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Phosphorus dynamics in the North Pacific subtropical gyre

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ABSTRACT: Phosphorus (P) dynamics were studied during several research cruises to Stn ALOHA and in the 'Climax region' of the North Pacific subtropical gyre (NPSG) in 1996–1997. The aim of this study was to: (1) investigate the coupled uptake and regeneration of inorganic phosphate (P_i) and the production of dissolved organic phosphorus (DOP), (2) quantify the size of the biologically available P (BAP) pool, and (3) estimate the relative bioavailability of select organic P compounds to the natural microbial assemblages. At all stations, the microbial community was dominated by prokaryotes (>99.5% of total cell numbers); *Prochlorococcus* spp. was the dominant pigmented group (>97% by numbers), comprising 20 to 30% of the total prokaryotic population. Phosphate uptake rates were 3.0 to 8.2 nM d^{-1} (median = 3.5 nM d^{-1}) and P pool turnover times ranged from 2 to 40 d (median = 9 d). The BAP pool generally exceeded the P_i pool, suggesting rapid turnover of at least a portion of the much larger DOP pool. The net production of DOP was approximately 10 to 40% of the net P uptake. Both the dissolved and the particulate organic matter pools were enriched in carbon (C) and nitrogen (N) relative to P, compared to the Redfield molar stoichiometry of 106C:16N:1P. The half-saturation constant, K_m, values for P_i uptake were higher than the ambient P_i pool concentrations, and uptake rates were positively correlated with exogenous P_i additions over the range tested (P_i = 25 to 250 nM). The bioavailabilities of exogenous adenine and guanine nucleotides were generally higher than other organic P compounds we tested. The net P_i regeneration rate from nucleotides was up to 50 times higher than the net P_i uptake rates, indicating a large potential for the regeneration of P_i from specific organic compounds. These P pool dynamics observed in the NPSG are consistent with a microbial community currently under P control.

KEY WORDS: Phosphate uptake rates · Turnover rates · Bioavailable phosphorus · Bacteria · North Pacific

INTRODUCTION

More than a century ago, Liebig (1842) postulated that the one bioelement in the lowest total supply, with the lowest loading rate, or lowest availability relative to an organism's nutritional requirement ultimately would determine the productivity of the organism. In terms of ecosystem productivity, it is clearly of importance how various supply routes differ among the macro- and micronutrients, as it will affect the total inventories, and possibly the bioavailability of the respective bioelements. In oceanic waters nitrogen (N) has conventionally been viewed as the nutrient most often limiting net organic production (Thomas 1966, Ryther & Dunstan 1971, Eppley et al. 1973, Codispoti 1989). However, others have suggested that new production in marine environments may ultimately be limited by phosphorus (P) (Redfield 1958, Smith 1984), or trace elements (Falkowski 1997). This apparent contradiction arises from the implied role of biological fixation of dinitrogen (N_2) that could potentially relieve the ecosystem of N limitation (McCarthy & Carpenter 1983, Tyrell 1999).

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The term 'new production' has most often been used to describe the portion of the net production fuelled by the influx of allochthonous nutrients as opposed to the portion that is supported by local nutrient regeneration (the new versus regenerated production paradigm; Dugdale & Goering 1967). By current experimental application in field-based oceanographic studies, new N is generally equated to nitrate and regenerated N to the sum of ammonium and urea (Harrison 1990). However, atmospheric deposition of fixed N and gas exchange at the air-sea interface may significantly contribute to the supply of new N and other nutrients in the upper ocean (Buat-Ménard 1986, Duce 1986), in contrast to P, which has negligible atmospheric exchange (Paerl 1993). The contribution from atmospheric sources of P to the total dissolved phosphorus (TDP) pool in the upper ocean has been estimated to less than 1% of the new P in the North Pacific gyre (Graham & Duce 1979, Duce 1986, Chadwick et al. 1999). Furthermore, the open ocean habitats are too far removed to receive any significant amounts of P from other terrestrial sources, such as river discharge or land run-off. Consequently, the sources of new P are limited relative to those of N in the open ocean, and as long as these ecosystems support the growth of N_2-fixing microorganisms, N should not limit primary production. Ultimately, other essential nutrients such as P may control oceanic productivity (Broecker 1974, Tyrell 1999).

