Marine phosphate oxygen isotopes and organic matter remineralization in the oceans

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We show that the isotopic composition of oxygen ($\delta^{18}O$) in dissolved inorganic phosphate (P_i) reveals the balance between P_i transport and biological turnover rates in marine ecosystems. Our $\delta^{18}Op$ of P_i ($\delta^{18}Op$) measurements herein indicate the importance of cell lysis in the regeneration of P_i in the euphotic zone. Depth profiles of the $\delta^{18}Op$ in the Atlantic and Pacific Oceans are near a temperature-dependent isotopic equilibrium with water. Small deviations from equilibrium below the thermocline suggest that P remineralization in the deep ocean is a byproduct of microbial carbon and energy requirements. However, isotope effects associated with phosphohydrolase enzymes involved in P remineralization are quite large and could potentially lead to significant disequilibration of P_i oxygen. The observed near equilibration of deep water P_i likely calls for continued slow rates of microbial uptake and release of P_i and/or extracellular pyrophosphatase-mediated oxygen exchange between water and P_i along the deep water flow path.

marine biogeochemistry | nutrient limitation | phosphorus cycle

The marine biogeochemical cycle of phosphorus (P) is inextricably 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 (P_i). Here, we show that the oxygen isotope composition of P_i 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.

P_i oxygen is bound tightly to P such that under Earth surface temperatures, inorganic exchange of oxygen between P_i and surrounding water is essentially negligible (1–3). Hydrothermal experiments have been used to assess the temperature dependence of inorganic P_i–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 P_i 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 P_i oxygen with ambient water only occurs rapidly through the enzyme-mediated formation and destruction of organophosphorus (P_{org}) 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 P_i 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), phosphoanyhydrides (P–O–P), or phosphonates (C–P), hydrolytic release of P_i appears to break an O–P or C–P bond in the substrate and incorporate a single oxygen atom into the resultant P_i from ambient water. Complete hydrolysis of a phosphodiester (C–O–PO_2–O–C) under natural conditions appears to incorporate two oxygen atoms (2, 6). Repeated intracellular synthesis and hydrolysis of P_{org} compounds thoroughly exchanges intracellular P_i oxygen with water oxygen. This exchange has been observed with laboratory cultures of bacteria metabolizing various P_{org} compounds (2, 7). Similarly, there is evidence from a study on an aquaculture system that environmental microbial communities promote rapid oxygen exchange between P_i and water (8).

Within cells, one enzyme in particular, pyrophosphatase (PPase), appears to impose a temperature-dependent isotopic equilibration between P_i oxygen and water (9). PPase is probably responsible for the recording of paleotemperature information as phosphate $\delta^{18}O$ values in bioapatite minerals. The temperature-dependent, equilibrium fractionation in the oxygen isotopic compositions of phosphate ($\delta^{18}Op$) and water ($\delta^{18}Ow$) can be written as

$$(\delta^{18}Op - \delta^{18}Ow) = (111.4 - T)/4.3,$$

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 $\delta^{18}Op$ variability in the oceans could be on the order of 7‰. Before the present study, analytical difficulties had prevented the accurate measurement of $\delta^{18}Op$ in marine ecosystems having low Pi concentration.

Methods

This study was possible because of a newly developed technique to measure $\delta^{18}Op$ 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 Enviromental 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; P_{org}, organophosphorus compounds.

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Table 1. $\delta^{18}O$ of P_i and H_2O for open ocean and LIS samples

