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## Phosphorus cycling in the Sargasso Sea: Investigation using the oxygen isotopic composition of phosphate, enzyme-labeled fluorescence, and turnover times

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[1] Dissolved inorganic phosphorus (DIP) concentrations in surface water of vast areas of the ocean are extremely low (<10 nM) and phosphorus (P) availability could limit primary productivity in these regions. We explore the use of oxygen isotopic signature of dissolved phosphate ( $\delta^{18}\text{O}_{\text{PO}_4}$ ) to investigate biogeochemical cycling of P in the Sargasso Sea, Atlantic Ocean. Additional techniques for studying P dynamics including  $^{33}\text{P}$ -based DIP turnover time estimates and percent of cells expressing alkaline phosphatase (AP) activity as measured by enzyme-labeling fluorescence are also used. In surface waters,  $\delta^{18}\text{O}_{\text{PO}_4}$  values were lower than equilibrium by 3–6‰, indicative of dissolved organic phosphorus (DOP) remineralization by extracellular enzymes. An isotope mass balance model using a variety of possible combinations of enzymatic pathways and substrates indicates that DOP remineralization in the euphotic zone can account for a large proportion of P utilized by phytoplankton (as much as 82%). Relatively short DIP turnover times (4–8 h) and high expression of AP (38–77% of the cells labeled) are consistent with extensive DOP utilization and low DIP availability in the euphotic zone. In deep water where DOP utilization rates are lower,  $\delta^{18}\text{O}_{\text{PO}_4}$  values approach isotopic equilibrium and DIP turnover times are longer. Our data suggests that in the euphotic zone of the Sargasso Sea, DOP may be appreciably remineralized and utilized by phytoplankton and bacteria to supplement cellular requirements. A substantial fraction of photosynthesis in this region is supported by DOP uptake.

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### 1. Introduction

[2] The oceanic phosphorus (P) cycle is closely coupled with the global carbon cycle through the role of P as a major nutrient supporting ocean primary productivity. The concentrations of dissolved inorganic phosphorus (DIP) in much of the open ocean surface waters are low and can

limit or co-limit primary production [Lomas *et al.*, 2004; Mills *et al.*, 2004; Vidal *et al.*, 2003; Wu *et al.*, 2000]. The dissolved organic phosphorus (DOP) pool in the surface ocean is often larger than the DIP pool and thus, utilization of this chemically heterogeneous pool as a P source for living organisms can potentially influence carbon sequestration in the ocean via the biological pump [Dyhrman *et al.*, 2006; Lomas *et al.*, 2010; Torres-Valdes *et al.*, 2009]. However, the complex biogeochemical cycling of P in the open ocean is poorly characterized and quantitative estimates of the bioavailability and utilization of DOP on large (ecologically relevant) spatial and temporal scales are lacking [Dyhrman *et al.*, 2007].

[3] A number of studies have shown that P is preferentially remineralized from dissolved organic matter relative to carbon, allowing efficient utilization of P in the euphotic surface layer of the ocean [Aminot and Kerouel, 2004; Clark *et al.*, 1998; Hopkinson and Vallino, 2005; Hopkinson *et al.*, 2002; Lomas *et al.*, 2010]. DOP constitutes a wide array of compounds and is present in various distinct pools with different degrees of reactivity and susceptibility for regeneration [Karl and Yanagi, 1997; Hopkinson and Vallino, 2005; Benitez-Nelson and Buesseler, 1999; Paytan and McLaughlin,

All supporting information may be found in the online version of this article.

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2007; White *et al.*, 2012]. Based on the distribution of DOP in the world's ocean, it has been suggested that different components within the DOP pool are recycled at different rates and thus may have different ages and residence times [Kolowitz *et al.*, 2001; Loh and Bauer, 2000; Loh *et al.*, 2004]. Indeed, it has been shown that individual molecules within the DOP pool may have unique cycling times, for example, turnover of nucleotide triphosphate pools can be up to five times faster than the bulk DOP pool [Bjorkman and Karl, 2005]. These data along with uptake experiments [Casey *et al.*, 2009; Orchard *et al.*, 2010; White *et al.*, 2012] suggest that some components in the DOP pool are biologically available and highly reactive; however, quantitative estimates of the contribution of DOP regeneration to carbon fixation in the open ocean is lacking.

[4] Several organisms (phytoplankton and heterotrophic bacteria) have adapted to utilize P from organic sources and from polyphosphate [Dyhrman *et al.*, 2006; Dyhrman and Haley, 2006]. Other species have lower cellular P requirements as a way to cope with low P levels [Christian, 2005; Van Mooy *et al.*, 2009]. The abundance of alkaline phosphatase (AP; an enzyme that catalyzes the cleavage of orthophosphate from orthophosphoric monoesters under alkaline conditions) activity in surface seawater at many locations has been interpreted as indicative of P deficiency and the utilization of DOP [Vidal *et al.*, 2003]. However, the utilization of DOP by different organisms is variable and could change considerably in space and time [Cembella *et al.*, 1984; Bjorkman and Karl, 1994; White *et al.*, 2012]. Indeed, enzyme-labeled fluorescence (ELF) analysis of AP in single cells revealed substantial variability in the degree of expression of this enzyme both among genera as well as within genera, suggestive of microscale differences in the nutrient status and the nutritional history of cells [Dyhrman and Palenik, 1999; Gonzalez-Gil *et al.*, 1998; Lomas *et al.*, 2004; Mackey *et al.*, 2007; Nicholson *et al.*, 2006; Ruttenberg and Dyhrman, 2005].

[5] Phosphorus uptake measurements also indicated that the DOP pool can be an important source of P in nutrient-depleted surface waters [Benitez-Nelson and Karl, 2002; Bjorkman and Karl, 2003, 2005], particularly in the P-depleted North Atlantic [Mather *et al.*, 2008]. Phosphorus turnover rates obtained using cosmogenic isotopes show that recycling rates of dissolved and particulate P in surface waters are rapid (less than a day to 2 weeks); suggesting that extensive recycling of P can result in low P concentrations supporting relatively high primary production [Benitez-Nelson and Buesseler, 1999; Benitez-Nelson and Karl, 2002]. Tracer experiment with radioactive P additions show that P recycling rates vary spatially and temporally [Sohm and Capone, 2010] and that picoplankton preferentially utilize certain DOP compounds to obtain P [Benitez-Nelson and Buesseler, 1999]. The radioactive P studies (natural or spiked experiments) indicate that DOP is an important source of P to organisms; however, estimating P cycling rates using radioactive P isotopes are complicated and/or not easy to use; thus, other ways to estimate P cycling are needed [Paytan and McLaughlin, 2007].

[6] DIP in seawater is predominantly present in the form of orthophosphate in which P is strongly bound to four oxygen (O) atoms. The PO bond in orthophosphate is resistant to inorganic hydrolysis and does not exchange O with

water without biological mediation under typical oceanic temperature and pH ranges [Blake *et al.*, 1997; Longinelli *et al.*, 1976; O'Neil *et al.*, 2003]. Accordingly, the oxygen isotopes in dissolved phosphate ( $\delta^{18}\text{O}_{\text{PO}_4}$ ) could be utilized to study P cycling [McLaughlin *et al.*, 2006; Paytan and McLaughlin, 2011 and reference therein, and supplementary material]. Specifically, significant exchange of oxygen isotopes between phosphate and water accompanies the enzyme-mediated hydrolytic cleavage and metabolism of both organically bound phosphate and inorganic orthophosphate [Blake *et al.*, 1997, 1998; Paytan *et al.*, 2002]. Laboratory observations indicate that P cycling by pyrophosphatase results in a temperature-dependent equilibrium oxygen isotope fractionation, which imparts the equilibrium  $\delta^{18}\text{O}_{\text{PO}_4}$  on phosphate recycled by this enzyme [Blake *et al.*, 2005]. In contrast, regeneration of DOP by enzymes such as alkaline phosphatase or 5' nucleotidase is accompanied by kinetic isotope effects and incorporation of water oxygen. The kinetic isotope fractionation depends on substrate type and the enzymes involved, and results in disequilibrium isotope effects [Blake *et al.*, 2005]. In general, these kinetic isotope effects in seawater drive the isotopic composition of the regenerated phosphate toward values that are lower than those expected from isotopic equilibrium [Paytan and McLaughlin, 2011; Goldhammer *et al.*, 2011].

