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Selective phosphorus regeneration of sinking marine particles: evidence from ^{31}P-NMR

Adina Paytan*, Barbara J. Cade-Menun[1], Karen McLaughlin[1], Kristina L. Faul[1,2]

Department of Geological and Environmental Science, Stanford University, Stanford, CA 94305-2115, USA

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Abstract

Phosphorus (P) regeneration and transformation in the oceanic water column and in marine sediments depends on the chemical nature of the sinking particulate P pool. For the first time, we have characterized the molecular composition of this pool, in various oceanic settings and water depths, using ^{31}P nuclear magnetic resonance (NMR) spectroscopy. Both inorganic P (orthophosphate, pyrophosphate, and polyphosphate) and organic P compounds (orthophosphate monoesters, orthophosphate diesters, and phosphonates) were identified. The inorganic P is present predominantly as orthophosphate with small amounts (< 10%) of pyro- and polyphosphates. These inorganic compounds may be at least partially of biological origin. The relatively high abundance of inorganic P suggests that considerable transformation from the organic to the inorganic pool occurs in the water column. Some of this inorganic P may be present in association with mineral phases (apatite, clays, and oxyhydroxides) and thus may not be bioavailable.

The distribution of organic P compounds in the sinking particulate matter pool is generally similar in composition to phytoplankton and significantly different than in the dissolved organic matter (DOM) pool. Results indicate that in most oceanic regions the majority of P regeneration occurs at very shallow depths. However, in the Ross Sea, a significant fraction of organic P is exported to depth below the euphotic zone. Hydrolysis of P compounds continues throughout the water column as indicated by a decrease in total particulate P with depth and a relative decrease in the organic P fraction at some sites. Orthophosphate monoesters dominate the organic P pool at all locations, followed by orthophosphate diesters. Phosphonates are present in a few samples but never contribute more than 6% of total extractable P compared to 25% abundance in the dissolved organic P (DOP) pool. This work shows that considerable spatial and temporal variability in the molecular composition of sinking particulate P exists. A more systematic study is needed to assess the different environmental parameters that affect the composition of particulate P and result in this variability.
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1. Introduction

Phosphorus is an essential nutrient utilized by all living organisms. It has been recognized as a limiting nutrient in some oceanic systems (Karl et al., 1995; Michaels et al., 1996; Cotner et al., 1997; Wu et al., 2000) and is possibly the ultimate limiting macro-

* Corresponding author. Fax: +1-650-725-0979.
E-mail addresses: apaytan@pangea.stanford.edu (A. Paytan), bjcm@pangea.stanford.edu (B.J. Cade-Menun), karenmcl@pangea.stanford.edu (K. McLaughlin), kfaul@stanford.edu (K.L. Faul).
[1] Fax: +1-650-725-0979.
[2] Current address: Chemistry and Physics Department, Mills College, Oakland, CA 94613, USA.

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nutrient for marine productivity over long time scales (Delaney, 1998; Toggweiler, 1999; Tyrrell, 1999; Benitez-Nelson, 2000). Primary producers obtain most P as dissolved orthophosphoric acid, although P from dissolved organic compounds may also be used (Lobban and Harrison, 1994; Bjorkman and Karl, 1994). The regeneration of dissolved inorganic P (DIP) and dissolved organic P (DOP) from particulate organic P (POP), and upwelling of these dissolved constituents to the euphotic zone, is the most important P source supporting biological productivity in the ocean. Thus, POP regeneration is a critical step in regulating P availability and biological productivity (Delaney, 1998; Benitez-Nelson, 2000). The organic P compounds that escape regeneration at shallow depths and are exported to the deep ocean are similarly important, because these compounds are the P resource for the deep ocean benthic community. In addition, the sinking particulate P pool is the source of P to marine sediments. These sediments are a sink that at steady state is equated with input fluxes to the ocean, which in turn affects oceanic productivity and organic carbon burial (Broecker, 1982; Raymo, 1994; Delaney, 1998).

Relatively little is known about the concentrations, turnover rates, transport, and fate of particulate P in the ocean and practically nothing is known about the composition and changes in composition of this pool with age/depth and location in the ocean. The processes controlling the extensive biodegradation of sinking particulates remain unclear, partly because no information exists with regard to the composition and distribution of the residual organic matter at depth (Hedges et al., 2001). Particulate organic matter (POM) in seawater, as a direct derivative of living and dead organisms, should contain a wide range of chemical compounds. The structural characteristic of POP compounds is the most important parameter controlling the ''reactivity'' of these compounds, which in turn influences their turnover rates (the ease of conversion to DIP and DOP). Accordingly, identification of P compounds in oceanic particulate matter and knowledge of changes in the makeup of the particulate phosphorus pool in space and time are relevant for understanding the origin, transformations, and regeneration of P (Ingall et al., 1990; Clark et al., 1999). In addition, since this is the major source of P to the sediment, the nature of the particulate phosphorus pool (quantity and composition) will dictate the amount of P burial and potential post-depositional transformations (Ruttenberg and Berner, 1993; Delaney, 1998).

Considerable temporal variability in soluble reactive P (SRP) concentrations (Karl and Tien, 1997) and in the sinking, suspended, and dissolved organic P pools (Loh and Bauer, 2000) has been observed and attributed to depth-specific variations in the chemical composition of the P pools and/or to differences in the relative reactivity of compounds within these pools. Similarly, the turnover rates of P within the dissolved and particulate pools were determined to be rapid and variable over seasonal time scales, using cosmogenically produced ^{32}P and ^{33}P (Lee et al., 1991; Benitez-Nelson and Buesseler, 1999). This variability was also attributed to preferential regeneration of certain P compounds. However, the specific compounds that make up the POP pool, the spatial and temporal variability in POP composition, and how this may affect P regeneration efficiency have yet to be fully described or quantified. A comparison between the distribution of different organic P compounds in living plankton and the residual material that is present in sediment traps and ultimately in marine sediments will help recognize if, and identify which, organic P compounds are preferentially remineralized.

One tool with the potential to characterize the POP pools is ^{31}P nuclear magnetic resonance (NMR) spectroscopy. ^{31}P-NMR is a well established and widely used spectroscopic technique that exploits the magnetic properties of the atomic nucleus, thereby providing information about the bonds of P and therefore the P species present in the sample (Condron et al., 1997). Because of the large gyromagnetic ratio of ^{31}P and its 100% natural abundance, it is easily detected by NMR spectroscopy. Solid-state ^{31}P-NMR has been used to identify the classes of organic P compounds present in high molecular weight dissolved organic matter (DOM) (Clark et al., 1998, 1999; Kolowith et al., 2001), while solution ^{31}P-NMR has been used for extracts of marine and lake sediments (Ingall et al., 1990; Carman et al., 2000; Sundareshwar et al., 2001). While solid-state ^{31}P-NMR allows samples to be examined directly with little preparation, solution ^{31}P-NMR gives a much better spectral resolution, thereby allowing the identification of more P species. However, it has generally required 10–30 g of material for extraction (Sundareshwar et al., 2001; Ingall et al.,

1990), making it impractical for very small samples such as sediment trap material.

