Marine phosphate oxygen isotopes and organic matter remineralization in the oceans

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We show that the isotopic composition of oxygen (δ18O) in dissolved inorganic phosphate (Pi) reveals the balance between Pi transport and biological turnover rates in marine ecosystems. Our δ18Op of Pi (δ18Op) measurements herein indicate the importance of cell lysis in the regeneration of Pi in the euphotic zone. Depth profiles of the δ18Op in the Atlantic and Pacific Oceans are near a temperature-dependent isotopic equilibrium with water. Small deviations from equilibrium below the thermocline suggest that Pi remineralization in the deep ocean is a byproduct of microbial carbon and energy requirements. However, isotopic effects associated with phosphohydrolyase enzymes involved in Pi remineralization are quite large and could potentially lead to significant disequilibrium of Pi oxygen. The observed near equilibrium of deep water Pi likely calls for continued slow rates of microbial uptake and release of Pi and/or extracellular pyrophosphatase-mediated oxygen exchange between water and Pi along the deep water flow path.

The marine biogeochemical cycle of phosphorus (P) is intricately linked to carbon fluxes and the partitioning of major and trace elements within the ocean through the role of P as an essential nutrient for life. In many near surface ocean environments, P concentrations are sufficiently low to limit or colimit (e.g., with N or trace metals) primary production. This limitation results in tight coupling between P remineralization and biological uptake in the surface ocean. However, the ecological and biochemical mechanisms of P regeneration from microbial biomass are not well understood. Inorganic phosphorus appears in nature dominantly as orthophosphate (Pi). Here, we show that the oxygen isotope composition of Pi in a midlatitude coastal environment contrasts significantly with that of the deep ocean and provides information on the marine P cycle and its links to the marine carbon cycle.

Pi oxygen is bound tightly to P such that under Earth surface temperatures, inorganic exchange of oxygen between Pi and surrounding water is essentially negligible (1–3). Hydrothermal experiments have been used to assess the temperature dependence of inorganic Pi–water oxygen exchange (3). Extrapolations to ocean intermediate and deep water temperatures indicate that, in the absence of biological cycling, it would take >6,000 years for 10% of Pi oxygen to exchange with ambient water at 10°C and >35,000 years for 10% exchange at 2°C. These time scales are substantially longer than the ~1,000 years for ventilation of the deep ocean.

At typical Earth surface temperatures, the exchange of Pi oxygen with ambient water only occurs rapidly through the enzyme-mediated formation and destruction of organophosphorus (Porg) and condensed polyphosphate phases (2, 4, 5). Phosphorus is integral to cellular function as an important constituent of cell membranes (phospholipids), genetic material (DNA and RNA), and energy transporters (ATP, ADP, and AMP). The hydrolytic regeneration of Pi from these phosphoesters and phosphoanhydrides involves the enzyme-mediated transfer of a nucleophilic oxygen from a water molecule to phosphorus. In the case of phosphomonoesters (C–O–P), phosphoanhydrides (P–O–P), or phosphonates (C–P), hydrolytic release of Pi appears to break an O–P or C–P bond in the substrate and incorporate a single oxygen atom into the resultant Pi from ambient water. Complete hydrolysis of a phosphodiester (C–O–P–O–C) under natural conditions appears to incorporate two oxygen atoms (2, 6). Repeated intracellular synthesis and hydrolysis of Porg compounds thoroughly exchanges intracellular Pi oxygen with water oxygen. This exchange has been observed with laboratory cultures of bacteria metabolizing various Porg compounds (2, 7). Similarly, there is evidence from a study on an aquaculture system that environmental microbial communities promote rapid oxygen exchange between Pi and water (8).

Within cells, one enzyme in particular, pyrophosphatase (PPase), appears to impose a temperature-dependent isotopic equilibration between Pi oxygen and water (9). PPase is probably responsible for the recording of paleotemperature information as phosphate δ18O values in bioapatite minerals. The temperature-dependent, equilibrium fractionation in the oxygen isotopic compositions of phosphate (δ18Op) and water (δ18Ow) can be written as

\[ \delta^{18}O_{\text{w}} - \delta^{18}O_{\text{p}} = (114.4 - T) / 4.3, \]

where T is temperature in degrees Celsius (5, 10–12). The range of water temperatures with latitude in the surface ocean and with depth suggest that δ18Ow variability in the oceans could be on the order of 7‰. Before the present study, analytical difficulties had prevented the accurate measurement of δ18Op in marine ecosystems having low Pi concentration.

