan DJ, Feren K, Sweetsir T. 2005. Ecotypic variation in phosphorus-acquisition mechanisms within marine picocyanobacteria. *Aquat. Microb. Ecol.* 39:257–69

Moutin T, Karl DM, Duhamel S, Rimmelin P, Raimbault P, et al. 2008. Phosphate availability and the ultimate control of new nitrogen input by nitrogen fixation in the tropical Pacific Ocean. *Biogeosciences* 5:95–109

Mulholland MR, Bronk DA, Capone DG. 2004. Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS101. *Aquat. Microb. Ecol.* 37:85–94

Nowack B. 2003. Environmental chemistry of phosphonates. *Water Res.* 37:2533–46

O'Brien PJ, Herschlag D. 2001. Functional interrelationships in the alkaline phosphatase superfamily: phosphodiesterase activity of *Escherichia coli* alkaline phosphatase. *Biochemistry* 40:5691–99

Orchard ED, Benitez-Nelson CR, Pellechia PJ, Lomas MW, Dyhrman ST. 2010. Polyphosphate in *Trichodesmium* from the low-phosphorus Sargasso Sea. *Limnol. Oceanogr.* 55:2161–69

Orchard ED, Webb EA, Dyhrman ST. 2009. Molecular analysis of the phosphorus starvation response in *Trichodesmium* spp. *Environ. Microbiol.* 11:2400–11

Orrett K, Karl DM. 1987. Dissolved organic phosphorus production in surface seawaters. *Limnol. Oceanogr.* 32:383–95

Palenik B, Brahamsha B, Larimer FW, Land M, Hauser L, et al. 2003. The genome of a motile marine *Synechococcus*. *Nature* 424:1037–42

Parekh P, Follows MJ, Boyle EA. 2005. Decoupling of iron and phosphate in the global ocean. *Glob. Biogeochem. Cycles* 19:GB2020

Paytan A, Kolodny Y, Neori A, Luz B. 2002. Rapid biologically mediated oxygen isotope exchange between water and phosphate. *Glob. Biogeochem. Cycles* 16:1013

Paytan A, McLaughlin K. 2007. The oceanic phosphorus cycle. *Chem. Rev.* 107:563–76

Perry MJ. 1972. Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method. *Mar. Biol.* 15:113–19

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Perry MJ. 1976. Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.* 21:88–107

Polovina JJ, Howell EA, Abecassis M. 2008. Ocean's least productive waters are expanding. *Geophys. Res. Lett.* 35:L03618

Quin LD. 1965. The presence of compounds with a carbon-phosphorus bond in some marine invertebrates. *Biochemistry* 4:324–30

Quinn JP, Kulakova AN, Cooley NA, McGrath JW. 2007. New ways to break an old bond: the bacterial carbon-phosphorus hydrolases and their role in biogeochemical phosphorus cycling. *Environ. Microbiol.* 9:2392–400

Redfield AC. 1934. On the proportions of organic derivatives in seawater and their relation to the composition of plankton. In *James Johnstone Memorial Volume*, ed. RJ Daniel, pp. 177–92. Liverpool: Univ. Press Liverpool

Redfield AC. 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46:205–22

Redfield AC, Ketchum BH, Richards FA. 1963. The influence of organisms on the composition of sea-water. In *The Sea*, Vol. 2, *The Composition of Sea-Water: Comparative and Descriptive Oceanography*, ed. MN Hill, pp. 26–77. New York: Wiley

Rivkin RB, Anderson MR. 1997. Inorganic nutrient limitation of oceanic bacterioplankton. *Limnol. Oceanogr.* 42:730–40

Rothstein LM, Cullen JJ, Abbott M, Chassignet EP, Denman K, et al. 2006. Modeling ocean ecosystems: the PARADIGM program. *Oceanography* 19(1):22–51

Rudakow KJ. 1929. Die Reduktion der mineralischen Phosphate auf biologischem Wege. *Cent. Bakteriol. Parasitenkd. Infekt.* 79:229–45

Rusch DB, Halpern AL, Sutton G, Heidelberg KB, Williamson S, et al. 2007. The *Sorcerer II* Global Ocean Sampling expedition: Northwest Atlantic through Eastern Tropical Pacific. *PLoS Biol.* 5:e77

Ruttenberg KC. 2013. The global phosphorus cycle. In *Treatise on Geochemistry*, Vol. 8, *Biogeochemistry*, ed. DM Karl, WH Schlesinger. Oxford, UK: Elsevier. 2nd ed. In press

Saba VS, Friedrichs MAM, Carr M-E, Antoine D, Armstrong RA, et al. 2010. Challenges of modeling depth-integrated marine primary productivity over multiple decades: a case study at BATS and HOT. *Glob. Biogeochem. Cycles* 24:GB3020