The objective of our research was to develop an extraction procedure for solution ^{31}P-NMR spectroscopy that could use very small samples, thereby allowing us to survey the chemical composition of POP and characterize, for the first time, the P forms of POP in sediment trap samples. We chose to adapt the NaOH–EDTA extraction procedure of Cade-Menun and Preston (1996). This extractant is widely used for ^{31}P-NMR of soil samples, and has been successfully used for ^{31}P-NMR of estuary sediments (Sundareshwar et al., 2001). Our specific objectives were to: (i) develop an extraction procedure for samples of 1 g or less; (ii) determine if solution ^{31}P-NMR spectroscopy on extracts of these small samples would be sensitive enough to determine differences in P species among POP in trap samples collected from different locations; (iii) determine if solution ^{31}P-NMR spectroscopy on extracts of these small samples would be sensitive enough to determine temporal or depth differences in P species among POP trap samples from a single location; and (iv) use this technique to survey, for the first time, the compositional makeup of P in sinking particulate matter in the ocean.

2. Methods

2.1. Samples used

We used sediment trap samples from several oceanic regimes, including coastal, open-ocean, and polar environments (Table 1). These represent a wide range of settings, characterized by differences in temperature, nutrient loading, productivity, ecosystem structure, and hydrography. These samples have been previously studied for estimating regional and temporal fluctuations of carbon flux to depth (Dymond and Collier, 1988; Dymond and Lyle, 1994; Honjo et al., 1995; Dunbar et al., 1998; Baldwin et al., 1998; Pilskaln et al., 1996). The wide range of samples used here allowed us to capture the global range of compositions and to identify the common and distinct features represented by these samples. Additionally, we also analyzed samples from different depths in the water column, and samples corresponding to different sampling seasons and years (Table 1). This allowed us to evaluate the depth dependence of the relative abundance of P compounds in sinking particulate matter and to identify seasonal changes in this composition that may arise from changes in plankton speciation and abundance and/or physical characteristics of the water column (e.g. temperature, mixing depth, etc.).

The sediment trap samples were not acquired for this particular study and were collected by different groups using different sampling and preservation protocols (see references in Table 1). In particular, in some sediment traps (Ross Sea, Palmer Deep, EP2), buffered formalin was used as the poisoning solution while in other studies mercuric chloride (Monterey Bay, Point Conception) or sodium azide (MP C3, M-T4) were used. The storage temperatures in the different studies were also different, but in all studies, samples were kept cold. Although these differences in sample handling may impact P preservation and bias our results, we were able to compare spectra obtained for samples from the same site and similar (but not identical) sampling intervals that were either stored in a cold room in the sediment trap cup solutions or split and dried immediately after recovery and kept at room temperature (Monterey Bay, S2-98 7-7 and S 203). Although the spectra for these samples are not identical, because these are not homogenized splits, the observed differences in the distribution of P compounds between these samples are small. We could not compare samples from the same trap that had different poison treatments since such samples were not available to us (typically only one poison solution is used in each trap deployment). However, when comparing samples from different sites where different poisons were used, a systematic trend in distribution of compounds among treatments was not identified. For example, Ross Sea, Palmer Deep, and EP2 traps were handled similarly but still show considerable differences in P concentrations and distributions. In fact, the largest differences in our samples are captured in these three sites. Similarly, samples from MP C3 and M-T4 are different from each other despite the use of sodium azide in both sites. Because no consistent relations between POP makeup and sediment trap preservation or storage protocols were evident, we interpret all observed variability as arising from differences in

Table 1
Sample location, depth, sampling time, and sampling protocol

Sample name	Longitude latitude, depth (m)	Trap depth (m)	Sampling dates (month/day/year)	Poison storage temperature (°C)	Contact and references
Monterey Bay					
S2-98 1–3	36.66N 122.37W, 1800	1200	2/1/98–3/12/98	3 mM $HgCl_2$; frozen	F. Chavez
S2-98 4–6	36.66N 122.37W, 1800	1200	3/13/98-4/20/98	3 mM $HgCl_2$; frozen	1
S2-98 7–9	36.66N 122.37W, 1800	1200	4/20/98–6/2/98	3 mM $HgCl_2$; frozen	
S 203	36.66N 122.37W, 1800	1200	4/28/98–5/6/98	3 mM $HgCl_2$; frozen	
S2-00 1–2	36.66N 122.37W, 1800	1200	2/1/00–2/28/00	3 mM $HgCl_2$; frozen	
S2-00 3–5	36.66N 122.37W, 1800	1200	2/28/00–3/26/00	3 mM $HgCl_2$; frozen	
S3-00 1–4	36.50N 122.93W, 3000	2400	2/1/00–3/28/00	3 mM $HgCl_2$; frozen	
Point Conception					
Stn. M-118	34°5N 123°0W, 4100	4050	6/23/89–10/22/89	3 mM $HgCl_2$; frozen	K. Smith
Stn. M-327	34°5N 123°0W, 4100	4050	2/15/90–6/15/90	3 mM $HgCl_2$; frozen	2
Stn. M-621	34°5N 123°0W, 4100	4050	2/25/91–6/25/91	3 mM $HgCl_2$; frozen	
Ross Sea					
A-T1	76°4S 169°0W, 817	228	1/22/95–1/19/96	3% formalin; 0 °C	R. Dunbar
A-B2	76°4S 169°0W, 817	775	2/01/95–2/15/95	3% formalin; 0 °C	3, 4, 5, 6
A-B4	76°4S 169°0W, 817	775	4/01/95–6/01/95	3% formalin; 0 °C	
A-B5	76°4S 169°0W, 817	775	6/01/95–8/01/95	3% formalin; 0 °C	
Chin97 B8–11	76°2S 165°3E, 825	777	5/15/97–12/23/97	3% formalin; 0 °C	
Chin98 B6–7	76°2S 165°1E, 827	775	3/01/98–4/01/98	3% formalin; 0 °C	
Gen98 B1–2	76°2S 172°6W, 621	571	1/5/98–2/1/98	3% formalin; 0 °C	
Palmer Deep					
PD 99 B3	64°5S 64°1W, 1040	1010	6/1/99–8/1/99	3% formalin; 0 °C	R. Dunbar
PD 99 B4	64°5S 64°1W, 1040	1010	8/1/99–10/1/99	3% formalin; 0 °C	
Equatorial Pacific					
MP C3 1–3	1°06N 138°98W, 4450	1083	12/20/83–7/10/84	0.3% Na azide; 4 °C	B. Collier
MP C3 4–5	1°06N 138°98W, 4450	1083	7/10/84–2/1/85	0.3% Na azide; 4 °C	7,8
MP C3 3	1°06N 138°98W, 4450	1883	4/1/84–7/10/84	0.3% Na azide; 4 °C	
MP C3 4	1°06N 138°98W, 4450	1883	7/10/84–10/20/84	0.3% Na azide; 4 °C	
MP C3 1–3	1°06N 138°98W, 4450	2908	12/20/83–7/10/84	0.3% Na azide; 4 °C	
MP C3 4–5	1°06N 138°98W, 4450	2908	7/10/84–2/1/85	0.3% Na azide; 4 °C	
MP C3 annual	1°06N 138°98W, 4450	4220	1984	0.3% Na azide; 4 °C	
MP C3 annual	1°06N 138°98W, 4450	4390	1984	0.3% Na azide; 4 °C	
EP2 1–14	0°09N 139°71W, 4360	1024	2/1/93–9/27/93	3% formalin; 4 °C	9
EP2 1–21	0°09N 139°71W, 4360	1926	2/1/93–1/24/94	3% formalin; 4 °C	
EP2 1–21	0°09N 139°71W, 4360	3650	2/1/93–1/24/94	3% formalin; 4 °C	
Central Pacific Gyre					
M T4	41°53N 131°99W, 3710	1500	9/1/89–8/30/90	0.3% Na azide; 4 °C	B. Collier
Plankton					
M1 505 μm	36°75N 122°03W	200	4/27/00	Formalin, room temp	F. Chavez
SG #69 505 μm	53°40S 36°25W	70	19/1/1989	Formalin, room temp	V. Loeb
MB 75 μm	36°75N 122°03W	Surface	5/9/00	Filtered, dry 50 °C	A. Paytan
LVFS					
R412 1–53 μm	36°05N 39°6–64°9W	193	10/03/1982	H_2O spray rinse, dry	J. Bishop
Sediments					
TTN013-69	0°11N 139°72W, 4360	1–3 cm	Holocene	Cold room 4 °C	B. Conard
W8709-10BC	42°08N 125°84W, 2778	0–2 cm	Holocene	Cold room 4 °C	
W8709-01BC	41°54N 131°95W, 3680	1–3 cm	Holocene	Cold room 4 °C	
BNTH III–08	1°06N 138°97W, 4450	1–3 cm	Holocene	Cold room 4 °C	