Throughout the oligotrophic subtropical oceans, recent evidence suggests that the presence of both free-living and symbiotic diazotrophic organisms, including cyanobacteria (*Trichodesmium* spp. and *Synechococcus* spp.) and other unidentified N_2-fixing *Bacteria*, may contribute significantly to new production (Mitsui et al. 1986, Carpenter & Romans 1991, Karl et al. 1992, 1997, Letelier & Karl 1996, Capone et al. 1997, 1998, Zehr et al. 1998). In the North Pacific subtropical gyre (NPSG), N derived from N_2 fixation may contribute between 30 and 50% of the new N required to offset particulate N losses from the euphotic zone (Karl et al. 1997), and similar values have been estimated for the North Atlantic based on subeuphotic zone nutrient ratios (Michaels et al. 1996, Gruber & Sarmiento 1997). These data suggest either that the quantitative importance of N_2 fixation in open ocean nutrient cycles has been underestimated in the past or that recent habitat changes have selected for diazotrophic organisms in contemporary oligotrophic oceans, or both. Recently, Broecker & Henderson (1998) proposed that the N:P stoichiometry of marine organic matter would shift between glacial and interglacial periods and that conditions favoring N_2 fixation should lead to increased N:P ratios. In the surface waters of the NPSG both the C:P and N:P stoichiometries of the dissolved and particulate matter pools are well in excess of the classical Redfield ratios of 106:1 and 16:1 respectively (Karl et al. 1995, 2000b, Karl & Tien 1997, Hebel & Karl 2000). These observations, and others involving dissolved organic matter production (Karl et al. 1998), have been interpreted as evidence for P control.

In view of these recent reports, it has been hypothesized that the subtropical North Pacific has undergone a regime shift over the past few decades from a predominantly N-controlled system, supporting a traditional eukaryotic food web, to an ecosystem where biogeochemical processes revolve around bacterial metabolism, and where production ultimately is controlled by the availability of P (Karl 1999, Karl et al. 2000a).

In a system undergoing such shifts on decadal time scales, it becomes paramount to resolve the changes in nutrient dynamics and community structure to be able to make relevant predictions about the productivity of Earth's largest biome. However, a persisting problem in nutrient dynamic studies is the analytical challenge to describe the ecologically relevant nutrient pools and fluxes among them. As long as we are unable to identify the chemical constituents of these pools, and to what degree they are available to the extant microbial communities, nutrient dynamics will remain poorly constrained. This restriction will consequently affect the interpretation of ecosystem productivity and carbon (C) flux.

By comparison to the N cycle, studies of P cycling in the NPSG are limited. Furthermore, relatively few tracer studies have been conducted in marine environments compared to work on the P cycle in freshwater ecosystems (Schindler 1977, Berman 1988, Tarapchak & Moll 1990). There are some studies that have investigated uptake and regeneration of inorganic P (P_i) in oceanic waters (Perry & Eppley 1981, Cuhel et al. 1983, Sorokin 1985, Harrison & Harris 1986, Cotner et al. 1997), and others focused on the utilization of the dissolved organic P (DOP) pool (Kuentzler 1970, Orrett & Karl 1987, Björkman & Karl 1994). However, it is currently uncertain to what extent analytically defined pools correlate to the biologically available P (BAP) pool, which is clearly the pool of greatest interest from an ecological and biogeochemical perspective.

In this study, conducted between July 1996 and August 1997 in the NPSG, the results from experiments performed to quantify P dynamics in the upper water column are presented. The primary objectives were to: (1) investigate the coupled uptake and regeneration of P_i and the production of DOP, (2) quantify the size of the BAP pool, and (3) estimate the relative bioavailability of select organic P compounds.

MATERIALS AND METHODS

Locations and seawater sampling. The majority of the seawater samples were collected at Stn ALOHA (22.75° N, 158.00° W) during several Hawaii Ocean Time-series (HOT) cruises in 1996 and 1997. Additional experiments were conducted at a station within the 'Climax region' (Venrick et al. 1987) of the NPSG (28.11° N, 155.36° W) in July 1996 and 1997 (Fig. 1). Seawater samples for incubation experiments and analyses were routinely collected from a depth of approximately 5 m using either Go-Flo or Niskin type polyvinyl chloride bottles, except on the July 1997 cruise, when a peristaltic pump fitted with Tygon tubing was used. The seawater was pooled into a large carboy prior to subsampling for organic and inorganic nutrients, chlorophyll *a* (chl *a*) and cell enumeration and population composition determinations by flow cytometry. Seawater for particulate carbon (PC), nitrogen (PN) and phosphorus (PP) was collected separately, but within a few hours of the initial water collection.