Sample station and sample ID	Latitude, d.d.	Longitude, d.d.	Date	Depth, m	Temp., °C	$[P_i]$, μM	$\delta^{18}O$ of H_2O, ‰, V-SMOW	$\delta^{18}Op$, ‰, V-SMOW			
								Measured	SD	*n*	Equil. model
HOT Sta. ALOHA											
H113-2-13-11	22.68 N	158.04 W	29-Mar-00	300	13.6	0.69	−0.2	23.37	n/a	1	22.54
H113-2-13-8	22.68 N	158.04 W	29-Mar-00	500	7.2	2.02	−0.2	23.21	0.15	2	24.03
H113-2-13-7	22.68 N	158.04 W	29-Mar-00	600	5.8	2.63	−0.2	24.17	0.97	2	24.36
H113-2-7-6A	22.75 N	157.98 W	29-Mar-00	700	5.3	2.98	−0.2	24.47	0.31	2	24.47
H113-2-7-5A	22.75 N	157.98 W	29-Mar-00	800	5.0	2.92	−0.2	24.54	n/a	1	24.54
H113-2-13-2	22.68 N	158.04 W	29-Mar-00	900	4.2	3.08	−0.2	24.01	0.26	2	24.73
H113-2-15-11	22.75 N	157.97 W	30-Mar-00	2,000	2.2	2.81	−0.2	24.15	0.05	2	25.20
H113-2-15-9	22.75 N	157.97 W	30-Mar-00	3,000	1.6	2.63	−0.2	24.07	0.46	2	25.33
BATS site and Spatial Sta. 3											
B146-21	32.15 N	64.02 W	16-Nov-00	900	7.3	1.55	0.1	23.83	0.24	2	24.31
B146-23	32.15 N	64.02 W	16-Nov-00	2,000	3.5	1.25	0.1	23.73	n/a	1	25.19
B146-5-20	31.56 N	64.19 W	14-Nov-00	3,000	2.6	1.35	0.1	25.07	0.48	3	25.40
B146-5-23	31.56 N	64.19 W	14-Nov-00	4,000	2.2	1.45	0.1	24.54	0.37	4	25.50
LIS, CT-DEP LISS A4, CT-DEP LISS J2											
LISS-A4-1A	40.873 N	73.734 W	5-Dec-01	2	11.5	2.98	−2.38	21.37	0.29	3	20.86
LISS-J2-1AB	41.182 N	72.458 W	4-Dec-01	2	11.9	1.53	−1.79	21.30	0.27	2	21.35

The $\delta^{18}O$ values of H_2O for the HOT and BATS samples were equated to the values measured at nearby stations in the GEOSECS program (14). The $\delta^{18}O$ values of H_2O for the LIS samples were measured at the University of California Davis stable isotope facility. The $\delta^{18}Op$ equilibrium model values are calculated using the phosphate–water paleotemperature equation from Longinelli and Nuti (9) and have an uncertainty (95% confidence interval about regression line) of ≈0.25‰. d.d., decimal degrees; V-SMOW, Vienna–Standard Mean Ocean Water; Equil. model, $\delta^{18}Op$ equilibrium model value; n/a, not applicable; Sta., station.

The HOT and BATS samples ranged in volume from 1.6 to 4.8 liters. Particulate phosphorus concentrations were insignificant (<2% of P_i) 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.

P_i in each sample was isolated in the laboratory as Ag_3PO_4 by using a sequence of $Mg(OH)_2$–PO_4 coprecipitations followed by anion resin chromatography (Bio-Rad AG50-×8, HCO_3^- form, 0.15M $NaHCO_3$ eluent), cation removal (Bio-Rad AG50-×8, H^+ form), and Ag_3PO_4 microprecipitation. $Mg(OH)_2$–PO_4 coprecipitations (13) were modified from Karl and Tien (17) and Thomson-Bulldis and Karl (18). Ag_3PO_4 microprecipitations (13) were modified from Wenzel *et al.* (19) and O'Neil *et al.* (20).

Ag_3PO_4 then was analyzed for oxygen isotope composition by means of online high-temperature thermal decomposition (21) using a ThermoChemical Elemental Analyzer (TC/EA) coupled with a Delta + XL continuous-flow isotope ratio mass spectrometer (Finnigan-MAT, Bremen, Germany). Oxygen liberated by the decomposition of Ag_3PO_4 at 1450°C in the TC/EA combines with graphitic and glassy carbon in the TC/EA reactor to form CO, which is analyzed for mass 30/28 ratio against a reference CO gas. Three or four different Ag_3PO_4 isotopic standards were run in triplicate or quadruplicate at the beginning of each day's analysis to calibrate the system. Table 1 lists means and SDs for $\delta^{18}Op$ analyses on each natural P_i sample. The typical precision for replicate unknowns is ±0.2–0.3‰ (1SD).