1: Pilskaln et al. (1996); 2: Baldwin et al. (1998); 3: Collier et al. (2000); 4: Dunbar et al. (1998); 5: Dunbar et al. (in press); 6: Langone et al. (in press); 7: Dymond and Collier (1988); 8: Dymond and Lyle (1994); 9: Honjo et al. (1995).

POP input and regeneration. However, more work should be done in the future to specifically address these issues.

2.2. Phosphorus extraction from particulate material

One gram of sediment trap material was extracted with 20 ml of a 1:1 mixture of 0.25 N NaOH and 0.05 M Na_2EDTA at room temperature for 16 h and centrifuged. A 1-ml aliquot was removed for determination of extractable P concentration, and the remaining supernatant was concentrated by lyophilization immediately after extraction. Total P in the supernatant aliquot was determined after a 1:10 dilution by inductively coupled plasma (ICP) spectroscopy (Thermo Jarrell Ash, Franklin, MA). Extraction yields for sediment trap samples (total P extracted/total P in sample) were 35–99% of the total P concentration of the unextracted sample, and were equal to or greater than 100% of the organic P concentration of the unextracted sample (total P extracted/organic P in sample; see Table 2). The extraction procedure was developed to recover organic P and may not quantitatively extract P associated with mineral phases such as fluorapatite or clay minerals, resulting in the lower than 100% extraction efficiency of the total P (Cade-Menun and Preston, 1996). Indeed, samples with higher organic P content typically show higher extraction yields while lower extraction yields are recorded in those samples with a significant detrital input (close to the sediment water interface) (Table 2). Total P and organic P concentrations in splits of the unextracted samples were determined by the sequential extraction method of Ruttenberg (1992) as modified by Anderson and Delaney (2000).

2.3. Solution nuclear magnetic resonance spectroscopy (NMR)

For an overview of the principles of NMR spectroscopy in general and ^{31}P-NMR in particular, please see Nanny et al. (1997), and papers therein. To prepare the samples for NMR spectroscopy, the lyophilized powder from each extracted sample was re-dissolved in 2.6 ml H_2O and 0.4 ml of 10 M NaOH. The NaOH was added here to increase and standardize the pH, for optimal peak separation (Crouse et al., 2000). Spectra were acquired, immediately after preparation, at 202.45 MHz on a GE Omega 500 MHz NMR spectrometer equipped with a 10-mm broadband probe. Running parameters were: 17000 scans; 25 °C; 90° pulse; 0.68 s acquisition time; and 4.32 s relaxation delay. Identification of compounds by ^{31}P-NMR is based on their chemical shift relative to an external H_3PO_4 standard. Chemical shift is defined by:

$$\frac{Vs - Vr}{Vr} \times 10^6$$

where Vs and Vr are the frequencies of the sample and reference standard, relative to that of the applied magnetic field (Wilson, 1987). Chemical shift values are dimensionless and expressed in parts per million (ppm) with the external standard set at 0 ppm. The assignment of peaks from the NMR spectra to specific P forms was based on chemical shift values from the literature.

After the NMR data were collected, the spectra were processed using NUTS–NMR Utility Transform software (Acorn NMR). A line broadening of 20 Hz was used, which enhanced the signal-to-noise ratio without reducing peak resolution. NUTS software was also used for baseline correction, peak picking, and to calculate peak areas by integration. The NMR spectra processing procedure we used recognizes peaks if their intensity is at least 1% that of the tallest peak. Peaks were accepted if they fit three criteria: (i) a peak should be identified by the NUTS software, (ii) evaluated and recognized as such by us after visual inspection of the expanded spectra, and (iii) consist of at least 1% of the total integrated area of the spectra. We adopted conservative criteria in our identification and acceptance of peaks. Peaks representing only 1% of the total area are easily recognized in spectra with high signal to noise. The sensitivity of the NMR is specific to each sample; because our samples contain few paramagnetic ions, the procedure is relatively sensitive and we can resolve P compounds with concentrations of 1–2 ppm in the NMR tube. Analysis of splits of the same sample that were extracted separately (M-T4) resulted in highly reproducible spectra; differences in calculated abundances of the different P compounds were less than 1% (see Table 3).