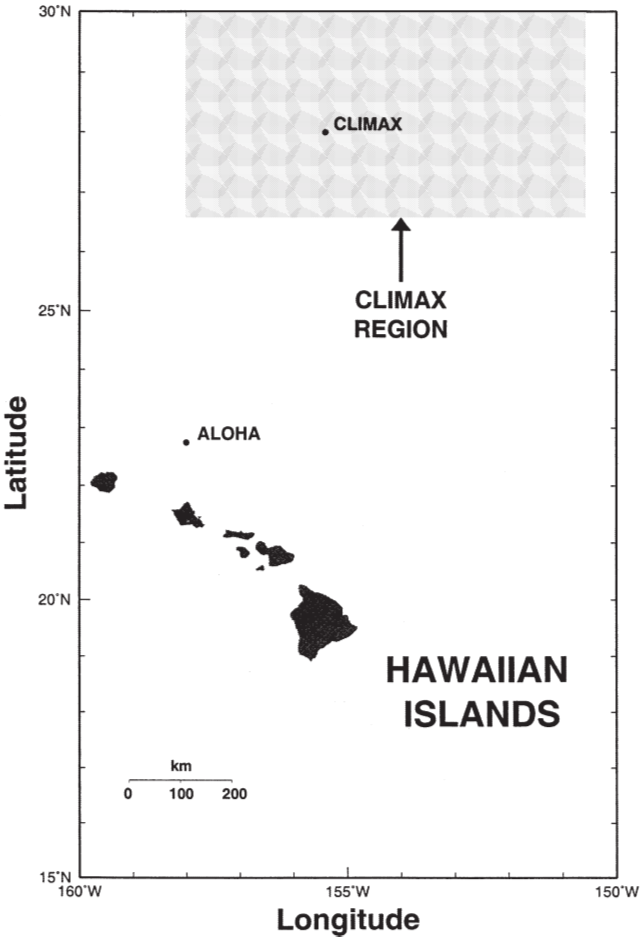

Fig. 1. Map of the sampling sites in the North Pacific subtropical gyre (NSPG). Stn ALOHA (22.75° N, 158.00° W) and Stn Climax (28.11° N, 155.36° W) within the Climax region (shaded box)

Ancillary analytical measurements. Samples for nutrient determinations were placed into high-density polyethylene bottles and stored frozen (Dore et al. 1996) for later analysis of: (1) nitrate plus nitrite [NO_3^- + NO_2^-], (2) soluble reactive phosphorus (SRP), (3) P_i, and (4) TDP and DOP concentrations (i.e., DOP = TDP – SRP). Samples for [NO_3^- + NO_2^-] were analyzed using an Antek model 720 chemiluminescence nitrogen oxide analyzer, according to the procedure described by Dore & Karl (1996) after Garside (1982). SRP analyses were performed using the magnesium-induced coprecipitation method (MAGIC; Karl & Tien 1992), and P_i analyses by the modified MAGIC procedure (Thomson-Bulldis & Karl 1998). TDP concentrations were determined using the wet persulfate oxidation procedure (Menzel & Corwin 1965) with modifications by Thomson-Bulldis & Karl (1998). All P analyses were corrected for arsenate interference (Johnson 1971) and measured by absorption spectrophotometry (880 nm; Beckman DU 640). For particulate matter analyses (PC, PN and PP), 4 l seawater was filtered through combusted glass fiber filters (Whatman GF/F) for PC/PN or combusted, acid-rinsed GF/F filters for PP (Karl et al. 1991). The PC/PN samples were analyzed using a Europa automated C and N analyzer. The PP concentrations were measured spectrophotometrically as dissolved P samples, following combustion (450°C, 4.5 h) and acid hydrolysis.

For fluorometric chl *a* determinations, triplicate 150 ml samples were filtered onto GF/F filters extracted in 100% acetone for 7 d in the dark at –20°C, then measured using a Turner model AU-10 fluorometer. Reagent chl *a* (#C-6144, Sigma Chemical Co., St Louis, MO) was used as the primary standard.

For particulate ATP determinations, triplicate 0.5 to 1.0 l samples were filtered onto GF/F filters and immediately extracted in 5 ml boiling Tris buffer (20 mM, pH 7.4). These samples were stored frozen for subsequent laboratory analysis by the firefly bioluminescence peak height method (Karl & Holm-Hansen 1978). ATP concentrations were extrapolated to total living microbial biomass C by assuming a C:ATP ratio by weight of 250:1 (Holm-Hansen 1973, Karl 1980).