[7] Distinct isotope effects are associated with specific regeneration pathways depending on the substrate and enzymes involved. For example, the hydrolysis of phosphomonoesters by alkaline phosphatase, an abundant enzyme that attacks a wide range of phosphomonoesters, involves an isotopic fractionation of  $-30\text{‰}$ , while 5' nucleotidase which targets nucleotides exclusively, involves a fractionation of  $-10\text{‰}$  [Liang and Blake, 2006]. The hydrolysis of phosphodiesterase is a two step process involving the activity of phosphodiesterase followed by a phosphomonoesterase and results in the exchange of two oxygen atoms [Liang and Blake, 2009]. The fractionation associated with phosphodiesterase is substrate dependant; for DNA, it is  $-20\text{‰}$ , and for RNA, it is  $+20\text{‰}$  [Liang and Blake, 2009]. It is important to note that the DOP pool in seawater consists of additional compounds (e.g., phosphonates) [Clark *et al.*, 1998, 1999; Kolowitz *et al.*, 2001; Young and Ingall, 2010] and that these DOP compounds as well as DIP compounds other than orthophosphate (e.g., polyphosphate) may also be utilized by organisms [Dyhrman and Palenik, 1999; Ilikchyan *et al.*, 2009; Scanlan and Wilson, 1999; Van Mooy *et al.*, 2009] in processes that likely involve yet unknown isotopic fractionation. However, isotopic fractionation data is available for some of the most common enzymes (pyrophosphatase, alkaline phosphatase, 5' nucleotidase, and phosphodiesterase) and potential substrate groups (monoesters and diesters) in seawater [Azam *et al.*, 1983; Cotner and Biddanda, 2002; Paytan and McLaughlin, 2007; Young and Ingall, 2010].

[8] In this study, we use the  $\delta^{18}\text{O}_{\text{PO}_4}$  distribution in the water column in order to obtain quantitative estimates of the fraction of DIP that is regenerated from DOP by extracellular enzymes, and to describe how this fraction varies with depth. The fraction of DIP that originated from enzymatic hydrolysis of DOP represents the degree of DOP utilization by phytoplankton and bacteria. In addition, we determined the percent expression of AP activity using

**Table 1.** Field and Laboratory Results for Samples Collected at Stations 1–6

Station	Lon	Lat	Depth (m)	Temp (°C)	Salinity	$\delta^{18}\text{O}_p$ (‰)	Equilib $\delta^{18}\text{O}_p$ (‰)	Chl <i>a</i> (mg/L)	SRP ( $\mu\text{M}$ )	% ELF	Turnover (hrs)	% From DOP
1	74.5494	37.3814	5	9.39	34.57	20.7	23.7	1.54	0.36	38.4	-	22.6
			25	9.68	34.71	22.5	23.7	2.27	0.37	44.1	-	8.9
			50	10.40	35.05	22.9	23.5	1.15	0.62	62.0	-	4.5
			75	10.55	35.08	-	-	0.35	0.58	68.8	-	-
			125	10.66	35.15	21.0	23.4	0.26	0.80	-	-	18.1
2	64.3488	31.9812	175	10.65	35.16	23.7	23.4	0.22	0.85	-	-	BDL
			5	19.91	36.70	-	-	0.12	0.03	79.7	-	-
			20	19.86	36.70	-	-	-	0.03	97.4	-	-
			50	19.84	36.69	17.1	21.3	0.14	0.03	76.7	-	32.6
			100	19.84	36.69	-	-	-	0.02	75.3	-	-
			150	19.61	36.67	-	-	-	0.05	57.7	-	-
3	58.7219	26.3658	600	15.06	36.02	20.0	22.4	-	0.35	-	-	10.7
			950	7.65	35.14	24.0	24.1	-	0.31	-	-	0.7
			5	24.00	37.09	-	-	0.018	0.02	51.3	16	-
			50	22.71	37.09	-	-	-	0.02	49.4	6	-
			100	22.60	37.08	-	-	-	0.03	63.6	35	-
			120	22.62	37.09	16.7	20.6	0.02	0.03	57.6	45	31.1
			150	21.54	36.91	15.4	20.9	-	*0.07	41.5	160	43.2
4	53.5569	20.8913	250	18.70	36.60	20.8	21.6	-	*0.15	-	107	6.2
			500	15.54	36.12	21.0	22.3	-	*0.37	-	133	9.9
			5	25.80	37.17	-	-	0.04	0.01	0.0	-	-
			20	25.26	37.17	14.2	20.0	0.04	0.07	10.4	4	46.8
			50	25.24	37.17	-	-	-	0.07	10.5	3	-
			115	24.20	37.23	14.5	20.3	0.05	0.09	4.8	17	46.1
			140	22.34	37.07	-	-	-	-	2.8	4	-
			200	19.98	36.82	17.4	21.3	-	*0.08	-	18	30.4
5	50.8272	10.7355	500	13.08	35.77	23.1	22.9	-	-	-	49	BDL
			5	25.78	36.32	-	-	0.035	0.03	22.5	5	-
			25	25.76	36.33	-	-	-	0.08	49.2	6	-
			60	25.78	36.59	15.1	19.9	0.02	0.09	39.5	7	38.5
			90	25.87	37.00	13.8	19.9	0.02	0.07	35.4	5	49.0
			100	25.85	37.00	-	-	-	-	43.2	-	-
			130	22.81	37.00	-	-	-	0.10	31.9	6	-
			200	16.71	36.13	19.4	22.0	-	-	-	17	20.3
6	50.1644	10.7355	500	8.39	34.87	22.1	24.0	-	-	-	16	14.1
			5	26.18	36.40	-	-	0.13	0.01	26.0	7	-
			50	26.11	36.43	18.7	19.8	0.22	0.03	22.5	9	9.4
			75	26.12	36.44	-	-	0.21	0.07	36.8	18	-
			100	21.65	36.87	17.6	20.9	0.15	0.36	44.4	57	25.9
			150	14.84	35.80	19.3	22.5	0.03	0.50	-	19	24.4
			500	7.75	34.80	24.4	24.1	-	1.28	-	24	BDL
			1000	5.12	34.73	23.2	24.7	-	1.34	-	83	11.0
			3800	2.39	34.91	25.4	25.4	-	1.38	-	69	BDL
4200	2.28	34.90	24.7	25.4	-	1.35	-	100	5.1			

The dash indicates the parameter was not measured. Not all parameters were measured at all sites.

\*SRP data that measured at this station on Cruise X0804, 14 May 2008 (provided by M. Lomas)

Chl *a* and %ELF were only measured in the upper water column.

The equilibrium value was calculated using the following equation: equilibrium  $\delta^{18}\text{O}_{\text{PO}_4} = ((\text{Temperature} - 111.4)/(-4.3)) + \delta^{18}\text{O}_w$

% P Utilized from DOP based on hydrolysis of monoesters by AP. Additional calculation for % P utilized from DOP is given in Table 3.

enzyme-labeled fluorescence (ELF) and  $^{33}\text{P}$  uptake derived P turnover times as additional proxies for DOP utilization and DIP deficiency, respectively. The number of cells expressing ELF activity is expected to increase with increased utilization of DOP since AP is used for DOP hydrolysis.  $^{33}\text{P}$  turnover, a measure of the time it takes for the residence biomass to use all available DIP, is expected to be short if the DIP pool is small and effectively utilized. Collectively, these independent measurements can provide an appreciation for DIP availability compared to DIP demand by the biota, or a measure of DIP limitation. Analyses were made on samples from different depths in the ocean collected at sites with relatively high nutrient concentrations in near-shore environments as well as in open ocean oligotrophic environments.

## 2. Methods

### 2.1. Study Site

[9] We collected water samples from various depths in the water column at six stations along a transect from the shelf break off the coast of Virginia (USA) through the Sargasso Sea (Figure S1 in the supporting information) on March 2004. Profiles ranged in depth from 200 m (at the shelf break) to 4200 m at Station 6. Station 2 (31.9812°N, 64.3488°W) approximately coincides with the existing Bermuda Atlantic Time Series (BATS) Station (34.667°N, 64.167°W).