Table 2
Sediment trap mass fluxes, C, P, and extractable P contents, and extraction yields

Sample name	Total mass flux (mg m^{-2} $year^{-1}$)	Total organic C (mmol g^{-1})	Total P (μmol g^{-1})	Total organic P (μmol g^{-1})	Total extracted P (μmol g^{-1})	Extracted P/ total P (%)	Extracted P/ organic P (%)
Monterey Bay							
S2-98 1–3	963	4.17	29.8	12.4	13.3	44	107
S2-98 4–6	753	3.51	42.3	31.9	41.9	99	134
S2-98 7–9	1490	3.33	51.9	34.8	45.0	87	129
S 203	1154	5.12	40.6	17.1	19.6	49	115
S2-00 1–2	395	3.46	39.2	15.4	23.0	59	149
S2-00 3–5	617	3.27	33.4	11.8	11.8	35	101
S3-00 1–4	317	NA	32.0	15.0	22.0	69	147
Point Conception							
Stn. M-118	219	4.91	21.0	9.6	15.0	71	156
Stn. M-327	137	4.76	24.9	8.8	13.7	55	157
Stn. M-621	418	4.38	26.1	12.6	19.3	74	153
Ross Sea							
A-T1	82	6.93	21.7	10.9	18.9	87	173
A-B2	1297	5.33	14.1	10.1	11.5	81	113
A-B4	846	2.82	12.2	6.6	6.7	55	101
A-B5	440	2.63	15.3	7.8	8.1	53	104
Chin97 B8–11	NA	NA	17.0	7.8	12.5	74	160
Chin98 B6–7	1949	3.26	11.9	6.7	7.8	66	116
Gen98 B1–2	1160	4.52	19.5	8.5	9.2	47	108
Palmer Deep							
PD 99 B3	2500	1.13	24.5	4.8	6.7	27	139
PD 99 B4	1999	0.98	22.5	3.9	14.2	63	368
Equatorial Pacific							
MP C3 1–3	10	4.68	18.0	5.9	17.8	99	303
MP C3 4–5	10	4.10	18.6	6.1	14.9	80	243
MP C3 3	22	4.78	13.1	5.3	11.1	85	209
MP C3 4	14	3.31	13.0	5.0	9.9	77	199
MP C3 1–3	11	3.73	10.7	4.5	10.5	98	235
MP C3 4–5	14	3.48	10.2	4.8	10.2	99	213
MP C3 annual	11	3.51	10.2	5.4	5.6	54	102
MP C3 annual	11	3.36	12.6	4.7	6.4	51	134
EP2 1–14	8	5.57	30.5	8.9	11.9	39	133
EP2 1–21	5	4.38	12.8	6.5	8.2	64	126
EP2 1–21	10	3.82	11.1	6.0	7.2	65	119
Central Pacific Gyre							
MT4	NA	NA	26.0	7.6	17.7	68	233
MT4 (duplicate)	NA	NA	25.3	7.7	16.4	65	214

Total flux and organic C values are from the respective sources in Table 1. Total P and organic P were determined using the Ruttenberg (1992) sequential extraction protocol as modified by Anderson and Delaney (2000). Total extractable P is determined on an aliquot of each sample after the NaOH–EDTA extraction (see text). Percent extracted P (columns 7 and 8) is calculated as the percent extracted P of the total P and of organic P, respectively (e.g. extracted P divided by total P or organic P). Analytical error on the P concentrations is less than 10%.

Table 3
Phosphorus compounds distribution in marine particulate matter and sediments

Sample name	Orthophosphate (%)	Pyrophosphate (%)	Polyphosphate (%)	Phosphonates (%)	Orthophosphate monoesters (%)	Orthophosphate diesters (%)	Inorganic P (%)	Organic P (%)
Monterey Bay								
S2-98 1–3	64	2	0	0	28	6	66	34
S2-98 4–6	58	3	0	0	31	8	61	39
S2-98 7–9	66	2	0	0	30	2	68	32
S 203	66	2	0	0	26	6	68	32
S2-00 1–2	55	3	0	5	26	11	58	42
S2-00 3–5	64	2	0	0	29	5	66	34
S3-00 1–4	47	3	2	6	31	11	52	48
Point Conception								
Stn. M-118	44	5	0	1	37	13	49	51
Stn. M-327	52	4	0	3	33	12	56	44
Stn. M-621	48	7	0	0	33	12	55	45
Ross Sea								
A-T1	20	1	0	0	68	11	21	79
A-B2	30	2	0	0	55	13	32	68
A-B4	18	1	0	1	68	12	19	81
A-B5	55	0	1	2	33	9	56	44
Chin97 B8–11	50	2	2	4	37	5	54	46
Chin98 B6–7	39	0	0	0	50	11	39	61
Gen98 B1–2	29	2	0	0	65	4	31	69
Palmer Deep								
PD 99 B3	75	2	1	0	18	4	78	22
PD 99 B4	78	0	0	1	19	2	78	22
Equatorial Pacific								
MP C3 1–3	49	6	0	2	32	11	55	45
MP C3 4–5	51	7	0	2	30	10	58	42
MP C3 3	47	5	1	0	34	13	53	47
MP C3 4	49	5	0	1	37	8	54	46
MP C3 1–3	37	8	0	0	38	17	45	55
MP C3 4–5	38	8	0	1	37	16	46	54
MP C3 annual	42	5	0	3	33	17	47	53
MP C3 annual	44	4	0	0	37	15	48	52
EP2 1–14	56	4	2	0	32	6	62	38
EP2 1–21	56	6	0	1	30	7	62	38
EP2 1–21	60	5	0	0	34	1	65	35
Central Pacific Gyre								
M T4	60	6	0	2	23	9	66	34
M T4 (duplicate)	60	5	0	1	24	10	65	35
Plankton								
M1 505 μm	7	1	0	0	73	19	8	92
SG #69 505 μm	4	0	0	0	84	12	4	96
MB 75 μm	45	2	0	0	50	4	47	53
LVFS								
R412-1–53 μm	53	5	0	0	42	0	58	42
Sediments								
TTN013-69	67	4	0	1	28	0	71	29
W8709-10BC	58	0	0	0	26	16	58	42
W8709-01BC	68	0	0	0	29	4	68	32
BNTH III-08	60	2	0	1	30	7	62	38

All the data in the table are the percent of total extractable P determined from the relative peak areas as calculated by integration. Peaks are identified only if their area consists of at least 1% of the total integrated area of the spectrum (see text). The total inorganic fraction is the sum of the percent orthophosphate, pyrophosphate, and polyphosphate, and the organic P percent is the sum of the orthophosphate monoesters and diesters and the phosphonates.