Samples for flow cytometry were preserved in paraformaldehyde (0.2% final concentration) and frozen in liquid N_2 immediately after sampling (Campbell & Vaulot 1993). The samples were stained with Hoescht 33342 (1 µg ml^{-1}) and analyzed using a dual beam Coulter EPICS 753 flow cytometer (250 mW 306 nm, 1 W 488 nm; Monger & Landry 1993) The samples were analyzed for total microbial cell numbers and community composition as represented by non-pigmented prokaryotes (*Bacteria* and *Archaea*),

Synechococcus spp., *Prochlorococcus* spp. and pico-eukaryotic cells. These are operational definitions based on size, pigmentation and nucleic acid content. Cell numbers were extrapolated to biomass C using the following relationships: 10 fg C cell^{-1} for non-pigmented prokaryotes, 30 fg C cell^{-1} for *Prochlorococcus* spp., 100 fg C cell^{-1} for *Synechococcus* spp., and 495 fg C cell^{-1} for pico-eukaryotic cells (Christian & Karl 1994, Chavez et al. 1996, Zubkov et al. 1998). Further information on the HOT program sampling and analysis protocols can be found at the Website: http://hahana.soest.hawaii.edu/hot/hot_jgofs.html.

Radiotracer experiments. The rates of P_i uptake and regeneration under a variety of conditions were determined using exogenous $^{32}P_i$ as a tracer (orthophosphoric acid, carrier free; ICN Radiochemicals, #64014 L). Seawater samples were dispensed into acid-washed, sample-rinsed, 500 ml polycarbonate incubation bottles and, depending on the experiment, spiked with $^{32}P_i$ to a final activity between 9 and 28 MBq l^{-1}. The bottles were placed in an on-deck incubator maintained at ambient sea surface water temperature. A similar design was used in a variety of experiments, as described below.

During each HOT cruise a single dawn to dusk (10 to 14 h depending on season) ^{14}C-bicarbonate *in situ* primary production experiment was conducted according to the methods described previously (Karl et al. 1996).

Light and dark uptake of $^{32}P_i$ and production of DO^{32}P. To investigate the effect of irradiance on P_i uptake, replicate sample bottles were incubated either in continuous darkness or under natural light conditions (day-night). A 100 ml portion from each sample treatment (conducted in duplicate) was collected immediately after $^{32}P_i$ addition and filtered through a 0.2 µm polycarbonate membrane filter (Nuclepore) to serve as the time zero measurement for ^{32}P-particulate activity. Subsequent samples were collected at various intervals, usually 4 to 6 times, over an 18 to 24 h period. The filters were placed into borosilicate scintillation vials for later liquid scintillation counting (LSC) and the 0.2 µm filtrate was collected and immediately frozen for subsequent analyses of total SRP and DOP concentrations, and $^{32}P_i$ labeled DOP (DO^{32}P).

In related P_i uptake experiments a sterile-filtered (0.2 µm) phosphate (KH_2PO_4) stock solution (125 µM) was added to seawater samples to effect an exogenous P_i addition of 25, 50, 75, 125 and 250 nM. The final P_i concentrations were determined by laboratory analyses, as described above. The P_i additions were made immediately after the $^{32}P_i$ spike had been added. Subsamples were taken 4 times over a 12 h incubation period.

BAP experiments. Compounds investigated as potential P sources for microbial metabolism were obtained from the Sigma Chemical Company and prepared as 2 mM P stock solutions in sterile filtered (0.2 µm) distilled water. Aliquots were stored at –20°C to minimize hydrolysis. The compounds used were: glucose-1-phosphate (G-1-P; #G-9380), glycerophosphate (GYP; #G-6014), phosphoenol pyruvate (PEP; #P-7002), ribulose-1,5-bisphosphate (RuBP; #R-0878), adenosine-5′-triphosphate (ATP; #A-5394), guanosine-5′-diphosphate (GDP; #G-8877) and ribonucleic acid (RNA; #R-7125). The stock solutions were added to the incubation bottles immediately after the addition of the $^{32}P_i$ to achieve a final concentration of approximately 250 nM P. Unsupplemented seawater served as a negative control, and seawater with an addition of P_i equivalent to that of the exogenous organic P treatments (250 nM P) served as a positive control for BAP. To measure ^{32}P particulate radioactivity, triplicate 10 ml subsamples were collected at approximately 4 to 6 h intervals over a 24 h incubation period and were filtered onto 0.2 µm polycarbonate filters (Nuclepore). Unfiltered subsamples were also collected for total $^{32}P_i$ radioactivity. Following the 24 h sampling period the remaining seawater was collected for endpoint SRP and TDP analysis. An identical set of incubations without the $^{32}P_i$ radiotracer was run in parallel to monitor changes in the SRP concentration over the incubation period. At t = 0, 12 and 24 h 100 ml portions were collected from the non-radiolabeled series for SRP and TDP measurements. All samples were immediately frozen and stored at –20°C for subsequent laboratory analyses.