Methods

This study was possible because of a newly developed technique to measure δ18Op in fresh water and sea water that reduced sampling requirements to a few micromoles of phosphate while retaining a precision (1 SD) of 0.2–0.3‰ (see detailed description in ref. 13). Table 1 includes sample collection information. Deep-ocean samples were collected by using Niskin-type poly(vinyl chloride) bottles on a conductivity, temperature, and depth recorder rosette. Water was returned from different depths in and below the thermocline at Station ALOHA in the North Pacific subtropical gyre (Hawaii Ocean Time-series (HOT) cruise no. 113, R/V Ka‘imikai-o-Kanaloa) and at the Bermuda Atlantic Time-series Study (BATS) site in the North Atlantic subtropical gyre (BATS cruise no. 146, R/V Weatherbird II). The Long Island Sound (LIS) samples were collected with the help of the Connecticut Department of Environmental Protection on a Long Island Sound Study monitoring cruise aboard the R/V John Dempsey.

Abbreviations: APase, alkaline phosphatase; BATS, Bermuda Atlantic Time-series Study; HOT, Hawaii Ocean Time-series; LIS, Long Island Sound; POM, particulate organic matter; PPase, pyrophosphatase; Porg, organophosphorus compounds.

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The HOT and BATS samples ranged in volume from 1.6 to 4.8 liters. Particulate phosphorus concentrations were insignificant (<2% of P) at 500 m and declined with greater depth (refs. 15 and 16 and http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html) in these samples, so that filtration was deemed unnecessary. Ocean samples were frozen at -20°C immediately after collection to halt microbial activity. Samples were thawed in the laboratory just before processing. LIS samples had substantial particulate loads and were filter-sterilized through 0.2-μm polypropylene, nylon, or polyethersulfone membrane filters shortly after collection, just before the onset of sample processing.

Pᵦ in each sample was isolated in the laboratory as Ag₃PO₄ by using a sequence of Mg(OH)₂–PO₄ coprecipitations followed by anion resin chromatography (Bio-Rad AG50–×8, H⁺ form), cation removal (Bio-Rad AG50–×8, H⁺ form), and Ag₃PO₄ microprecipitation. Mg(OH)₂–PO₄ coprecipitations (13) were modified from Karl and Tien (17) and Thomson-Bulldis and Karl (18). Ag₃PO₄ then was analyzed for oxygen isotope composition by using laboratory standards that this technique separates Pᵦ from sea water and recovering the initial, unaltered Pᵦ oxygen isotope composition. We also verified by using laboratory standards that this technique separates Pᵦ from a wide range of P₀-org compounds and does so without hydrolyzing the phosphoryl groups (13). This technique ensures that our Pᵦ samples were not contaminated by remineralization of P₀-org during sample processing.

**Results and Discussion**

The results for our measurements of δ¹⁸O in LIS and the Pacific and Atlantic Oceans are presented in Table 1.

**Table 1. δ¹⁸O of Pᵦ and H₂O for open ocean and LIS samples**

<table>
<thead>
<tr>
<th>Sample station and sample ID</th>
<th>Latitude, d.d.</th>
<th>Longitude, d.d.</th>
<th>Date</th>
<th>Depth, m</th>
<th>Temp., °C</th>
<th>[Pᵦ], μM</th>
<th>δ¹⁸O of H₂O, %o</th>
<th>δ¹⁸Op, %o, V-SMOW</th>
<th>Measured SD</th>
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<th>Equil. model</th>
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<tr>
<td>H113-2-13-11</td>
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<td>2.81</td>
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<tr>
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<td>1.45</td>
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<td>2</td>
<td>21.35</td>
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The δ¹⁸O values of H₂O for the HOT and BATS samples were equated to the values measured at nearby stations in the GEOSECS program (14). LIS measurements characterize the nature of Pᵦ inputs, reflecting the oxygen isotope composition of the Pᵦ inputs, which was constrained by using an internal lab KH₂PO₄ oxygen isotope standard to 1-liter samples of coastal water from Milford, CT (salinity of 26.0), coastal sea water from Woods Hole, MA (salinity of 32.6), or artificial sea water (Instant Ocean, salinity of 34.5), filtered through 0.2-μm polyethersulfone membrane filters. The Milford and Woods Hole coastal samples had natural Pᵦ concentrations of <100 nM, and the artificial sea water had undetectable (<20 nM) Pᵦ. Sample processing was completed as described above. Isotopic standards were recovered with the original Pᵦ oxygen isotope composition (13). This result indicates that the technique used by this study is effective for isolating Pᵦ from sea water and recovering the initial, unaltered Pᵦ oxygen isotope composition.