3. Results and discussion

3.1. Solution ^{31}P-NMR spectroscopy of POP samples

We used solution ^{31}P-NMR for our analyses for the following reasons: (1) samples can be concentrated by lyophilization after extraction to maximize the P concentration in the NMR tube, reducing the total sample size required and improving the quality of the spectrum by improving the signal-to-noise ratio; and (2) solution NMR methods have significantly higher resolution than solid-state methods, and thus have the potential to identify more compounds.

We have identified six major groups of P compounds that may be present in the sinking particulate matter; not all P groups are present in each sample (Fig. 1, Table 1). These groups include both inorganic P (orthophosphate, pyrophosphate, and polyphosphate) and organic P compounds (orthophosphate monoesters, orthophosphate diesters, and phosphonates).

It is commonly assumed that all of the sinking particulate P is in the form of organic compounds derived from living and dead organisms. Our results, however, suggest that a significant fraction of sinking particulate matter P is inorganic (i.e. does not contain a C moiety). Some of this inorganic P may indeed be biogenic, perhaps as vacuolar orthophosphate or intracellular compounds (Ratcliffe, 1994). Inorganic P, and in particular orthophosphate and polyphosphates, has been observed in natural phytoplankton samples (Bieleski, 1973) as well as in ^{31}P-NMR studies of fixed or in vivo plankton, algae, bacteria, and higher plants, where no extractions were employed (Feuillade et al., 1995; Deslauriers et al., 1980; Ratcliffe, 1994). This suggests that at least some of the inorganic P in our samples is biogenic. Using solid-state ^{31}P-NMR, the orthophosphate peak is not resolved from the ester peaks. Accordingly, the broad peak observed in bacterial cultures and ultrafiltered particulate material (0.1–60 μm) by Kolowith et al. (2001) may include two-components, one of which is in the orthophosphate and monoester region and the other in the diester region, thus potentially consistent with the above observations (J. Stebbins, personal communication). Although ultrafiltration followed by diafiltration as used by Kolowith et al. (2001) will remove any orthophosphate in solution, it will not remove intracellular inorganic P unless the cells are lysed. Optical observations of ultrafiltered POM suggest the cells are not lysed in the process (Benner et al., 1997).

The methodological and analytical evidence thus far suggests that there was no significant hydrolysis of the sample during the extraction procedure, despite the use of alkaline reagents. In a recent study, Turner et al. (2003) tested more than 40 commercially available P compounds for peak shifts and hydrolysis in NaOH–EDTA extracts, for ^{31}P-NMR spectroscopy. With the exception of the diesters RNA and phosphatidyl choline (a lipid), they found little or no hydrolysis of organic P compounds under the same analytical conditions that we used. RNA and phosphatidyl choline, however, degrade rapidly to orthophosphate monoesters in the alkaline solution used for extraction. Therefore, if these compounds exist in the POP pool, following our extraction, they will contribute to the monoester peaks (at 3 to 6 ppm chemical shift) and will not be detected in the diester chemical shift region (− 1 to 2 ppm) (Turner et al., 2003). RNA is amongst the most labile macromolecules present in cells and phosphatidyl choline, like other mono unsaturated fatty acids, also degrades very rapidly (80–90% remineralization in less than 2 weeks; Sun et al., 1997). Accordingly, we do not expect that RNA and phosphatidyl choline will comprise a significant portion of sinking POP collected below the euphotic zone. Regardless, since we do not know what fraction of the total organic P in the sediment traps is attributable to these compounds, the potential effect of hydrolysis of these compounds in our extraction protocol should be kept in mind.

The low orthophosphate content (3.9% and 6.7%) of the zooplankton samples (SG #69 and M1, respectively) that were processed using the same protocol as for sediment trap samples suggests minimal hydrolysis of organic compounds to orthophosphate. A sequential leaching extraction (Ruttenberg, 1992) of our sediment trap samples indicates that in some cases, particularly in coastal regions, up to 30% of the P in sinking particulate matter is associated with detrital clays or mineral compounds such as carbonate fluorapatite, and additional P is associated with Fe oxyhydroxides (Faul et al., 2002). These inorganic P compounds may have been partially digested or leached during our extraction and thus could have partially contributed to the orthophosphate observed