### 2.2. Sampling Approach and Sample Analyses

[10] Water samples were collected from Niskin bottles mounted to a 24-place rosette. Water samples at each depth

**Table 2.** Additional Measurements Relevant for P Dynamics Obtained in the Upper Water Column at Some Stations Where  $\delta^{18}\text{O}_{\text{PO}_4}$  Was Also Measured During This Cruise

Stn #	Depth (m)	Temp (°C)	Sal	SRP ( $\mu\text{M}$ )	TDP ( $\mu\text{M}$ )	Chl <i>a</i> ( $\text{mg m}^{-3}$ )	ELF (%)	Bulk APA ( $\text{nmol } \mu\text{gchl}^{-1} \text{h}^{-1}$ )	PO <sub>4</sub> Uptake ( $\text{nmol } \mu\text{gchl}^{-1} \text{h}^{-1}$ )	Turnover (h)	$\delta^{18}\text{O}_{\text{PO}_4}$ (‰)	$\delta^{18}\text{O}_{\text{PO}_4}$ (‰)	DOP Utilization (%)
1	5	9.39	34.60	0.36	-	1.54	38	-	-	-	20.7	23.7	22.6
2	50	19.85	36.69	0.03	0.20	0.14	77	17.6	25.0	-	17.1	21.3	32.5
3	120	22.62	37.09	0.03	-	0.02	57	83.9	61.1	45	16.7	20.6	31.1
4	20	25.26	37.24	0.07	0.17	0.14	50	6.5	154.9	4	14.2	20.0	46.8
5	60	25.78	36.59	0.09	0.21	0.21	39	-	74.4	7	15.1	19.9	38.4
6	100	21.65	36.59	0.36	-	0.15	44	3.1	69.5 and 43.8	57	17.6	20.9	25.7

DOP was determined from the difference between total dissolved P (TDP) and SRP.

Bulk APA = bulk AP activity from *Sohm and Capone* [2006] collected on the same cruise within the euphotic zone.

PO<sub>4</sub> uptake rate data from *Sohm and Capone* [2006] collected on the same cruise within the euphotic zone. PO<sub>4</sub> uptake at station 6 was measured twice.

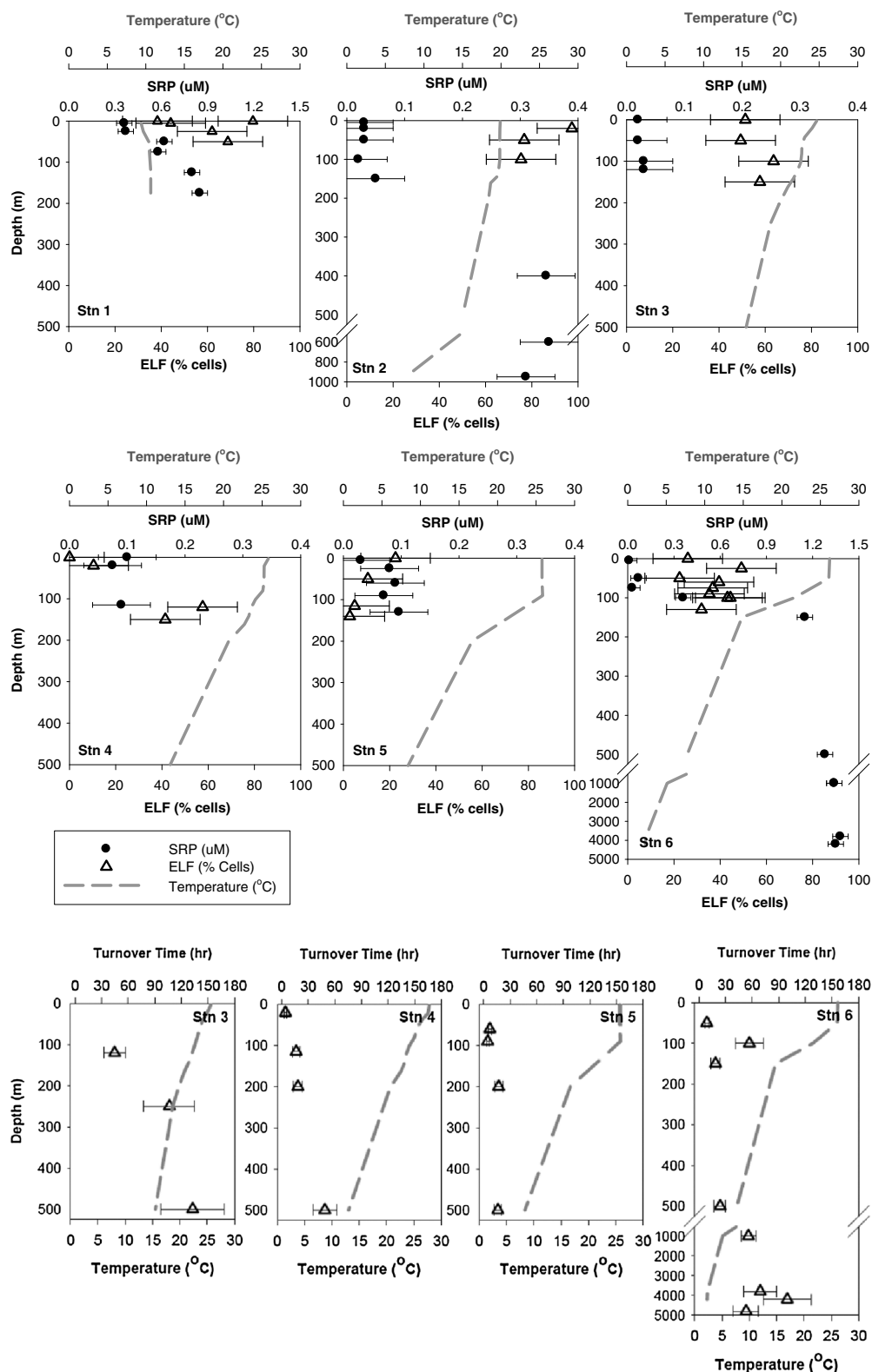
$\delta^{18}\text{O}_{\text{EOC}} = \delta^{18}\text{O}_{\text{PO}_4}$  expected for equilibrium with temperature and  $\delta^{18}\text{O}_{\text{w}}$  [Longinelli and Nuti, 1968]: equilibrium  $\delta^{18}\text{O}_{\text{P}} = ((\text{Temperature} - 111.4)/(-4.3)) + \delta^{18}\text{O}_{\text{w}}$ .

% P Utilized from DOP based on hydrolysis of monoesters by AP see text and Table 3 for additional calculations.

were analyzed for  $\delta^{18}\text{O}_{\text{PO}_4}$  and soluble reactive phosphate (SRP) concentration. SRP is defined as the dissolved P fraction that reacts in an acid solution containing molybdate ions to form a phosphomolybdate complex which when reduced with ascorbic acid forms a colored molybdenum blue complex. This fraction in seawater is composed primarily of DIP, but it may also include some easily hydrolyzable inorganic and organic forms of P [Monaghem and Ruttenberg, 1999]. Surface water samples from some stations were also analyzed for total dissolved phosphate (TDP) concentrations, chlorophyll *a*, and % ELF. Phosphorus turnover time was assessed using samples from Stations 3 through 6.

[11] Approximately 50 mL of water was collected for SRP analysis. These samples were concentrated by the Magnesium-Induced Coprecipitation (MAGIC) method of Karl and Tien [Karl and Tien, 1992] and analyzed on an Alpkem autoanalyzer. To reduce the interference from arsenate, absorbance was read within 10 min of adding the mixed reagent, reducing any arsenate interference to less than 4% because reduction of the arsenomolybdate complex is much slower than that of phosphomolybdate [Johnson, 1971] (e.g., at a typical arsenate concentration of 15 nmol/L, the maximum interference would be 0.6 nmol/L that is below the method's detection limit of 0.5 nmol/L for a 50 mL sample). TDP was quantified using the high temperature/acid persulfate oxidation technique and DOP determined by subtracting SRP from TDP [Lomas *et al.*, 2010]. Surface waters (1 L) were collected for alkaline phosphatase (AP) enzyme-labeled fluorescence (ELF) [Dyhrman and Palenik, 1999]. This involved collecting plankton samples on a 0.22  $\mu\text{m}$  filter (by low-vacuum filtration; <15 kPa), resuspending the sample in an ethanol solution, adding the label (ELF-97), and transferring the sample to an Epi tube. Samples are stored in the dark at 4°C until analysis. Cell counts are performed using a Nikon epifluorescent microscope (DAPI filter set, excitation at 350 nm, and maximum ELF emission at 520 nm) to estimate ELF labeling as well as with standard illumination. Slides are scanned, and each identifiable cell is tallied as either positive or negative for ELF labeling, a positive tally is given to any cell that had a considerable amount (>10% of cell area) of visible fluorescent ELF labeling. At least 300 individual cells were tallied for each sample and fraction of cells labeled from total number counted determined. We note that the ELF procedure is not a quantitative measure of enzyme activity but rather an account of the percentage of cells that have expressed the enzyme over the past week or two [Dyhrman and Palenik, 1999; Lomas *et al.*, 2004].