We also verified by using laboratory standards that this technique separates Pᵦ from a wide range of P₀-org compounds and does so without hydrolyzing the phosphoryl groups (13). This technique ensures that our Pᵦ samples were not contaminated by remineralization of P₀-org during sample processing.

**LIS**. The LIS measurements characterize the nature of Pᵦ oxygen cycling in an ocean-surface environment with relatively rapid biological turnover of the ambient Pᵦ pool. In the LIS samples, Pᵦ is in isotopic equilibrium with ambient water within the uncertainty of the measurement and the equilibrium model (Table 1). This state is despite the fact that Pᵦ inputs to LIS are distinctly disequilibrated from LIS conditions. The Pᵦ source to LIS is dominated by sewage treatment plant effluent, either directly entering the LIS or through effluent entering the East River and the Connecticut River (22). Effluent Pᵦ characteristic of LIS inputs had δ¹⁸Op values on the order of 5‰ lower than the LIS samples presented here (13). The observed isotopic equilibration of Pᵦ in LIS represents a complete resetting of the oxygen isotope composition of the Pᵦ inputs, reflecting the rapidity of microbiological turnover of the Pᵦ pool. The maintenance of temperature-dependent equilibrium fractionation between phosphate and water oxygen isotope compositions despite significant seasonal temperature variation in the LIS waters constrains the biological turnover rates (i.e., rates of cellular uptake of Pᵦ, intracellular cycling, and release of Pᵦ) for...
with the determination of Pi turnover times of regenerated Pi. The sinking POM derives mainly from the sites of deep-water formation, POM is the dominant source of the possible exception of oceanic regions located near the few dissolved organic matter from deep-water source regions. With that Pi biological turnover rates in the coastal ocean can be as regions of deep water formation, or (ii) the regeneration of Pi from POM likely results in the inheritance of isotopically light oxygen from the phosphoryl groups of the source P$_{org}$ molecules; and (iii) the regeneration of Pi from POM incorporates oxygen from water that is substantially lighter than equilibrium predictions. Each effect is explained below; the combined effect is sufficiently great that it requires slow but nonnegligible rates of Pi processing in the deep ocean to prevent $\delta^{18}$O values from being lower than we observe. This Pi processing probably involves extracellular PPase enzymes and/or microbial uptake and release.

**Isotopically light preformed Pi from seasonal temperature shifts.** The LIS data support the hypothesis that preformed Pi, equilibrated with the temperature and the water and the $\delta^{18}$O of its surface ocean source (i.e., the site of deep-water formation). High-latitude regions, including sources for deep-water formation, exhibit a strong seasonality in primary production rates and concomitant heterotrophic activity (29–31). It is possible that the preformed Pi equilibrates isotopically with source water during warmer spring and summer months and retains this composition as the water cools and biological activity slows during the darker winter months that more closely correspond to periods of deep-water formation. In this case, a 1–2°C decline in water temperature for the high-latitude euphotic zone from summer, high-productivity conditions to winter, low-productivity, downwelling conditions would shift the equilibrium $\delta^{18}$O to values higher than the summer equilibrated $\delta^{18}$O by 0.25–0.5‰.

**Inheritance of isotopically light phosphoryl oxygen during Pi remineralization.** The enzymatic hydrolysis of P$_{org}$ results in Pi with two oxygen sources, phosphoryl oxygen inherited from the source P$_{org}$ and oxygen incorporated from ambient water. The isotopic composition of phosphoryl oxygen in marine organic matter is poorly understood (6). In the open ocean, the bulk of sinking POM originates in the euphotic zone as fecal pellets, marine snow particles, and phytodetritus aggregates. Such particulates should carry P$_{org}$ that has isotopically light P-bound oxygen due to equilibration with warmer, near-surface waters. Thus, the inheritance of phosphoryl oxygen during Pi release from sinking POM should result in the production of isotopically light Pi relative to equilibrium $\delta^{18}$O values of $-25.0$ to $-25.5$‰ for the deep ocean. If sinking POM has phosphoryl oxygen that reflects equilibrium with surface water temperatures, then a euphotic zone with 0.0‰, 10°C water would produce phosphoryl oxygen with a $\delta^{18}$O value of 23.6‰. Euphotic zone water that is 0.0‰ and 20°C would export phosphoryl oxygen with a $\delta^{18}$O value of 23.5‰.