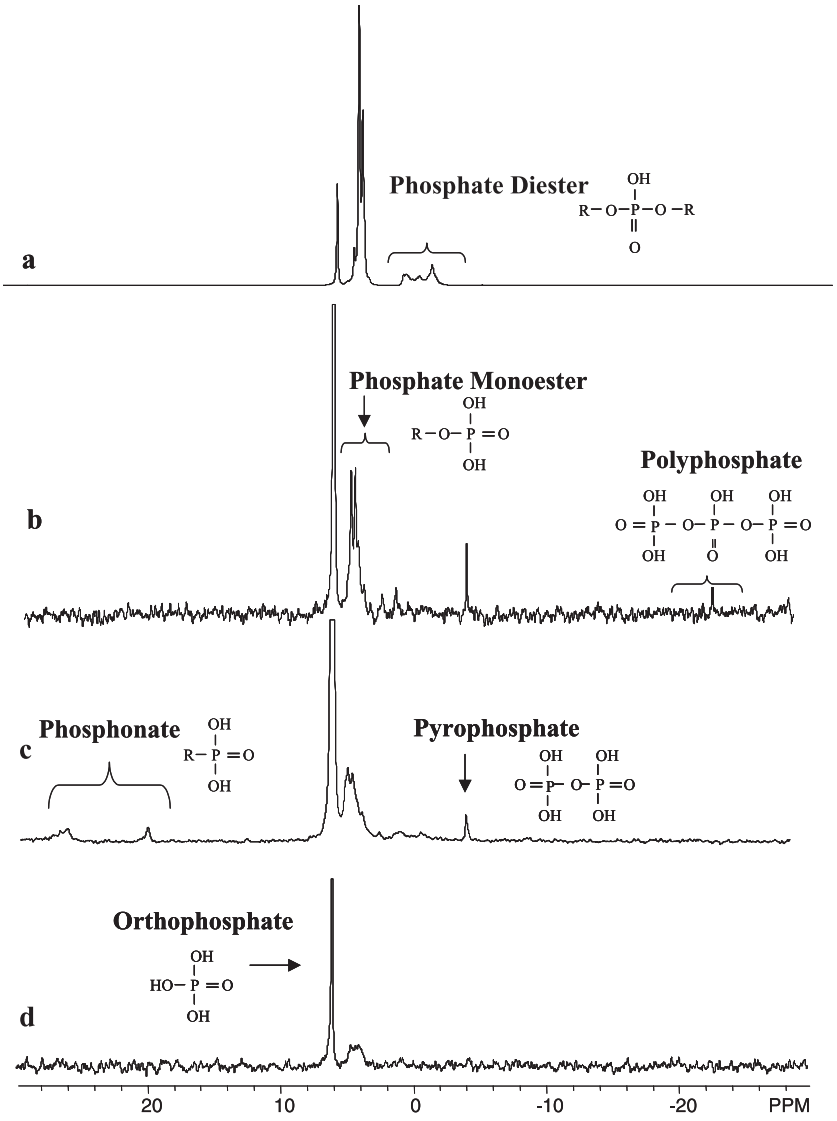

Fig. 1. ^{31}P-NMR spectra of sediment trap material. Peaks corresponding to different compounds are labeled. The spectra were acquired at 202.45 MHz on a GE Omega 500 MHz spectrometer equipped with a 10-mm broadband observe probe (see Methods). Chemical shifts were measured relative to an external orthophosphate standard. Peak areas were calculated by integration. The spectra presented are for (a) plankton tow sample M1, 505 μm; (b) Equatorial Pacific Station EP2, 1024 m; (c) Santa Barbara Basin (SBB-8 #7), 540 m, sampling period July 10–24, 1997; and (d) core top sediments TTN013-69. Note that spectra are plotted for best fit and are not to scale.

in our samples. It is possible, however, that hydrolysis of labile organic P forms occurred within the sediment trap cups or during processing of the samples before we obtained them. Indeed, it has been shown that in shallow traps a significant fraction of the total P is found in the cup solutions, while in deep traps, a much smaller fraction (<10%) is contained in the cup solution (R. Collier, personal communication). Orthophosphate was readily detected by ^{31}P-NMR in solutions obtained from the trap cups that we tested (Monterey Bay samples), but no organic P forms were seen. This P, which is so readily regenerated, is not expected to remain on the sinking particulate matter in the ocean, and thus its loss into the cup solution

should not affect the conclusions of this study, which is focused on the residual sinking particulate P.

3.2. P species in samples from different locations

As stated above, our results indicate that P exists in sinking particulate matter in a multitude of chemical forms, both inorganic and organic. The total particulate P concentrations differ in the different oceanic regions represented in our study. The highest concentrations were found in Monterey Bay (30–50 μmol g^{-1}), followed by Point Conception, Palmer Deep, and station M-T4 (20–25 μmol g^{-1}), and the lowest concentrations were in the Equatorial Pacific and Ross Sea traps (10–20 μmol g^{-1}). The total (and P) fluxes in the Equatorial Pacific traps are also significantly lower than at the other sites.

The inorganic P compounds that are observed in all samples (including zooplankton and phytoplankton) are partially (orthophosphate) or fully (pyro- and polyphosphate) of biological origin (Bieleski, 1973). In the open ocean and Ross Sea, a larger fraction of the orthophosphate may be attributed to biological sources as suggested by the higher extraction yields in these samples and the smaller authigenic and detrital P content in particulate matter at these sites compared to coastal locations (Faul et al., 2002). The inorganic pool mostly consists of orthophosphate at all regions with small amounts of pyrophosphate (<8% of total extractable P) existing in most samples and longer chain polyphosphates (<2% of total extractable P) observed in only a few samples. The average inorganic fraction of the total extracted P in the sediment trap samples is 55%, and the different oceanic regions cluster about this value. In the majority of oceanic regions and depths sampled, the inorganic P fraction was between 45% and 65%. Notable differences were observed in the Ross Sea, where the inorganic fraction averages only 36%, and in the Palmer Deep, where the inorganic fraction consists more than 70% of the total extracted P. The relatively high inorganic content in the Palmer Deep trap may result from re-suspension and addition of sedimentary material to the bottom trap, which is located only 30 m above the sediment water interface. However, such high inorganic fractions are not observed in traps deployed close to the seabed at other sites. Samples from shallower traps at Palmer Deep will be needed to evaluate whether this is typical of the whole water column or just the deep traps. It is interesting to note that traps within the same general oceanic region (the Equatorial Pacific, MP C3 and EP2) show some significant differences. The fraction of orthophosphate in the EP2 samples is higher than in the MP C3 samples, while the orthophosphate diesters are more abundant in MP C3. It is not clear if this is typical for each site or has to do with temporal changes within this region, because sampling intervals were different (1983–1984 and 1993–1994 for MP C3 and EP2, respectively). Further investigation into this is warranted.

Pyrophosphate was present in all but two sediment trap samples. It is interesting that this compound is present in higher abundance in all open ocean Pacific sites (average ~6%) than in coastal or high latitude samples (1–3%). This difference may be attributed to more extensive synthesis of this compound by open ocean biota or to warmer surface water temperatures. Comparison of the distribution of P compounds in natural plankton assemblages collected from different oceanic regions may shed light on this hypothesis. The possibility that the primary intracellular biosynthesis of P compounds may vary among species or depend on growth conditions is interesting since this may imply that changes in faunal assemblages as a result of climate or environmental change may influence the regeneration rate of P in the water column. Sundareshwar et al. (2001) observed very high pyrophosphate concentrations in estuary samples, and have attributed them to human impact. However, the pyrophosphate concentrations in particulate trap samples are much lower that those reported by Sundareshwar et al. (2001), and are most likely naturally occurring.

Although considerable variability in the POP makeup between samples from different sites is observed, some common features are evident. At all sites and depths, orthophosphate monoesters, which include mononucleotides, sugar phosphates, and inositol phosphate (and in our samples may potentially include contribution from the diesters RNA and phosphatidyl choline), are the major components in the sinking POP pool (18–68% of total P), followed by orthophosphate diesters (0–17%), which includes phospholipids and DNA. Although phosphonates typically comprise only a minor fraction (0–2%), in a few samples, they contributed up to 5% of the total

particulate P extracted. No regional trend in the abundance of phosphonates is observed in our samples and the few plankton samples we analyzed did not have measurable phosphonate concentrations. However, it is interesting that both samples that had a relatively higher phosphonate content are from Monterey Bay. This suggests that regional trends may be better identified with more samples from coastal regions.

The diester-to-monoester ratios in the trap samples (excluding one sample from Monterey Bay (S2 98 7–9) and one from the Ross Sea (Gen98) are between 0.15 and 0.45, with most samples clustering between 0.3 and 0.4. Keeping in mind that any degraded RNA and phosphatidyl choline may contribute to the monoester peaks, these ratios represent minimum values. If, however, we assume that the contribution of these compounds is negligible, or at least constant in our samples, then the variability in the diester-to-monoester ratios could be related to degradation processes affecting this ratio. Orthophosphate diesters, with two C moieties per orthophosphate group, are very labile (Condron et al., 1997; Dai et al., 1996; Cade-Menun and Preston, 1996; Cade-Menun et al., 2002). Orthophosphate monoesters have one C moiety per orthophosphate group. The high charge density of orthophosphate monoesters, particularly inositol phosphates, allows them to form relatively insoluble complexes with cations, protecting them from degradation (Celi et al., 1999). Accordingly, we would expect lower diester-to-monoester ratios in more remineralyzed samples. In our samples, the Southern Ocean sediment traps have lower diester-to-monoester ratios (average ~ 0.20) suggesting more extensive degradation. However, the total organic fraction in these samples is high compared to other sites, thus the lower diester-to-monoester ratios could reflect differences in this ratio in the synthesized organic matter. Alternatively, this may be a result of a larger fraction of "fresh" organic P present in the more labile form of RNA and possibly phosphatidyl choline, enhancing the monoester peak. The cause for this lower ratio needs to be verified with more samples and in particular plankton samples from this region. Using analyses that can identify specific compounds in the POP pool and in particular the concentrations of RNA and phosphatidyl choline will certainly shed light on this issue.