Sakamoto CM, Karl DM, Jannasch HW, Bidigare RR, Letelier RM, et al. 2004. Influence of Rossby waves on nutrient dynamics and the plankton community structure in the North Pacific subtropical gyre. *J. Geophys. Res.* 109:C05032

Sannigrahi P, Ingall ED, Benner R. 2006. Nature and dynamics of phosphorus-containing components of marine dissolved and particulate organic matter. *Geochim. Cosmochim. Acta* 70:5868–82

Scanlan DJ, Mann NH, Carr NG. 1993. The response of the picoplanktonic marine cyanobacterium *Synechococcus* species WH7803 to phosphate starvation involves a protein homologous to the periplasmic phosphate-binding protein of *Escherichia coli*. *Mol. Microbiol.* 10:181–91

Scanlan DJ, Ostrowski M, Mazard S, Dufresne A, Garczarek L, et al. 2009. Ecological genomics of marine picocyanobacteria. *Microbiol. Mol. Biol. Rev.* 73:249–99

Schink B, Friedrich M. 2000. Phosphite oxidation by sulphate reduction. *Nature* 406:37

Seidel HM, Freeman S, Seto H, Knowles JR. 1988. Phosphonate biosynthesis: isolation of the enzyme responsible for the formation of a carbon-phosphorus bond. *Nature* 335:457–58

Seitzinger SP, Mayorga E, Bouwman AF, Kroeze C, Beusen AHW, et al. 2010. Global river nutrient export: a scenario analysis of past and future trends. *Glob. Biogeochem. Cycles* 24:GB0A08

Simpson FB, Burris RH. 1984. A nitrogen pressure of 50 atmospheres does not prevent evolution of hydrogen by nitrogenase. *Science* 224:1095–97

Slomp CP. 2011. Phosphorus cycling in the estuarine and coastal zones: sources, sinks, and transformations. In *Treatise on Estuarine and Coastal Science*, Vol. 5, *Biogeochemistry*, ed. E Wolanski, DS McLusky, pp. 201–29. Waltham, MA: Academic

Smith SV, Kimmerer WJ, Walsh TW. 1986. Vertical flux and biogeochemical turnover regulate nutrient limitation of net organic production in the North Pacific Gyre. *Limnol. Oceanogr.* 31:161–67

Sterner RW, Elser JJ. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton, NJ: Princeton Univ. Press

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Tamburini F, Bernasconi SM, Paytan A. 2012. Phosphorus in the environment. *Eos Trans. AGU* 93:405

Temperton B, Gilbert JA, Quinn JP, McGrath JW. 2011. Novel analysis of oceanic surface water metagenomes suggests importance of polyphosphate metabolism in oligotrophic environments. *PLoS ONE* 6:e16499

Ternan NG, McGrath JW, McMullan G, Quinn JP. 1998. Review: organophosphonates: occurrence, synthesis and biodegradation by microorganisms. *World J. Microbiol. Biotechnol.* 14:635–47

Thompson AW, Foster RA, Krupke A, Carter BJ, Muscat N, et al. 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. *Science* 337:1546–50

Thomson-Bulldis A, Karl DM. 1998. Application of a novel method for phosphorus determinations in the oligotrophic North Pacific Ocean. *Limnol. Oceanogr.* 43:1565–77

Torriani-Gorini A, Yagil E, Silver S, eds. 1994. *Phosphate in Microorganisms: Cellular and Molecular Biology*. Washington, DC: Am. Soc. Microbiol.

Tsubota G. 1959. Phosphate reduction in the paddy field I. *Soil Plant Food* 5:10–15

Twining BS, Nuñez-Milland D, Vogt S, Johnson RS, Sedwick PN. 2010. Variations in *Synechococcus* cell quotas of phosphorus, sulfur, manganese, iron, nickel and zinc within mesoscale eddies in the Sargasso Sea. *Limnol. Oceanogr.* 55:492–506

Tyrrell T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* 400:525–31

Van Mooy BAS, Devol AH. 2008. Assessing nutrient limitation of *Prochlorococcus* in the North Pacific subtropical gyre by using an RNA capture method. *Limnol. Oceanogr.* 53:78–88

Van Mooy BAS, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, et al. 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* 458:69–72

Van Mooy BAS, Hmelo LR, Sofen LE, Campagna SR, May AL, et al. 2011. Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia. *ISME J.* 6:422–29

Van Mooy BAS, Rocap G, Fredricks HF, Evans CT, Devol AH. 2006. Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments. *Proc. Natl. Acad. Sci. USA* 103:8607–12

Wanner BL. 1994. Molecular genetics of carbon-phosphorus bond cleavage in bacteria. *Biodegradation* 5:175–84

Wanner BL. 1996. Phosphorus assimilation and control of the phosphate regulon. In Escherichia coli *and* Salmonella: *Cellular and Molecular Biology*, Vol. 1, ed. FC Neidhardt, pp. 1357–81. Washington, DC: Am. Soc. Microbiol. 2nd ed.