**Acquisition of isotopically light oxygen from water through fractionation during hydrolytic Pi remineralization.** Recent laboratory experiments have shown that various phosphohydrolase enzymes preferentially transfer $^{18}$O over $^{16}$O from water molecules during hydrolytic release of Pi from P$_{org}$ compounds (6, 9). In experiments using sterile solutions containing a range of phosphatase, nuclease, and substrate combinations, the hydrolysis of phosphonooesters incorporates oxygen to the resultant Pi that differs by $-8$‰ to $-38$‰ (median $-30$‰) from the ambient water oxygen; the complete hydrolysis of phosphodiester incorporates oxygen to the resultant Pi that differs by $+5$‰ to $-15$‰ (median $-5$‰) from the ambient water oxygen (6).

The Central North Pacific and Central North Atlantic Oceans. Samples from Station ALOHA (HOT) in the Pacific and the BATS site in the Atlantic reveal that Pi in the deep ocean is near isotopic equilibrium with ambient water (Fig. 1). We observe that deep samples acquired from below the thermocline are shifted slightly toward isotopically lighter compositions than equilibrium predictions. Pi in the deep ocean derives from two sources (Fig. 2): (i) “preformed” Pi is advected to depth from surface water in regions of deep water formation, or (ii) “regenerated” Pi is released from the hydrolysis of sinking particulate organic matter (POM), either directly or through a sequence of hydrolysis reactions that produce a dissolved organic phosphorus intermediate. Also there may be a small component of regenerated Pi, derived from the hydrolysis of coadverted surficial dissolved organic matter from deep-water source regions. With the possible exception of oceanic regions located near the few sites of deep-water formation, POM is the dominant source of regenerated Pi. The sinking POM derives mainly from the euphotic zone. By using apparent oxygen utilization, we calculate for samples from depths of ≥1,000 m that 43–65% of the Pi in deep waters at Station ALOHA and 23–49% at BATS is regenerated (Fig. 2) (see Supporting Text, which is published as supporting information on the PNAS web site).

Although deep-ocean Pi oxygen is near isotopic equilibrium with ambient water, $\delta^{18}$O values are consistently shifted by 0.5–1.25‰ to values lower than predicted by equilibrium models (Fig. 1). There are three likely contributions to this observed shift as follows: (i) the preformed Pi may be isotopically lighter than equilibrium predictions due to a temperature difference between the period when Pi is cycled biologically in deep-water formation regions and the period when downwelling occurs; (ii) the regeneration of Pi from POM likely results in the inheritance of isotopically light oxygen from the phosphoryl groups of the source P$_{org}$ molecules; and (iii) the regeneration of Pi from POM incorporates oxygen from water that is substantially lighter than equilibrium predictions. Each effect is explained below; the combined effect is sufficiently great that it requires slow but nonnegligible rates of Pi processing in the deep ocean to prevent $\delta^{18}$O values from being lower than we observe. This Pi processing probably involves extracellular PPase enzymes and/or microbial uptake and release.
This hydrolytic effect has the potential to exert significant influence over deep-water $\delta^{18}O_p$. As an example, let us assume that phosphoryl oxygen in sinking POM has a $\delta^{18}O$ value of 23‰ and that the fractionations between bulk water oxygen and the oxygen atoms used to hydrolyze $P_{org}$ are $-30‰$ and $-5‰$ for phosphomonoesters and phosphodieters, respectively (the medians of the observed fractionations as discussed above.) Then, $P_t$ derived from hydrolysis of a phosphomonoester and phosphodiester in sea water (assigning a $\delta^{18}O$ of 0‰) would have $\delta^{18}O$ values of +9.75‰ and +9.0‰, respectively, compared with the original phosphoryl oxygen that had a $\delta^{18}O$ of 23‰.