The observed POP makeup is very different from the relative distribution of P compounds found in ultrafiltered dissolved organic matter (DOM). Solid-state ^{31}P-NMR showed the POP in ultrafiltered DOM to consist of phosphoesters (75%) and phosphonates (25%), with little variability in the relative abundance of these compounds throughout the world's oceans (Ingall et al., 1990; Clark et al., 1998, 1999; Kolowith et al., 2001). The composition of ultrafiltered DOM is clearly distinct from fresh plankton, suggesting extensive modification from the original composition due to degradation of specific compounds. The differences between the ultrafiltered DOM and sinking POP attest to the more reactive nature of sinking POP relative to DOP and may indicate different sources, or processes, involved in the regeneration of these distinct organic P pools in seawater. These results may also imply that DOP is a more refractory, older reservoir, which is well mixed throughout the ocean.

The higher organic P content in Ross Sea samples (with an average of 64% compared to other sites with $< 50\%$ on average) suggests that, in this region, much of the P is exported to depth below the euphotic zone as organic P, particularly in areas dominated by diatom production (A traps). This could be attributed to shallower sampling depths at these sites. However, the depth difference between the two extremes in organic P content, e.g. most of the Ross Sea traps (~ 775 m) and the Palmer Deep traps (1010 m), is only 235 m, and the difference in depth between the Ross Sea traps and the shallow traps at MP C3 (1083 m) is only about 300 m. It is more likely that the sinking rate (fast sinking after bloom events) and mechanisms (aggregation) of particulate matter formation in the Ross Sea result in fast transport of fresh particulate matter (DiTullio et al., 2000), which contains a larger fraction of organic P compounds.

3.3. Temporal and depth differences in P species

Our results show that, in all sites where samples from different depth are available, the particulate P content decreases with depth. This confirms previous sediment trap research (Loh and Bauer, 2000). Both the organic and inorganic fractions get depleted with depth, as suggested by the decrease in the absolute amount in both fractions (Table 2). At sites EP2 and Ross Sea-A, where we have data from several sam-

pling depths in the water column, the relative fraction of extractable inorganic P is somewhat higher in the deeper traps (Fig. 2c). At site MP C3, on the other hand, no such trend is observed. At all three sites, the organic fraction in the trap samples is lower than the relative fraction observed in plankton, and higher than the underlying sediments, suggesting more extensive P release to solution from the organic compounds at shallower depths than the deployed traps. These observations indicate that although much of the P regeneration occurs in the upper water column, hydrolysis of P compounds continues throughout the water column. It is likely that the processes affecting specific compound regeneration are similar throughout the water column but happen more extensively at shallower depths. The general similarity in the com-

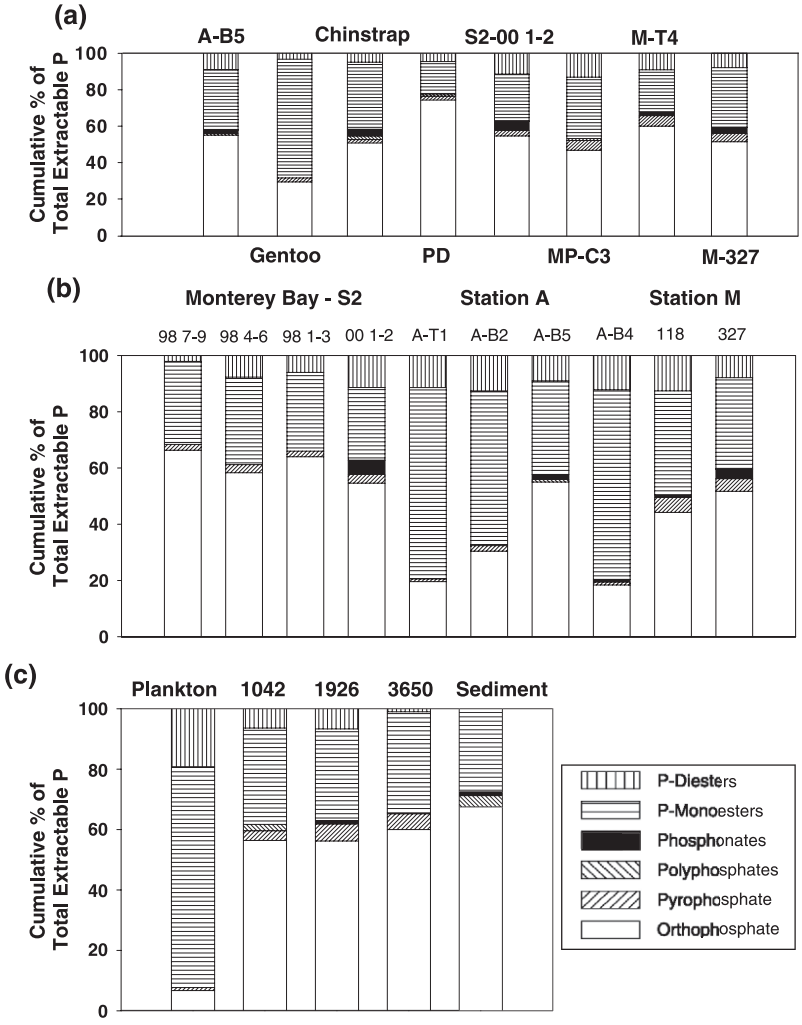

Fig. 2. Phosphorus compounds distribution in sediment trap samples determined by ^{31}P-NMR. Data are presented as cumulative percentages of total extractable phosphorus as calculated from the peak areas for each compound. Samples are grouped to represent (a) spatial variability: Ross Sea Stn. A–B5; Gentoo; Chinstrap; Palmer-Deep; Monterey Bay S2-00 1–2; Equatorial Pacific MP C3; Central Pacific-M-T4; Point Conception Stn. M-327; (b) Temporal variability: Monterey Bay S2, samples collected during different months in 1998 and 2000; Ross Sea Stn. A, samples collected during different months in 1995; Point Conception Station M, samples collected in 1989 and 1990. (c) Change with depth in the Equatorial Pacific: Plankton, station EP2 (the numbers correspond to the water depth in meters from which the sample was collected), and core top sediment sample TTN013-69. All of the data are listed in Table 1 although not all the data from Table 1 are plotted.

pounds present in traps, plankton, and a high volume filtration sample (R 412) supports this hypothesis.