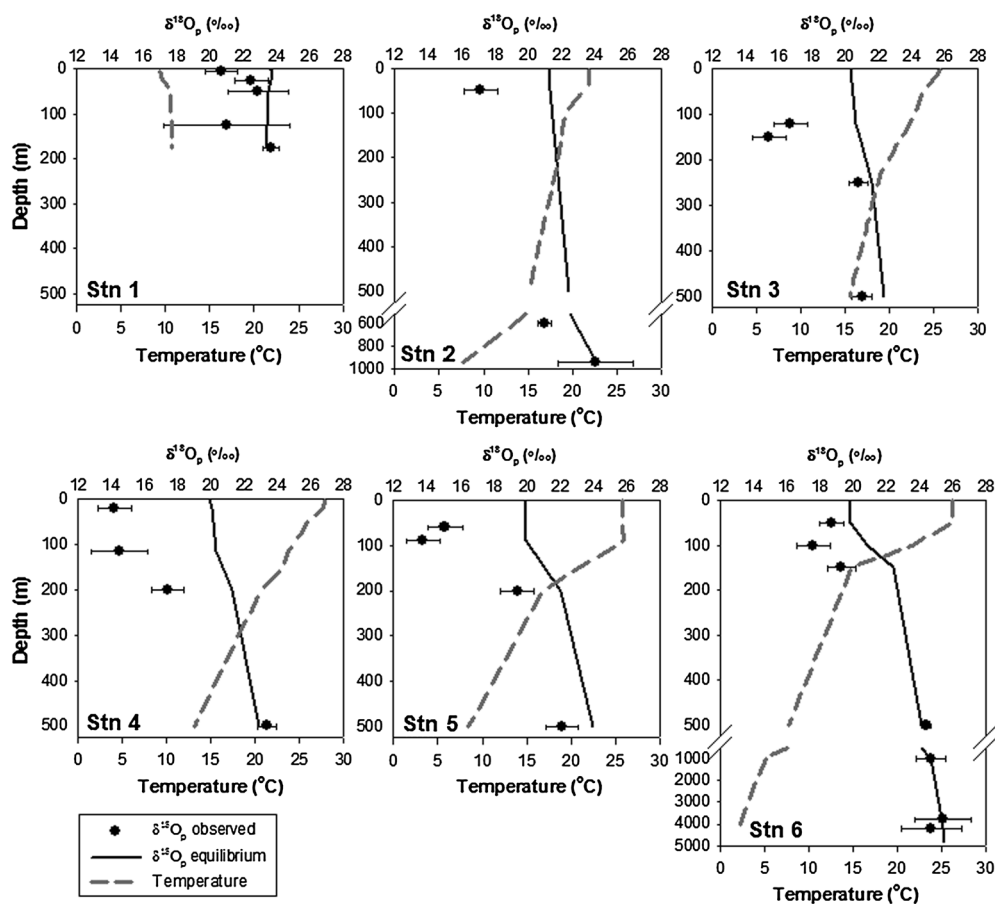
[12] Approximately 40 L of water was collected from each depth for oxygen isotope analysis of phosphate ( $\delta^{18}\text{O}_{\text{PO}_4}$ ) in high density polyethylene (HDPE) acid washed, sample rinsed Jerrycans (two 25 L containers per depth). DIP was stripped from seawater using the MAGIC procedure by adding 200 mL of 1 mol L<sup>-1</sup> sodium hydroxide (Fisher Scientific, American Chemical Society grade NaOH pellets) to each Jerrycan immediately after collection and rigorously shaking [Thomson-Bulldis and Karl, 1998]. Because two Jerrycans were collected per depth, each container was treated as a separate sample for the initial precipitation. The magnesium hydroxide floc was allowed to settle in each container for 2 h in a cold room (4°C) before supernatant was siphoned off, leaving approximately 5 L of floc in



**Figure 1.** (a) Depth profiles of SRP concentration and percent cells labeled for AP expression based on the ELF labeling method. (b) Turnover times and temperature profiles measured at stations 3–6.

seawater. The floc from both Jerrycans was then combined into a single container and floc was allowed to continue to settle. After another hour, additional supernatant was siphoned off leaving approximately 1–2 L of floc in

seawater. This was then stored in a 2 L HDPE bottle and frozen until analysis for  $\delta^{18}\text{O}_{\text{PO}_4}$  [McLaughlin *et al.*, 2004]. The SRP recovery in the floc was 100% based on analysis of SRP in the supernatant after floc formation; any DOP



**Figure 2.** Depth profiles of  $\delta^{18}\text{O}_{\text{PO}_4}$  along with the calculated equilibrium values and temperature. The solid line represents the expected equilibrium  $\delta^{18}\text{O}_{\text{PO}_4}$  calculate based on the oxygen isotope value of seawater and the temperature at the respective depth using the equation for equilibrium [Longinelli and Nuti, 1968].

collected in the MAGIC step was removed in the following purification steps that include precipitation of cerium phosphate, cation exchange, and a final precipitation as silver phosphate [Paytan and McLaughlin, 2011]. Phosphate blanks for all reagents used in the process as well as whole procedure blanks were measured and at no time were above detection limits or yielded any amount of silver phosphate [McLaughlin et al., 2004 and Figure S2]. Isotopic analyses were conducted on a Eurovector Elemental Analyzer coupled to a mass spectrometer at the U.S. Geological Survey in Menlo Park, California. Two calibrated (via fluorination by Dr. Torsten Vennemann, at the Universität Tübingen, see McLaughlin et al. [2004] for details) internal silver phosphate standards, STDH ( $\delta^{18}\text{O}_{\text{PO}_4} = 19.8\text{‰}$ ) and STDL ( $\delta^{18}\text{O}_{\text{PO}_4} = 11.3\text{‰}$ ), were analyzed throughout each mass spectrometer run for calibration and drift correction. Results from field replicate analyses of water samples (collected at the same depth and time thus including natural variability and processing reproducibility) were typically identical within analytical uncertainty ( $\pm 0.3\text{‰}$ ), although for one sample, these replicates differed by 3‰. Water samples were also collected in 20 mL HDPE-depressed cap scintillation vials with no headspace for  $\delta^{18}\text{O}_{\text{w}}$  analysis.  $\delta^{18}\text{O}_{\text{w}}$  was determined using a Finnigan MAT 251 mass spectrometer also at the U.S. Geological Survey in Menlo Park.  $\delta^{18}\text{O}_{\text{PO}_4}$  isotopic values defined as the ratio of  $^{18}\text{O}/^{16}\text{O}$  in phosphate and water oxygen

isotope values are reported in standard delta notation ( $\delta^{18}\text{O}$ ) relative to Vienna standard mean ocean water.