During the remineralization of phosphorus from POM, any oxygen isotope effects in $P_t$ associated with either the inheritance of phosphoryl oxygen or the preferential incorporation of isotopically light water oxygen will only influence $\delta^{18}O$ of the deep ocean if the remineralized $P_t$ remains unaltered. If the remineralized $P_t$ were released by membrane-bound or periplasmic space-associated enzymes followed by cellular uptake, then the oxygen isotope effects discussed above would be overprinted by rapid enzyme-mediated oxygen exchange during intracellular phosphorus cycling.

In the deep ocean, cellular uptake of remineralized $P_t$ should be minor. Bacterial growth in the mesopelagic (32), the oligotrophic, and ultraoligotrophic (33–36) ocean requires that $>95\%$
of the carbon in consumed organic matter is respired to CO₂, whereas only ~5% is used in the synthesis of new cellular material. This bacterial growth efficiency of ~5%, when coupled with the C/P stoichiometry of consumed organic matter and bacterial cell biomass (37), suggests that only 5–20% of consumed Porg is needed for generation of new bacterial biomass. Hence, potentially 80–95% of remineralized P is not needed by bacteria for growth and may be released directly to the water column after hydrolysis without ever passing through a cell.

Our δ¹⁸Op measurements coupled with the above calculation on bacterial phosphorus demand imply that bacteria and archaea in the deep ocean are carbon and energy limited rather than phosphorus limited. This finding is consistent with a recent interpretation of the paradox of high measured alkaline phosphatase (APase) activities in the deep ocean (38, 39). APase is a widely assayed phosphomonooesterase whose detection in aquatic ecosystems traditionally has been interpreted as a sign of phosphorus stress resulting from low-nutrient P concentrations. APase enables microbes to use a range of dissolved organic phosphorus compounds as sources of P, thus diminishing P stress. Our δ¹⁸Op data require that APase expressed by the deep-ocean microbial community is not a response to P availability but rather is used in dephosphorylation as a necessary step before cellular uptake of carbon in the targeted Porg compounds.

Given that the degradation of Porg produces an excess of P over the requirements for synthesis of new microbial biomass, it is likely that the P reservoir in the deep ocean is not used extensively by the heterotrophic microbial population. It is this biochemical inactivity of deep-water P that enables oxygen isotope disequilibrium to persist until the deep water is returned to the euphotic zone in regions of active upwelling. Yet if >80% of the remineralized P acquired the light isotopic compositions attendant to the hydrolytic effect, we would expect deep-ocean δ¹⁸Op to be offset by several permil to values lower than equilibrium. In fact, the δ¹⁸Op in deep-water samples is within 1.5‰ of equilibrium (Fig. 2). This result requires that either remineralized P is taken up by cells at a rate significantly higher than predicted by growth demands alone or that a slow rate (e.g., 10% P pool per 1,000 years) of biological or extracellular PPase turnover of the deep-water P pool continues.

Pacific vs. Atlantic results. Our first-order assumption is that regions in the deep ocean with a higher percent regenerated P would show greater deviation in δ¹⁸Op from equilibrium. The average deviation from equilibrium for δ¹⁸Op values we have measured on samples 900 m and deeper is larger in the Pacific than in the Atlantic. This finding agrees with the greater fraction of regenerated P in the Pacific; however, the interocean differences are not at present statistically significant. As discussed above, several factors influence the isotopic composition of remineralized P, including inherited phosphorus oxygen and fractionation during the transfer of oxygen from water to the phosphorus group. Furthermore, slow rates of ectoenzymatic and cellular P cycling in the deep ocean would noticeably alter deep-water δ¹⁸Op over time. At present, it is difficult to assess how these processes might proceed at different rates from one ocean basin to another or from one deep-ocean current to another.

Comparison with Other Studies. An earlier attempt to measure the δ³⁴S of marine P was made as part of the GEOSECS expeditions in the early 1970s (40). The GEOSECS 8°Op data appear to be an artifact of sample storage and handling. Our technique contrasts with that used during GEOSECS. In that study, P was extracted from splits of ferric (oxy)hydroxide-coated acrylic fibers that had been deployed at various depths in the ocean primarily to adsorb cosmogenic ³²Si as part of the natural tracer work on ocean circulation and biogeochemistry in GEOSECS (40–42). After sample collection, the fibers were stored in plastic bags within 55-gallon drums on the research vessel’s deck without refrigeration (41). That collection approach was suitable for the study of cosmogenic ³²Si, but we now know it to be inappropriate for maintaining the isotopic integrity of phosphate oxygen because of likely continued microbial P cycling and enzymatic exchange of Pi oxygen with residual seawater in the fibers. No evidence was provided to show that the full GEOSECS 8°Op sample collection and processing protocol were verified with standards (40). Furthermore, the use of ferric (oxy)hydroxide fibers would have physically filtered a fraction of the suspended particulate Porg. Trapping of particulate matter was shown to be a factor when these fibers were analyzed for ³²Si (42). The ferric (oxy)hydroxide-coated fibers also would have quantitatively adsorbed dissolved Porg (43), compromising the specificity of the measurement.