The sample processing and measurement technique was validated by adding 4.5–5.0 μmol of an internal lab KH_2PO_4 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, made to salinity of 34.5), filtered through 0.2-μm polyethersulfone membrane filters. The Milford and Woods Hole coastal samples had natural P_i concentrations of <100 nM, and the artificial sea water had undetectable (<20 nM) P_i. Sample processing was completed as described above. Isotopic standards were recovered with the original P_i oxygen isotope composition (13). This result indicates that the technique used by this study is effective for isolating P_i from sea water and recovering the initial, unaltered P_i oxygen isotope composition. We also verified by using laboratory standards that this technique separates P_i from a wide range of P_{org} compounds and does so without hydrolyzing the phosphoryl groups (13). This technique ensures that our P_i samples were not contaminated by remineralization of P_{org} during sample processing.

Results and Discussion

The results for our measurements of $\delta^{18}Op$ in LIS and the Pacific and Atlantic Oceans are presented in Table 1.

LIS. The LIS measurements characterize the nature of P_i oxygen isotope cycling in an ocean-surface environment with relatively rapid biological turnover of the ambient P_i pool. In the LIS samples, P_i 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 P_i inputs to LIS are distinctly disequilibrated from LIS conditions. The P_i 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 P_i characteristic of LIS inputs had $\delta^{18}Op$ values on the order of 5‰ lower than the LIS samples presented here (13). The observed isotopic equilibration of P_i in LIS represents a complete resetting of the oxygen isotope composition of the P_i inputs, reflecting the rapidity of microbiological turnover of the P_i 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 P_i, intracellular cycling, and release of P_i) for

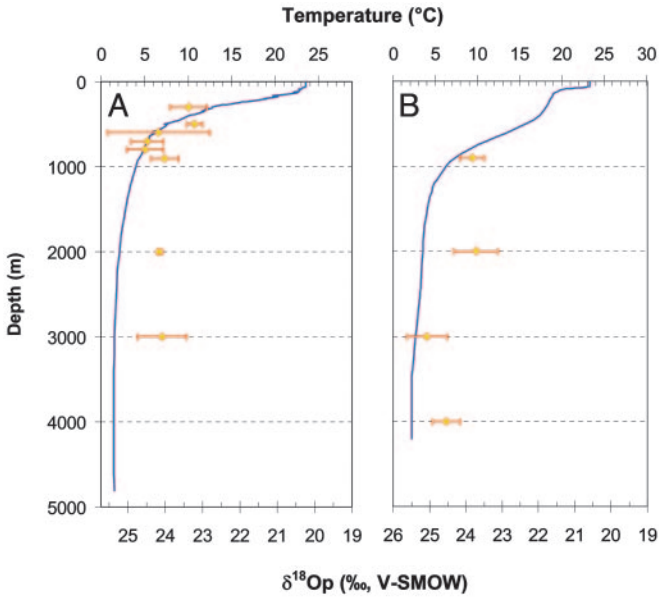


Fig. 1. δ^{18}Op and temperature profiles at Station ALOHA (HOT) and the BATS site. Temperature profiles are plotted (blue lines) for Station ALOHA, HOT Cruise 113 (*A*), and the BATS site, BATS Cruise 146 (*B*). The Longinelli and Nuti (10) phosphate–water paleothermometer is used to map the temperature scale to a δ^{18}Op scale for P_i equilibrated with subtropical gyre water. Therefore, the temperature profiles may be read off the bottom horizontal axis as predicted equilibrium δ^{18}Op values. GEOSECS data for nearby stations provide the δ^{18}O values for the subtropical gyre water at depth [North Pacific = −0.2‰; North Atlantic = +0.1‰ (14)]. Measured δ^{18}Op values from the present work are plotted (yellow and orange diamonds) against this bottom axis. Error bars represent 95% confidence intervals based on replicate mass spectrometric analyses of single samples.

the entire P_i pool to be <2 weeks (13). This finding is consistent with the determination of P_i turnover times of <10 days in coastal waters in the Gulf of Maine by using cosmogenic ^{32}P and ^{33}P (23). Radiotracer ^{32}P and ^{33}P bottle incubations have shown that P_i biological turnover rates in the coastal ocean can be as short as hours in P_i-stressed ecosystems (24) and in the surface open ocean are generally on the order of days to several weeks (25–28). The LIS δ^{18}Op data indicate that if the mechanisms of P_i remineralization in the open ocean are similar to those operating in LIS, then these rapid turnover rates should equilibrate P_i oxygen isotopically with open ocean surface waters.