[13] Phosphate turnover times were measured following methods described in Sohm and Capone [2010]. Duplicate 50 mL samples of seawater were placed in 60 mL acid washed polycarbonate bottles with 0.5–2  $\mu\text{Ci}$  of  $\text{H}_3^{33}\text{PO}_4^{3-}$  and incubated in 40% light or in a dark incubator (samples > 200 m) on deck or in the dark at 4°C in an incubator (samples > 1000 m) for 60–90 min [Sohm and Capone, 2010]. Samples were collected at fixed intervals, and only samples within the linear range of increase of isotope in the cell were used for calculating an instantaneous uptake rate. Incubated samples were filtered onto 0.2  $\mu\text{m}$  polycarbonate filters placed into 6 mL plastic scintillation vials which were rinsed with filtered seawater. To control for abiological adsorption, control samples amended with glutaraldehyde were also incubated. Activity of  $^{33}\text{P}$  was measured in a scintillation counter after addition of 5 mL of scintillation cocktail. The turnover time of the phosphate pool in a sample is calculated as  $T = R_i \times t / (R_f - R_k)$ , where  $t$  is the incubation time and  $R_i$ ,  $R_f$ , and  $R_k$  are the radioactivity (in counts per minute) of the total pool added, the filter, and the killed control, respectively. SRP uptake rates were measured for several of the samples [reported by Sohm and Capone, 2006] and were normalized to chlorophyll *a* (Chl *a*) concentration ( $\text{mg chl m}^{-3}$ ). Chl *a* was measured by a modified fluorometric procedure in which water is filtered

**Table 3.** % DIP from DOP Hydrolysis for Different Combinations of Substrates and Enzymes

Station	Depth (m)	Mid Range Estimate		High Estimate		RNA		Low Estimate		Error (%)
		Monoesters	Alk-P	Monoesters	5'nucleo	Alk-P	5'nucleo	DNA	Alk-P	
1	5	22.6		35.6		32.1	20.9	15.5	12.3	5.25
	25	8.9		14.2		12.8	8.4	6.2	4.9	5.25
	50	4.5		7.2		6.5	4.2	3.12	2.5	9.25
	125	18.1		28.7		26.1	17.0	12.5	9.9	19.25
	175	BDL		BDL		BDL	BDL	BDL	BDL	-
2	50	32.6		53.4		51.3	31.8	23.0	18.1	5.25
	600	10.7		17.3		16.1	10.2	7.5	5.9	2.25
	950	0.7		0.8		0.7	0.5	0.4	0.3	11.5
3	120	31.1		51.0		50.0	30.5	21.9	17.1	5.25
	150	43.2		71.2		69.2	42.5	30.6	24.0	5.25
	250	6.2		10.1		9.6	6.0	4.4	3.4	3.25
	500	9.9		16.1		15.0	9.5	7.0	5.5	3.45
4	20	46.8		77.3		77.3	46.4	33.1	25.8	5.25
	115	46.1		76.6		75.8	45.9	32.9	25.6	8.75
	200	30.4		49.8		47.9	29.7	21.5	16.9	5.25
	500	BDL		BDL		BDL	BDL	BDL	BDL	-
5	60	38.5		64.2		64.4	38.6	27.5	21.4	5.25
	90	49.0		81.6		81.9	49.0	35.0	27.2	5.25
	200	20.3		32.5		30.6	19.3	14.1	11.1	5.25
	500	14.1		22.4		20.0	13.1	9.7	7.8	5.25
6	50	9.4		14.8		14.9	8.9	6.3	4.9	3.75
	100	25.9		42.7		41.5	25.5	18.4	14.4	5.25
	150	24.4		39.4		36.6	23.3	17.1	13.5	4.4
	500	BDL		BDL		BDL	BDL	BDL	BDL	-
	1000	11.0		17.3		15.2	10.1	7.6	6.0	4.65
	3800	BDL		BDL		BDL	BDL	BDL	BDL	-
	4200	5.1		7.9		6.9	4.6	3.5	2.8	9.25

We assume that both DIP and DOP from cell lyses or excretion will have an equilibrium signature (equilibrium isotope value associated with pyrophosphatase P cycling at in situ temperature and  $\delta^{18}\text{O}_w$ ). During our cruise, equilibrium values range from +19.8‰ (surface) to +25.5‰ (deep water).

Error (%) was calculated based on the error associated from repeat mass spectrometer analysis of the isotope value for each specific DIP sample collected and is therefore the same for all percent DOP utilized calculations that are based on the same measured  $\delta^{18}\text{O}_{\text{PO}_4}$  (e.g., the same sample). The errors for the isotope analyses ranged from  $\pm 0.3\%$  to  $\pm 3\%$ . These errors represent analytical error and natural variability.

BDL = below detection limits. – Assigned when the measured and calculated equilibrium isotope values were identical within analytical error (0.3%)

Equations used for calculating the isotope mass balance:

Kinetic fractionation P monoesters hydrolysis by Alk-Pase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.75 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w - 30))$$

Kinetic fractionation P monoesters hydrolysis by 5'Nase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.75 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w - 10))$$

Kinetic fractionation RNA hydrolysis by PDase+Alk-Pase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.50 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w + 20)) + (0.25 \times (\delta^{18}\text{O}_w - 30))$$

Kinetic fractionation RNA hydrolysis by PDase+5'Nase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.50 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w + 20)) + (0.25 \times (\delta^{18}\text{O}_w - 10))$$

Kinetic fractionation DNA hydrolysis by PDase+Alk-Pase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.50 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w - 20)) + (0.25 \times (\delta^{18}\text{O}_w - 30))$$

Kinetic fractionation DNA hydrolysis by PDase+5'Nase

$$\delta^{18}\text{O}_{\text{PO}_4} = (0.50 \times \delta^{18}\text{O}_{\text{PO}_4 \text{ equil.}}) + (0.25 \times (\delta^{18}\text{O}_w - 20)) + (0.25 \times (\delta^{18}\text{O}_w - 10))$$

through 25 mm Whatmann GF/F filters, filters were extracted in 90% acetone in a freezer overnight, and Chl *a* is measured using a Turner Designs Model-10 fluorometer calibrated with a commercial Chl *a* standard. Turnover times are defined as the length of time that would be required for the resident biota to uptake and utilize all of the SRP in each sample (hours) and SRP uptake rates represent the amount of SRP utilized per unit time by the resident biomass (represented by Chl *a* concentrations) ( $\text{nmol SRP } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ ). The average coefficient of variation associated with the turnover time calculations based on analysis of replicates was 27%. For more detail, see Sohm and Capone [Sohm and Capone, 2006].

### 3. Results

[14] Samples were collected at six sites representing different oceanic settings from coastal to open ocean

(Figure S1). Data (temperature, salinity,  $\delta^{18}\text{O}_{\text{PO}_4}$  and calculated expected equilibrium  $\delta^{18}\text{O}_{\text{PO}_4}$  values, Chl *a*, SRP concentrations, % ELF labeling, and turnover time) for each discrete sample collected at different depths in each station are summarized in Table 1. Table 2 provides additional data relevant for P dynamics that was collected on the same cruise for only some of the surface samples for which  $\delta^{18}\text{O}_{\text{PO}_4}$  was measured (DOP, bulk AP activity, and phosphate uptake rates).  $\delta^{18}\text{O}_{\text{PO}_4}$  ranged from 14 to 26‰, % ELF labeling ranged from less than 5% to close to 100% of cells labeled and turnover rates ranged from 4 h to over 6 days. DIP uptake rates in the euphotic zone differed at different sites (between 25 and 150  $\text{nmol P } \mu\text{g chl }^{-1} \text{ h}^{-1}$ ). DOP concentrations in the euphotic zone, in the samples where DOP was measured, were higher than SRP concentrations (Table 2) consistent with long term data collected in the region (Figure 3). SRP concentrations ranged from



0.02 to 1.34  $\mu\text{M}$  (Table 1). Depth trends in SRP concentrations and % ELF labeling, and turnover time are shown in Figures 1a and 1b, respectively. Turnover time and SRP concentration were significantly, positively correlated with depth (Spearman's  $\rho$ ,  $p < 0.0001$ , and  $p = 0.0008$ , respectively), though percent of cells with ELF labeling was not significantly correlated with depth; however, ELF was only measured in the upper water column. Both SRP and % ELF-labeled cells were significantly positively correlated with turnover times (Spearman's  $\rho$ ,  $p = 0.0108$ , and  $p = 0.0371$ , respectively, Table S1). Figure 2 shows the measured  $\delta^{18}\text{O}_{\text{PO}_4}$  and the expected equilibrium  $\delta^{18}\text{O}_{\text{PO}_4}$  (calculated based on water temperature and  $\delta^{18}\text{O}_{\text{w}}$ ) for the various stations as well as temperature distribution with depth. Samples in the surface ocean show large offsets from expected equilibrium values (toward lower than equilibrium values), while the differences are small at depth (Figure 2). The calculated percent DIP that has been regenerated from DOP for each sample, using different combinations of enzymes and substrate types is given in Table 3, and the depth distribution of that fraction, at all the stations, for the range of possible end-member solutions is shown in Figure 3. The data illustrates that percent DIP that originates from extracellular remineralization of DOP was generally high in the surface ocean and decreases with depth in the water column (Spearman's  $\rho$ ,  $p = 0.0132$ , using the mid-range values). Percent of DIP from remineralized DOP was also significantly, negatively correlated with percent of ELF-labeled cells, and SRP (Spearman's  $\rho$ ,  $p = 0.0347$ , and  $p < 0.001$ , respectively, using the mid-range values). It is important to note that the calculated percent DIP that has been regenerated from DOP based on the mass balance isotope model used does not account for any phosphate that has been initially derived from DOP but has then achieved equilibrium within cells and was subsequently released to the environment. Rather, our calculation provides a minimum estimate of the fraction of phosphate derived from DOP. It is an estimate of what fraction of the standing stock of phosphate still carries a signature of its DOP origin and hydrolysis by extracellular enzymes.