Although extensive mineralization at shallow depths is the common feature in the ocean, in some samples (e.g. Ross Sea Station A, collected at 775 m, between Jun 1 1995 and August 1 1995, see Table 1), a "fresh" signal (high organic P) occurs at depths well below the euphotic zone, recording fast sinking/deposition events probably linked to specific phytoplankton blooms (DiTullio et al., 2000). Such events may be important for C sequestration from the euphotic zone, although this also sequesters P to the deep sea and makes it less available to phytoplankton.

The organic P fraction in the sediment traps is always higher than in core top sediments at the respective sites (where coupled samples were available). However, the differences in the relative inorganic to organic fraction in the traps and sediment are relatively small, with just a 3–10% decrease in the organic fraction in core tops. This indicates that transformations of P from the organic to the inorganic pool, as seen in sediments (Ruttenberg and Berner, 1993; Filippelli and Delaney, 1996), also occur within sinking particulate matter in the ocean. Including the inorganic P fractions when measuring total P to derive C/P ratios in particulate matter relative to Redfield ratios will result in lower estimates of C/P ratios than are relevant to the regeneration of organic matter, underestimating the preferential regeneration of P relative to C. The general positive correlation between the organic C and organic P content and the lack of correlation between organic C and total P in these traps further support this notion (Table 2).

The distributions of specific compounds observed in our sediment trap samples are in general similar to those detected in plankton tow samples collected in Monterey Bay and in the Southern Ocean using 505 and 75-μm nets (Table 3). This is not surprising since marine planktons are expected to be the main source of sinking particulate matter in the open ocean (Romankovich, 1984). The P in the 505-μm plankton samples, which consisted mostly of zooplankton (euphausids at Monterey Bay and juvenile krill and copepods at the South Georgia site), is over 90% organic; in contrast, the trap samples typically have lower organic P concentrations (average: 45%). The 75-μm plankton tow sample that was composed mainly of diatoms (Chaetoceros, Pseoudonitzschia and Thalassiosira; identified by microscopy) was more similar in composition and relative abundance of compounds to the particulate matter and had lower organic P content. The composition and relative distribution of all P compounds in a large volume filtration sample obtained from the center of Warm Core Ring 82H, in the Atlantic at a depth of 193 m (R 412), are also within the ranges observed in the sediment traps, possibly suggesting a similar origin.

There is considerable variability in the orthophosphate diester to orthophosphate monoester ratio in our samples (0.05–0.51), but no clear relation of this ratio with either depth or sampling period was identified (a possible spatial trend may be identified, as suggested in Section 3.2). The higher abundance of orthophosphate monoesters relative to orthophosphate diesters at all depths is expected when considering the more labile nature of diesters. Although the relative abundance of orthophosphate monoesters would be expected to increase with depth at the expense of diesters, no such trend is evident (Fig. 2c). The diester-to-monoester ratio in core top sediments is typically lower than in the traps at the respective sites, while the ratio in the zooplankton is higher, indicating that this expected preferential regeneration may be more prevalent in the euphotic zone and after burial in the sediments. Interestingly, in the two sites where depth profiles were obtained (MP C3 and EP2), the diester flux is lower in the deeper traps regardless of the absolute flux, which does not always decrease with depth (Table 1). A similar decrease is not observed for the monoesters.

Temporal differences in the composition of particulate P are observed at some sites but are less obvious at other sites. For example, the samples from Monterey Bay (S2-98) sampled at different seasons (winter and spring) in 1998 do not show much difference, but a sample from this station sampled in the winter of 2002 (S2-00 1–2) has a significant amount of phosphonates (5%) not observed in 1998 and a higher orthophosphate diesters content. Similarly, large temporal fluctuations in the ratio of organic to inorganic P in the Ross Sea traps at station A (A–B2, A–B4, A–B5) were detected, in which the inorganic fraction increased from 18% in the April/June period to 55% in June/July, possibly as a result of changes in overlying productivity. No significant differences were observed in traps sampling different time intervals at

Point Conception or the Palmer Deep. As mentioned above, traps from the Equator and from 1°N at 139°W in the Pacific (MP C3 and EP2), deployed at different years (1983–1984 and 1993–1994, respectively), show some significant differences. EP2 at the Equator has more inorganic orthophosphate and samples from MP C3 at 1°N have more orthophosphate diesters. Although the data we have are limited, we believe that this temporal variability results from changes in ecosystem assemblage. The abundance of different phytoplankton groups that synthesize different compounds and the zooplankton and bacteria that recycle these compounds in the euphotic zone may be important in controlling the composition of POP. These community changes may in turn be determined by local hydrographic conditions.

4. Summary

Work presented here is a survey of the chemical composition of P in sinking particulate matter. It highlights the temporal and spatial similarities and differences in the distribution of these compounds in the ocean and introduces a tool (solution ^{31}P-NMR) that has not been extensively utilized for oceanographic research. Results indicate that the distribution of different compounds in POP differs from that in DOP, that hydrolysis of organic P occurs throughout the water column, although it is more prevalent at shallow depths, that much of the sinking particulate P is inorganic, and that transformations of P from one pool to another occur in the water column and not only in the sediment.

The spatial and temporal variability in the POP composition suggests that the processes that govern production and regeneration of POP in the water column are not uniform. They may be affected by local environmental conditions such as compositional differences in the organic compounds synthesized by organisms, taxonomic differences in nutrient draw down, trophic structure, primary production rates, hydrographic conditions, particle sinking rates, "protection" effects by inorganic minerals, and more. The variability observed in POP composition is in contrast to recent observations of bulk organic matter composition using solid-state ^{13}C-NMR (Hedges et al., 2001). These results for C only show minimal changes in composition despite extensive biodegradation. For P, both extensive biodegradation with depth and changes in the relative abundance of P compounds are evident. The partial decoupling of C and P regeneration in the water column may provide a potential mechanism that would permit upwelling of P (and possibly other nutrients) to the euphotic zone while effectively removing C to depth. This process would increase the efficiency of the biological pump and C sequestration. Biosynthesis of different organic P compounds by different plankton groups may result in different regeneration rates, and thus recycling efficiency, in different oceanic ecosystems as a result of the variable susceptibility of different POP compound to hydrolysis. More work is currently underway to identify the processes that control the variability in oceanic sinking POP and to better characterize P regeneration in the ocean and P associations in POM.

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