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 P_i 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. P_i in the deep ocean derives from two sources (Fig. 2): (*i*) "preformed" P_i is advected to depth from surface water in regions of deep water formation, or (*ii*) "regenerated" P_i 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. There also may be a small component of regenerated P_i derived from the hydrolysis of coadvected 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 P_i. 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 P_i 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 P_i oxygen is near isotopic equilibrium with ambient water, δ^{18}Op 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 P_i may be isotopically lighter than equilibrium predictions due to a temperature difference between the period when P_i is cycled biologically in deep-water formation regions and the period when downwelling occurs; (*ii*) the regeneration of P_i 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 P_i 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 P_i processing in the deep ocean to prevent δ^{18}Op values from being lower than we observe. This P_i processing probably involves extracellular PPase enzymes and/or microbial uptake and release.

Isotopically light preformed P_i from seasonal temperature shifts. The LIS data support the hypothesis that preformed P_i equilibrated with the temperature and the water δ^{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 P_i 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 δ^{18}Op to values higher than the summer equilibrated δ^{18}Op by 0.25–0.5‰.

Inheritance of isotopically light phosphoryl oxygen during P_i remineralization. The enzymatic hydrolysis of P_{org} results in P_i 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 P_i release from sinking POM should result in the production of isotopically light P_i relative to equilibrium δ^{18}Op 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 δ^{18}O value of 23.6. Euphotic zone water that is 0.0‰ and 20°C would export phosphoryl oxygen with a δ^{18}O value of 21.3‰.