#### 4. Discussion

[15] The percentage of eukaryote cells expressing AP activity (as determined based on ELF labeling) in surface waters at all stations is high (up to 80%), and within the range reported by Lomas et al. (30–70%) for this region [Lomas et al., 2004]. The high expression of AP is also consistent with the observations of Mather et al. [2008], who found widespread AP activity in the North Atlantic. The ELF-based AP expression, which documents the percentage of cells expressing AP activity and not a cell-specific AP activity rate, however, is variable among sites, and no consistent relation between SRP concentrations and % ELF labeling is observed ( $p = 0.8538$ , Spearman's  $\rho$ ) (Figure 1A, Table 1, and Table S1). The lack of strong correspondence between % ELF labeling and SRP concentrations is not surprising because the capacity for phosphomonoester hydrolysis, and thus AP activity is highly variable between taxa and the phytoplankton composition varies considerably between stations and even with depth at any location [Ruttenberg and Dyrman, 2005; Dyrman and Ruttenberg, 2006; Nicholson et al., 2006; Mackey et al., 2007]. In the

absence of regulation information provided by culture studies and accounting for phytoplankton community change, the presence of AP activity cannot be unequivocally interpreted as evidence for P stress or P limitation, and simple correspondence to SRP is not expected [Dyrman and Ruttenberg, 2006]. Bulk AP activities in the euphotic zone reached 80  $\text{nmol } \mu\text{g chl } a^{-1} \text{ h}^{-1}$  (volumetric rate 2.13  $\text{nmol } \text{h}^{-1}$ ) (Table 1) [Sohm and Capone, 2006]. While comparison of AP activity data reported in other studies is not trivial because measured rates depend on the method used and different studies normalize the activity differently (e.g., activity per hour, per liter or per mass of C, or per number of cells), the activity in our samples was comparable to the high range of rates reported for the North Atlantic Subtropical Gyre (42–122  $\text{nmol } \mu\text{g chl } a^{-1} \text{ h}^{-1}$  in Spring and Autumn, respectively [Mather et al., 2008]). Similar values were also reported for the Sargasso Sea (maximum 70  $\text{nmol } \text{g chl } a^{-1} \text{ h}^{-1}$  [Lomas et al., 2010]). The prominent ELF labeling and AP activity found in our samples suggest utilization of the DOP pool by phytoplankton and bacteria, likely in response to the relatively low concentration of DIP in the euphotic zone (Figure 1A and Table 1). DOP concentrations were not measured for most samples collected in this cruise; however, data by M. Lomas at Station 2 [Figure S3; Bermuda Atlantic Time Series Station, Lomas et al., 2010] and data from Sohm and Capone [Sohm and Capone, 2006, 2010] at our station and several other stations in the area, as well as other published data in similar systems, indicate that DOP concentrations are consistently highest in surface waters (40–60 nM) and decrease with depth (<20 nM) [Hopkinson and Vallino, 2005; Van Mooy et al., 2009; Wu et al., 2000]. Specifically, the DOP concentrations in the upper 100 m of the water column in the Sargasso Sea are typically higher than SRP concentrations [Cavender-Bares et al., 2001; Lomas et al., 2010] and if utilized can constitute an important P source for phytoplankton and bacteria in the euphotic zone (Figure S3).

[16] Turnover times derived from  $^{33}\text{P}$  uptake are most rapid in the surface layer (typically 4–8 h, except at 5 m in Station 3) and increase with depth reaching up to 6 days in the deep water samples from Station 6 (Figure 1B, Tables 1, and 2). These turnover times are comparable to the shortest turnover rates recorded in the North Atlantic surface waters measured using the same methods (2–159 h) [Sohm and Capone, 2010] and to data from the Sargasso Sea (BATS, 9 h) [Cotner et al., 1997]. These rates are very short compared to estimates from surface water in other ocean basins (1–30 days) [see summary and discussion in Sohm and Capone, 2010]. Short turnover times generally indicate greater demand for DIP by the resident biomass, particularly in our samples where Chlorophyll *a* is fairly constant between stations (except for station 1), and thus potentially suggest greater deficiency [Zohary and Roberts, 1998; Flaten et al., 2005; Sohm and Capone, 2010]. Phosphate uptake reached rates of over 150  $\text{nmol } \mu\text{g chl } a^{-1} \text{ h}^{-1}$  in the euphotic zone at the time of our sampling (25–155  $\text{nmol } \mu\text{g chl } a^{-1} \text{ h}^{-1}$ ) (Table 2) [Sohm and Capone, 2006] These data are consistent with similar measurements in this region obtained at different times [Sohm and Capone, 2010]. Comparison of DIP turnover times and nutrient addition bioassays in the P limited Eastern Mediterranean Sea suggest that turnover times of less than 7 h indicate DIP deficiency [Zohary and

Roberts, 1998]. The near surface turnover times seen in this study are comparable to values seen in the DIP-limited area of the Eastern Mediterranean (2–7 h) [Zohary and Roberts, 1998; Flaten et al., 2005] and suggest low DIP availability compared to demand in the surface waters of the Sargasso Sea. These data are consistent with other reports for the region [Cotner et al., 1997; Ammerman et al., 2003; Van Mooy et al., 2009] and particularly consistent with data from Sohm and Capone [2010] for the Western Atlantic. Under low DIP concentration, it is likely that the more abundant DOP pool may be utilized to supplement cellular P needs. Hydrolysis of DOP throughout the euphotic zone by extracellular enzymes such as AP is also consistent with the presence of ELF expression and the high AP activities seen in our samples.

[17] Observed  $\delta^{18}\text{O}_{\text{PO}_4}$  values in the upper 200 m of the water column at most of the stations are lower than expected equilibrium values calculated based on the temperature and  $\delta^{18}\text{O}_w$  for each sample (Figure 2). This distribution implies that isotopic fractionation associated with extracellular enzyme hydrolysis which imparts a negative fractionation at the PO bond site, shifting the product (phosphate) isotopic composition toward lower than equilibrium values [Paytan

temperature, algal species, or nutritional state [Paytan et al., 2002]. We calculate a range of possible solutions for the fraction of phosphate in a water sample derived from extracellular DOP regeneration, by solving simple mass balance equations using different possible DOP substrate and enzyme combinations, and the reported isotope fractionation associated with each combination (Table 3). Each of the equations provides a solution for an end-member scenario which presumes that the whole DOP pool is composed of one substrate type (monoesters, DNA, and RNA) which is hydrolyzed by one set of enzymes (PDase, Alk-Pase, and 5'Nase) with the respective associated fractionations. The fractionation is applied to the number of oxygen atoms involved in the reaction (one for monoesters and two for diesters), the other oxygen atoms do not change (e.g., retain the equilibrium value). The equations indicate that the  $\delta^{18}\text{O}_{\text{PO}_4}$  values of the resulting DIP (the product of DOP remineralization) will be lower than the original equilibrium isotope value of the DOP, with variable offsets depending on the substrate and enzymes involved. The fraction of phosphate regenerated from DOP is then calculated from the measured  $\delta^{18}\text{O}_{\text{PO}_4}$  and the  $\delta^{18}\text{O}_{\text{PO}_4}$  values calculated from each equation as follows:

$$\% \text{ DOP utilized} = \frac{(\text{observed } \delta^{18}\text{O}_{\text{PO}_4} \text{ values} - \text{equilibrium } \delta^{18}\text{O}_{\text{PO}_4} \text{ values}) * 100}{(\text{calculated remineralized } \delta^{18}\text{O}_{\text{PO}_4} \text{ values} - \text{equilibrium } \delta^{18}\text{O}_{\text{PO}_4} \text{ values})}.$$

and McLaughlin 2011]. The thermocline is at ~50 m near the coastline (Station 1) and at ~150 m offshore in the Sargasso Sea (Stations 2 through 6). Below the thermocline,  $\delta^{18}\text{O}_{\text{PO}_4}$  approaches equilibrium values, similar to observations made by others [Colman et al., 2005]. The trend toward approaching isotopic equilibrium with depth corresponds to an increase in SRP concentration and longer phosphate turnover time (Figures 1, 2, and Table 1). The general trend in the open ocean sites of lower than equilibrium  $\delta^{18}\text{O}_{\text{PO}_4}$  values and short turnover times to coincide with low SRP concentrations is consistent with the hypothesis that in the absence of sufficient DIP, the biological communities utilize labile DOP. This hypothesis is also consistent with the relatively high alkaline phosphatase activity and the percent of cells showing ELF labeling in the waters we sampled throughout the euphotic zone (e.g., reported in Tables 1 and 2).