Wanner BL, McSharry R. 1982. Phosphate-controlled gene expression in *Escherichia coli* K12 using Mu*dl*-directed *lacZ* fusions. *J. Mol. Biol.* 158:347–63

Watkins-Brandt KS, Letelier RM, Spitz YH, Church MJ, Böttjer D, White AE. 2011. Addition of inorganic or organic phosphorus enhances nitrogen and carbon fixation in the oligotrophic North Pacific. *Mar. Ecol. Prog. Ser.* 432:17–29

White A, Björkman K, Grabowski E, Letelier R, Poulos S, et al. 2010a. An open ocean trial of controlled upwelling using wave pump technology. *J. Atmos. Ocean. Technol.* 27:385–96

White AE, Karl DM, Björkman KM, Beversdorf LJ, Letelier RM. 2010b. Production of organic matter by *Trichodesmium* IMS101 as a function of phosphorus source. *Limnol. Oceanogr.* 55:1755–67

White AE, Spitz YH, Letelier RM. 2006. Modeling carbohydrate ballasting by *Trichodesmium* spp. *Mar. Ecol. Prog. Ser.* 323:35–45

White AE, Spitz YH, Letelier RM. 2007. What factors are driving summer phytoplankton blooms in the North Pacific Subtropical Gyre? *J. Geophys. Res.* 112:C12006

White AK, Metcalf WW. 2004. The *htx* and *ptx* operons of *Pseudomonas stutzeri* WM88 are new members of the Pho regulon. *J. Bacteriol.* 186:5876–82

White AK, Metcalf WW. 2007. Microbial metabolism of reduced phosphorus compounds. *Annu. Rev. Microbiol.* 61:379–400

Wilkins AS. 1972. Physiological factors in the regulation of alkaline phosphatase synthesis in *Escherichia coli*. *J. Bacteriol.* 110:616–23

Wilson ST, Foster RA, Zehr JP, Karl DM. 2010. Hydrogen production by *Trichodesmium erythraeum*, *Cyanothece* sp. and *Crocosphaera watsonii*. *Aquat. Microb. Ecol.* 59:197–206

Annu. Rev. Marine. Sci. 2014.6:279-337. Downloaded from www.annualreviews.org
by University of Hawaii at Manoa Library on 01/06/14. For personal use only.

Wolfe-Simon F, Switzer Blum J, Kulp TR, Gordon GW, Hoeft SE, et al. 2011. A bacterium that can grow by using arsenic instead of phosphorus. *Science* 332:1163–66

Wu J, Sunda W, Boyle EA, Karl DM. 2000. Phosphate depletion in the western North Atlantic Ocean. *Science* 289:759–62

Yang K, Metcalf WW. 2004. A new activity for an old enzyme: *Escherichia coli* bacterial alkaline phosphatase is a phosphite-dependent hydrogenase. *Proc. Natl. Acad. Sci. USA* 101:7919–24

Young CL, Ingall ED. 2010. Marine dissolved organic phosphorus composition: insights from samples recovered using combined electrodialysis/reverse osmosis. *Aquat. Geochem.* 16:563–74

Zehr JP. 2013. Interactions with partners are key for oceanic nitrogen-fixing cyanobacteria. *Microbe* 8:117–22

Zehr JP, Kudela RM. 2011. Nitrogen cycle of the open ocean: from genes to ecosystems. *Annu. Rev. Mar. Sci.* 3:197–225

Zehr JP, Montoya JP, Short CM, Hansen A, Jenkins BD, et al. 2007. Experiments linking nitrogenase gene expression to nitrogen fixation in the North Pacific subtropical gyre. *Limnol. Oceanogr.* 52:169–83

Zehr JP, Waterbury JB, Turner PJ, Montoya JP, Omoregie E, et al. 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* 412:635–38

Zeleznick LD, Myers TC, Titchener EB. 1963. Growth of *Escherichia coli* on methyl- and ethylphosphonic acids. *Biochim. Biophys. Acta* 78:546–47

ZoBell CE. 1946. *Marine Microbiology: A Monograph on Hydrobacteriology*. Waltham, MA: Chronica Botanica

Zubkov MV, Mary I, Malcolm E, Woodward S, Warwick PE, et al. 2007. Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre. *Environ. Microbiol.* 9:2079–89

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