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

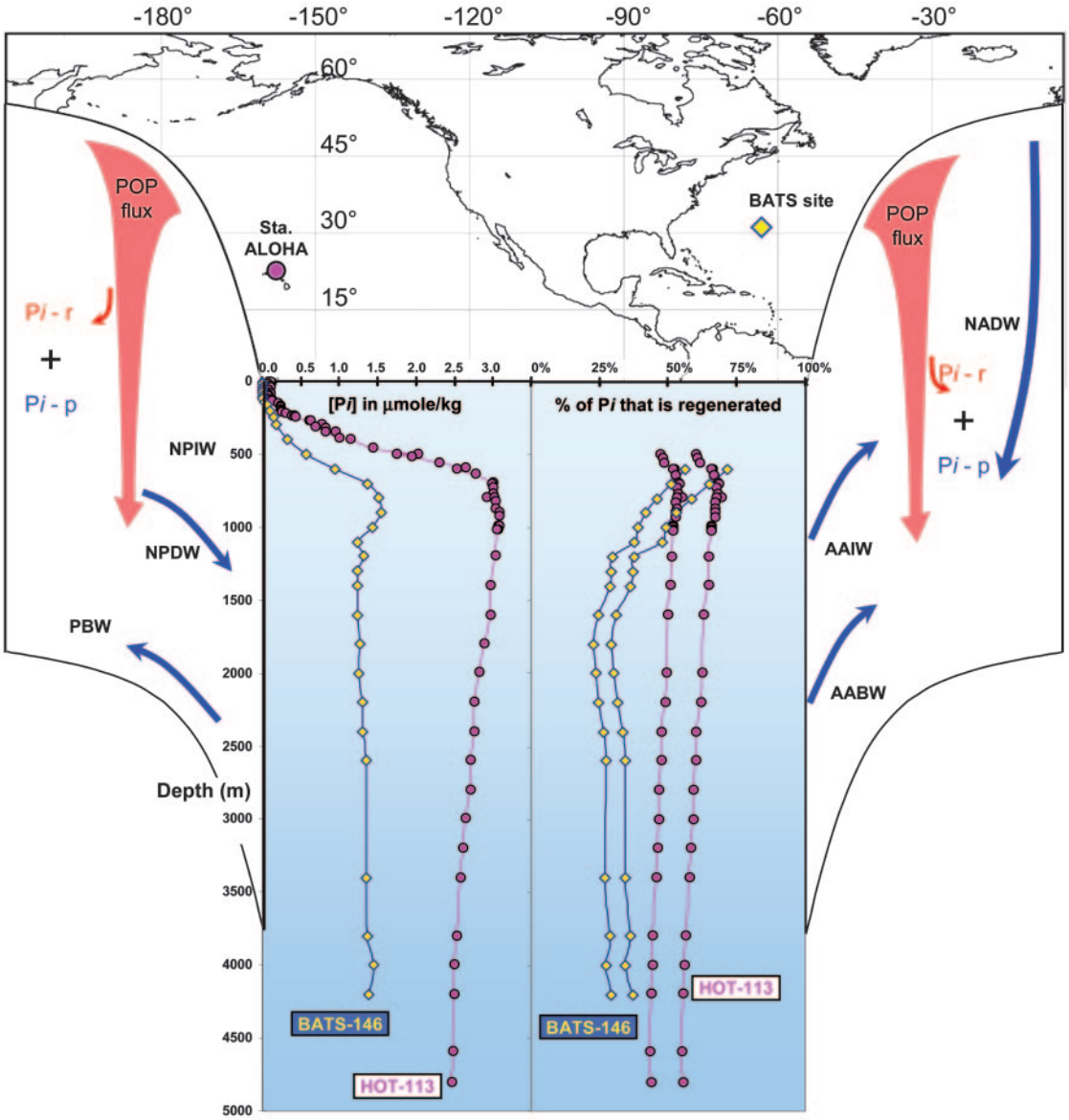


Fig. 2. Cutaway views of the North Pacific and North Atlantic indicate the major routes by which P_i is delivered to the deep ocean. Red arrows denote the flux of sinking particulate organic phosphorus, which yields dissolved P_i on remineralization. P_i thus produced is labeled P_i–r to represent regenerated P_i. Blue arrows denote advected P_i that descends to the deep ocean as a dissolved constituent of sea water in deep-water formation regions. Such P_i is labeled P_i–p to represent preformed P_i. Principal deep-water constituents in the North Atlantic are indicated as North Atlantic Deep Water (NADW), Antarctic Intermediate Water (AAIW), and Antarctic Bottom Water (AABW); correspondingly in the North Pacific are Pacific Bottom Water (PBW, a mixture of NADW and AABW deriving from the circum-Antarctic current), North Pacific Deep Water (NPDW), and North Pacific Intermediate Water (NPIW). The HOT and BATS sampling sites are identified on the top map-view surface. (*Insets*) Graphs show depth profiles for P_i concentrations (*Left*) and %P_i regenerated (*Right*), corresponding to the cruises on which samples were collected for this study. Pairs of P_i regeneration curves are plotted for each site pair: the left curve corresponds to a Redfield-type $\Delta O{:}\Delta P_i$ ratio of 175; the right curve corresponds to a $\Delta O{:}\Delta P_i$ ratio of 138 (see *Supporting Text*). P_i and dissolved O_2 concentration data are from the HOT (http://hahana.soest.hawaii.edu/hot/hot-dogs/interface.html) and BATS (http://www.bbsr.edu/cintoo/bats/bats.html).

This hydrolytic effect has the potential to exert significant influence over deep-water δ^{18}Op. As an example, let us assume that phosphoryl oxygen in sinking POM has a δ^{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 phosphodiesters, respectively (the medians of the observed fractionations as discussed above.) Then, P_i derived from hydrolysis of a phosphomonoester and phosphodiester in sea water (assigning a δ^{18}Ow of 0‰) would have δ^{18}Op values of +9.75‰ and +9.0‰, respectively, compared with the original phosphoryl oxygen that had a δ^{18}O of 23‰.