[18] Based on our knowledge of the fractionation associated with enzyme-mediated DOP hydrolysis [Liang and Blake, 2006, 2009], and the deviation from equilibrium of  $\delta^{18}\text{O}_{\text{PO}_4}$  in the water column, we used an isotope mass balance model to estimate the fraction of DIP derived from DOP remineralization. To obtain an estimate for the fraction of phosphate regenerated from DOP, we assume that the  $\delta^{18}\text{O}_{\text{PO}_4}$  of DOP is in isotopic equilibrium with ambient water; this is supported by  $\delta^{18}\text{O}_{\text{PO}_4}$  of living and sinking cells [Paytan et al., 2002], and the rapid and extensive turnover of P within cells [Blake et al., 2005]. Existing data on the  $\delta^{18}\text{O}_{\text{PO}_4}$  of cellular phosphate while limited suggest that the  $\delta^{18}\text{O}_{\text{PO}_4}$  signature of living and sinking cells is at or close to equilibrium regardless of growth rate,

[19] Notably, any phosphate that originated from DOP, was hydrolyzed, taken up by organisms, processed in the cells (by pyrophosphatase), and subsequently released to the environment would have an isotope value that is at or close to equilibrium, thus our calculation provides only a minimum estimate of fraction of DIP that originated from extracellular DOP hydrolysis. We realize that DOP is a chemically heterogeneous pool with many compounds, and that different enzymes are involved in the regeneration of DOP. Without precise knowledge of the molecular characterization of the DOP pool, and all the active enzymes, and the respective fractionation factors associated with each of the enzymes, it is impossible to calculate exact values for the fraction of DIP regenerated from DOP. However, the enzymes (PDase, Alk-Pase, and 5'Nase) and substrates (monoesters and diesters) for which the fractionation was characterized are abundant in the water column [Cotner and Biddanda, 2002; Kolowith et al., 2001; Young and Ingall, 2010; Duhamel et al., 2011 and references therein], and our calculations should provide first-order estimates of the fraction of the DOP pool that appears to be turning over. We expect that the actual regenerated DOP fraction (which is an estimate of the DOP utilization rate) is within the range values obtained from the end-member calculations (reported in Table 3) or even higher since we do not account for DIP that originated from DOP and was released after being processed within cells achieving an equilibrium isotope value. Moreover, our calculations do not take into account the dilution effect of DIP that originated from upwelling and typically will have a higher isotopic signature than that

of DIP in equilibrium with surface water due to the colder temperatures at depth, potentially reducing the effect of the disequilibrium fractionation.

[20] Our calculations indicate that DOP utilization can account for 5–80% of the observed DIP in the oligotrophic surface water column (e.g., Stations 2–5) depending on the substrate and enzyme combination used (Table 3). The estimate using monoesters and AP which is in the middle of the range of values is approaching 50% at these stations. Closer to shore (Station 1) DOP remineralization typically accounts for less than 20% of DIP (range 12–35% depending on substrate-enzyme combination) (Table 3). Calculations using phosphodiester RNA as substrate and the combination of phosphodiesterase and AP will give values similar to those with monoesters and AP. Using monoesters as substrate and the 5' nucleotidase enzyme, or a diester RNA substrate and the combination of phosphodiesterase and 5' nucleotidase enzymes will result in a higher percent of DOP utilized (results shown in Table 3 as “high estimate” values). If on the other hand all of the DOP is in the form of phosphodiester DNA, then the lower fractions of DOP utilized are obtained, with the minimum value calculated for the phosphodiester DNA substrate hydrolysis with phosphodiesterase and AP enzymes (results shown in Table 3 as “low estimate” values). Importantly, the isotope mass balance calculations we use do not require knowledge of absolute fluxes or concentrations of the DIP or DOP pools as only the relative (percent or fraction) of DIP in the water column that originated from remineralized DOP is determined. The DIP fraction that is not from DOP regeneration (e.g., the fraction that is in equilibrium with seawater) is either released from cells or expelled during cell lysis, or it could also be “new” DIP from external sources (upwelling, riverine, or atmospheric deposition).

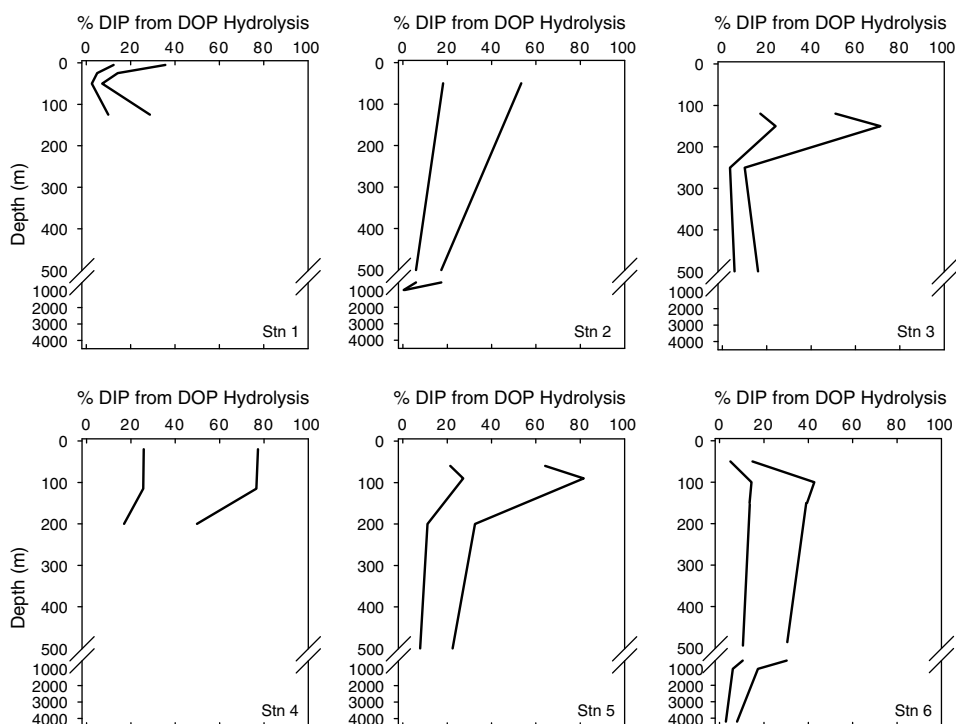
[21] While the data is limited (~10 data points collected during one cruise), with the exception of Station 1, the offsets from equilibrium in the upper water column (<200 m) do not seem to vary systematically between stations. This may indicate that the whole area (e.g., between 10° and 30° latitude in the North Pacific) represents a similar oceanographic regime in terms of P dynamics. Indeed, DOP and SRP concentrations which may be expected to affect the degree of P regeneration from DOP and subsequent utilization do not vary much between these stations [Lomas *et al.*, 2010; Sohm and Capone, 2006]. P uptake rates were also relatively similar within these latitudes in the Western Atlantic [Sohm and Capone, 2010]. Interestingly, the offset at stations 2 and 6, at the edges of the zone, are slightly lower than in the center (stations 3–5), though statistical evaluation of these patterns is not possible due to the limited data. During the time of our sampling (March 2004), Sohm and Capone [2006] measured slightly higher Chlorophyll *a* levels in the surface water of stations 2 and 6 (~0.12 compared to 0.018  $\mu\text{g L}^{-1}$  at the other sites). If the higher Chlorophyll *a* is indicative also of higher biomass, then this may reflect higher nutrient availability in the preceding few days that supported the biomass and possibly lower demand for DOP as a source of phosphate. The  $\delta^{18}\text{O}_{\text{PO}_4}$  signature represents conditions that are integrated over the residence time of DIP in the water sampled. Time series data may further illuminate and verify or dismiss this tentative observation.