Only one other study, conducted at the same time our study was executed, has measured 8°Op in marine samples deeper than 500 m in the ocean (44). One sample was collected in the California Current at a depth of 665 m, and the other was collected in the North Pacific (coordinates not reported) at a depth of 1,000 m. The reported 8°Op values for these samples were 20.2‰ and 20.3‰, and water temperatures were 4.9° and 3.9°C respectively. These values are 3–5‰ lower than the equilibrium values typical of our results for the thermocline. The focus of McLaughlin et al. (44) was to present a technique for 8°Op measurements. It is not clear from their discussion whether the biogeochemical regime in their continental margin samples was markedly different from our open ocean samples, although we suspect this regime accounts for the difference.

Our measurements of 8°Op on marine P provide a basis for comparison with measurements on phosphate extracted from pore waters and particulate phases in marine sediments, especially authigenic carbonate fluorapatite and P adsorbed onto and incorporated into ferric oxides and (oxy)hydroxides. For example, the phosphate scavenged from sea water onto metaliferous sediments associated with diffuse flow hydrothermal systems at Larson’s Seamounts and Seamount No. 5 near the East Pacific Rise exhibit 8°Op values ranging from 11.9‰ to 25.6‰ (45). These values diverge from the rather narrow range of 8°Op values we have measured on P in the deep ocean, showing a strong imprint from microbial phosphate cycling at temperatures ranging from the 2°C background temperature up to 60°C, consistent with hydrothermal system conditions.

Conclusions

P in the deep ocean is the largest reservoir of bioavailable phosphorus on Earth. Here, we present depth profile measurements of 8°Op in the Atlantic and Pacific Oceans, which allow us to infer the biochemical mechanisms involved in remineralization of P in the ocean water column. We observe that 8°Op values are near isotopic equilibrium with ambient water. At depths below the thermocline, 8°Op values range from equilibrium predictions to 1.5‰ lower than equilibrium. The acquisition and persistence of small deviations from oxygen isotopic equilibrium suggests that uptake of P by deep-water bacteria and archaea is extremely slow during the ~1,000-year transit time for water in the deep ocean.

Our measurements are consistent with two lines of evidence that suggest that P remineralization is incidental to the microbial consumption of organic matter as an energy and carbon source rather than to fulfill cellular P requirements. First, we calculated that deep marine heterotrophic microbes only require 5–20% of the P in remineralized organic matter to be used in the production of new microbial biomass. The satisfaction of the bacterial phosphorus demand by Porg substrate alone indicates that the P reservoir in the deep ocean is probably not used extensively by the heterotrophic bacteria population as a supplement to the substrate P. Second, our 8°Op data are consistent with the interpretation that APase...
expressed by the deep-ocean microbial community is used to remove excess P from the targeted POM/dissolved organic matter substrate (39). The apparent preferential use by bacteria and/or archaea of $P_{\text{org}}$ compounds over bulk particulate and dissolved organic matter suggests a correlation between P content and carbon bioavailability or energy content (39).

The biochemical inactivity of deep-water $P$ enables the measured oxygen isotope disequilibrium to persist until the deep water is returned to the euphotic zone in regions of active upwelling. However, unless mitigated by other processes, the large isotopic fractionations associated with phosphohydrolases (6, 9) would probably produce substantially larger deviations from equilibrium than we observe for deep-water $\delta^{18}$O$P$ values. We suggest that deep-water $\delta^{18}$O$P$ is modified by a partial turnover of the $P$ pool either through cellular uptake and release of $Pi$ or extracellular PPase-mediated formation and hydrolysis of pyrophosphate. Such continued biochemical processing of deep-water $P$ at a rate on the order of 10% of the $P$ pool per 1,000 years would be sufficient to maintain the isotopic disequilibrium that we have measured.

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