During the remineralization of phosphorus from POM, any oxygen isotope effects in P_i associated with either the inheritance of phosphoryl oxygen or the preferential incorporation of isotopically light water oxygen will only influence δ^{18}Op of the deep ocean if the remineralized P_i remains unaltered. If the remineralized P_i 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_i 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 CO_2, 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 P_{org} is needed for generation of new bacterial biomass. Hence, potentially 80–95% of remineralized P_i 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 δ^{18}Op 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 phosphomonoesterase whose detection in aquatic ecosystems traditionally has been interpreted as a sign of phosphorus stress resulting from low-nutrient P_i concentrations. APase enables microbes to use of a range of dissolved organic phosphorus compounds as sources of P, thus diminishing P_i stress. Our δ^{18}Op data require that APase expressed by the deep-ocean microbial community is not a response to P_i availability but rather is used in dephosphorylation as a necessary step before cellular uptake of carbon in the targeted P_{org} compounds.

Given that the degradation of P_{org} produces an excess of P over the requirements for synthesis of new microbial biomass, it is likely that the P_i reservoir in the deep ocean is not used extensively by the heterotrophic microbial population. It is this biochemical inactivity of deep-water P_i 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_i acquired the light isotopic compositions attendant to the hydrolytic effect, we would expect deep-ocean δ^{18}Op to be offset by several permil to values lower than equilibrium. In fact, the δ^{18}Op 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_i pool per 1,000 years) of biological or extracellular PPase turnover of the deep-water P_i pool continues.

Pacific vs. Atlantic results. Our first-order expectation is that regions in the deep ocean with a higher percent regenerated P_i would show greater deviation in δ^{18}Op from equilibrium. The average deviation from equilibrium for δ^{18}Op 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_i 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_i, including inherited phosphoryl oxygen and fractionation during the transfer of oxygen from water to the phosphoryl group. Furthermore, slow rates of ectoenzymatic and cellular P_i cycling in the deep ocean would noticeably alter deep-water δ^{18}Op 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 δ^{18}O of marine P_i was made as part of the GEOSECS expeditions in the early 1970s (40). The GEOSECS δ^{18}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 ^{32}Si 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 ^{32}Si, 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 P_i oxygen with residual seawater in the fibers. No evidence was provided to show that the full GEOSECS δ^{18}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 P_{org}. Trapping of particulate matter was shown to be a factor when these fibers were analyzed for ^{32}Si (42). The ferric (oxy)hydroxide-coated fibers also would have quantitatively adsorbed dissolved P_{org} (43), compromising the specificity of the measurement.

Only one other study, conducted at the same time our study was executed, has measured δ^{18}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 δ^{18}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 δ^{18}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 δ^{18}O on marine P_i 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_i 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 exihibit δ^{18}Op values ranging from 11.9‰ to 25.6‰ (45). These values diverge from the rather narrow range of δ^{18}Op values we have measured on P_i 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_i in the deep ocean is the largest reservoir of bioavailable phosphorus on Earth. Here, we present depth profile measurements of δ^{18}Op in the Atlantic and Pacific Oceans, which allow us to infer the biochemical mechanisms involved in remineralization of P_i in the ocean water column. We observe that δ^{18}Op values are near isotopic equilibrium with ambient water. At depths below the thermocline, δ^{18}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_i 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_i remineralization is incidental to the microbial consumption of organic matter as an energy and carbon source rather than to fulfill cellular P_i 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 P_{org} substrate alone indicates that the P_i reservoir in the deep ocean is probably not used extensively by the heterotrophic bacteria population as a supplement to the substrate P. Second, our δ^{18}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_{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_i 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 likely produce substantially larger deviations from equilibrium than we observe for deep-water δ^{18}Op values. We suggest that deep-water δ^{18}Op is modified by a partial turnover of the P_i pool either through cellular uptake and release of P_i or extracellular PPase-mediated formation and hydrolysis of pyrophosphate. Such continued biochemical processing of deep-water P_i at a rate on the order of 10% of the P_i pool per 1,000 years would be sufficient to maintain the isotopic disequilibrium that we have measured.

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