[22] Our results suggest that a significant fraction of DIP utilized in this area originates from DOP, and this implies strong coupling between P uptake and regeneration processes in the surface waters of this region. Specifically, since a significant fraction of DIP has originated from DOP hydrolysis, as implied by the isotope data, then this would indicate that enzymes are produced by organisms to hydrolyze the DOP, most likely for uptake and utilization of the phosphate released. The evidence for DOP regeneration (e.g., lower than equilibrium isotope ratios and expressed enzyme activities) along with the low SRP standing stock and lack of SRP accumulation in the water column and the fast turnover rates indicate that the DIP produced by hydrolysis is indeed utilized. Thus, it seems logical to conclude that the regeneration and uptake are coupled. This is expected for regions where DIP stress may be encountered, such as has been suggested for this region of the Atlantic Ocean [Sohm and Capone 2006, and references therein].

[23] The degree of deviation from equilibrium is related to the ratio of extracellular regeneration of DOP to phosphate in isotopic equilibrium released from cells. Thus, the  $\delta^{18}\text{O}_{\text{PO}_4}$  values in the surface waters consistently deviating from equilibrium toward lower values indicates significant utilization of the DOP pool. We conclude that the DIP available in the water column in this region does not satisfy the demand or needs of the biota, although this does not necessarily imply physiological limitation (cellular stress) because the required P is obtained from DOP regeneration (an energetically costly process that involves synthesis of enzymes).

[24] The depth trends in the percent DIP that originated from DOP remineralization (which should be proportional to DOP utilization) at the various stations, based on calculations using a range of possible enzymes shows a decrease in percent DOP utilization with depth (Figure 3, Table 3, and Table S1). Below ~200 m  $\delta^{18}\text{O}_{\text{PO}_4}$  values are closer to equilibrium, DIP concentrations are higher, and turnover times are longer. These results indicate that below the euphotic zone utilization of DOP is limited, though it does not stop completely, as also noted by Colman *et al.* [2005]. The offset from equilibrium at depth was interpreted as resulting from contribution of DIP from metabolism of sinking particulate matter by heterotrophic bacteria in the deep ocean using hydrolytic phosphoenzymes such as AP. Because of the high DIP concentrations in the deep ocean, it has been speculated that remineralization of organic P compounds occurs to meet organic C (or N) to sustain metabolic needs and not to supplement P requirements [Hoppe and Ullrich 1999; Van Wambeke *et al.*, 2002]. Additional processes can also contribute to the small offset observed.

[25] The  $\delta^{18}\text{O}_{\text{PO}_4}$  values of the deep water DIP pool may shed light on the relative contribution of different sources and cycling processes of DIP in deep water. Specifically, three processes can be considered (1) remineralization of sinking particulate organic matter from the surface waters using extracellular enzymes which would tend to shift  $\delta^{18}\text{O}_{\text{PO}_4}$  toward lower than equilibrium values, (2) DIP processed using pyrophosphatase by deep heterotrophic organisms and released to seawater, shifting  $\delta^{18}\text{O}_{\text{PO}_4}$  values toward equilibrium, and (3) preformed phosphate which will carry  $\delta^{18}\text{O}_{\text{PO}_4}$  signatures of the DIP pool at the site of deep water formation (e.g., high latitudes). Since deep ocean values approach the equilibrium isotopic composition,



**Figure 3.** Percentage of phosphate in the water column that was regenerated from DOP utilization for all stations as a function of depth. Data from Table 3, the two profiles represent the highest and lowest calculations, and the actual value is likely between the two extreme depth trends presented in the figure.

we can infer that extracellular remineralization rates (nonequilibrium processes) are less important compared to hydrolysis by pyrophosphatase (which imparts an equilibrium isotopic signature). Our turnover time data (e.g., turnover time 50–100 h, Table 1) indicates that DIP is taken up by the biota at much higher rates than the residence time of phosphate in the deep ocean, suggesting that the equilibrium values observed are at least in part achieved by microbial pyrophosphatase activity. However, the limited deep water data preclude an exhaustive interpretation of these processes.

[26] To provide a general (order of magnitude) estimate of the contribution of DOP to primary productivity in the Sargasso Sea, we estimate the fraction of primary production supported by DIP originating from DOP remineralization. This simple calculation assumes that DIP that originated from DOP is utilized by phytoplankton to support primary production. Net primary production in the Sargasso Sea, estimated by various transient tracer distributions, is approximately  $4.0 \text{ mol C m}^{-2} \text{ yr}^{-1}$  which, using the Redfield ratio of C:N:P=106:16:1, is equivalent to  $0.038 \text{ mol P m}^{-2} \text{ yr}^{-1}$  [McGillicuddy and Robinson, 1997]. Our  $\delta^{18}\text{O}_{\text{PO}_4}$  values indicate that the percent DOP utilized in surface waters (<200 m) for all stations in the Sargasso Sea (Stations 2–4) ranges between 18% and 77% depending on what enzyme substrate combination is used (average ~40%; Table 3). Thus, 18–77% of the productivity, corresponding to between  $0.72$  and  $2.8 \text{ mol C m}^{-2} \text{ yr}^{-1}$  ( $0.0065$  and  $0.029 \text{ mol P m}^{-2} \text{ yr}^{-1}$ ), is supported by DOP regeneration. This translates to between  $1.8 \times 10^9 \text{ mol yr}^{-1}$  and  $7 \times 10^9 \text{ mol yr}^{-1}$  C fixation supported by regenerated DOP utilization in surface waters of the Sargasso Sea. While this value is only a crude estimate based

on relatively few samples collected during one cruise, and the exact value will depend on the composition and bioavailability of different compounds in the DOP pool and specific enzyme fractionation effect during their hydrolysis, our results illustrate the importance of DOP in supporting ocean productivity and C uptake by phytoplankton.

## 5. Summary and Implications

[27] Using the oxygen isotopic signature of dissolved phosphate in the water column along with additional indicator of P dynamics (P turnover time, % ELF labeling, and APA), we determined that the Sargasso Sea is deficient in DIP such that the biological communities utilize extracellular enzymes to access the DOP pool. The importance of DOP as a source of phosphate in this region of the ocean is consistent with an increasing body of data [Cotner *et al.*, 1997; Ammerman *et al.*, 2003; Mills *et al.*, 2004; Mather *et al.*, 2008; Van Mooy *et al.*, 2009; Torres-Valdes *et al.*, 2009; Casey, *et al.*, 2009; Sohm and Capone, 2006, 2010; Orchard *et al.*, 2010; Lomas *et al.*, 2010]. We also provide a range of quantitative estimates for the contribution of DOP to P uptake in the water column based on the isotope data. The calculated range of DOP utilization in the euphotic zone, reported here based on  $\delta^{18}\text{O}_{\text{PO}_4}$  (average ~40%, range 17–82%) is consistent with other estimates for this region determined by a variety of different methods. Using APA as a proxy for DOP hydrolysis, Mather *et al.* [2008] suggested that DOP can fuel up to 30% of primary productivity in the North Atlantic; using uptake rates and kinetics of  $^{33}\text{P}$ ,  $^{33}\text{P}$ -ATP [Orchard *et al.*, 2010], and a P flux budget, Lomas *et al.* [2010] also suggest that the contribution of

DOP to total P uptake can be higher than 25%. Collectively, our data (e.g., turnover time, APA, %ELF labeling, and  $\delta^{18}\text{O}_{\text{PO}_4}$ ) demonstrate that the bioavailability of the DOP pool is critical for organisms in the study area. Specifically, our isotope data indicates that a large fraction of phosphate in these waters originates from extracellular enzymatic regeneration of DOP (rather than upwelling or recycling of DIP). These extracellular hydrolytic enzymes are produced by the biomass to supplement the low DIP concentrations (relative to demand) by utilizing the DOP pool.

[28] An implication of these results is that inorganic nitrogen (N) to DIP ratio (N:P) is not a suitable measure for nutrient limitation. Global assessments of nutrient limitation in oligotrophic systems should include estimates of all bioavailable nutrients including those from the DOM pools [Downing, 1997; Emerson et al., 2001; Klausmeier et al., 2004; Krom et al., 2004]. In addition, we suggest that in the absence of studies such as the one reported here, global estimates of maximum primary productivity that are based on inorganic nutrient availability would considerably underestimate actual productivity and related carbon uptake.

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