Intracellular ATP pool labeling experiments. To assess the size of the initial BAP pool the specific labeling of the γ position of ATP was used (Karl & Bossard 1985). On 4 occasions experiments were conducted to measure the kinetics of ATP pool labeling following the addition of $^{32}P_i$. Samples were collected 9 times over a 12 h incubation period, with more frequent sampling in the beginning, when the rate of change of the specific activity of the particulate ATP pool is expected to be the greatest. At each sampling time the content of an entire incubation bottle (450 ml) was filtered through a GF/F filter and extracted in boiling Tris buffer (see above). The extract was stored frozen until further processed following procedures described elsewhere (Karl & Bossard 1985, Bossard & Karl 1986, Karl et al. 1987, Karl 1993). Briefly, the Tris extract was concentrated by vacuum evaporation and the ATP purified using polyethyleneimine (PEI) thin-layer chromatography. The isolated ^{32}P-ATP was eluted from the PEI matrix and hydrolyzed with apyrase (Sigma Chemical Co., #A6132) to estimate the respective radioactivities of the α, β, and γ positions of ATP as a function of time. The data were fitted using a non-linear function formula, similar to PROCNLIN (Karl et al. 1987), to estimate the maximum specific radioactivitity of the γ P. This value was used to estimate the size of the BAP pool.

Laboratory analysis and calculations. Radioactivity was determined using a Packard Tri-Carb® scintillation counter. Aquasol II was used as the fluor, and the samples were quench corrected using Packard Instruments SIS protocols. Uptake rates of $^{32}P_i$ were determined from the slope of the ^{32}P-particulate activities versus incubation time, calculated from the least square regression analysis over a time period where the uptake was linear, generally the first 8 to 12 h of incubation. Turnover time (T, in days) was calculated as:

$$T = a/r \quad (1)$$

where a is the total $^{32}P_i$ activity (Bq l^{-1}) added to the samples and r is the uptake rate into the particulate fraction (Bq l^{-1} d^{-1}). The rate of phosphate uptake, expressed as nM SRP d^{-1}, was calculated from T and the measured concentration of the SRP. Note, however, that these uptake rates must be regarded as approximations as they are based on analytically derived values for phosphate pool concentrations and the rate estimate will depend on the choice of analysis. Theoretically, the potential BAP pool concentrations can range from free P_i to TDP.

In the experiments where known organic P compounds were tested, the net regeneration of P_i over an approximately 24 h incubation period, expressed as nM P d^{-1}, was termed the phosphorus regeneration potential (PRP). The PRP was calculated as the change in SRP concentration in the organic P amended samples over time and corrected for any changes in the control samples:

$$PRP = ([SRP_E] - [SRP_C])/t \quad (2)$$

where SRP_E and SRP_C are the net changes in SRP (nM) during the incubation period in the experimental and control samples respectively, and t is the incubation time in days.

The relative bioavailability of the different organic P compounds tested, the bioavailability factor (BF), was estimated as previously described (Björkman & Karl 1994). BF is the isotope dilution effect in $^{32}P_i$ uptake caused by a particular organic P compound, relative to the P turnover time in control incubations:

$$BF = (T_E - T_N)/(T_P - T_N) \quad (3)$$

where T_E is the P_i pool turnover time in the experimental treatment, T_N is the P_i pool turnover time in the negative control (no P addition), and T_P is the P_i pool turnover time in the positive control (P_i addition). In theory BF values can range from 0 to 1, where 0 represents a biorefractory substrate and 1 is a P compound with a bioavailability equal to that of P_i.

Production rates of DOP were measured using the modified MAGIC method (Thomson-Bulldis & Karl 1998). Analyses were performed using the filtrates collected from the light and dark P_i uptake experiments. A portion of each sample was placed into a 50 ml centrifuge tube and a 1 ml subsample was removed, for the measurement of the total ^{32}P activity, prior to an addition of a 0.5% vol/vol ratio of NaOH (1 M). The samples were mixed, then centrifuged for 1 h at $1000 \times g$ to separate $^{32}P_i$, which is co-precipitated with brucite ($Mg(OH)_2$), from the organic P compounds that largely remain in solution (Thomson-Bulldis & Karl 1998). A portion of the supernatant was analyzed for ^{32}P activity by LSC. The rate of $DO^{32}P$ production was determined from the increase in ^{32}P activity over time during the time period where the production was linear (usually 0.5 to 1 d). The calculation of DOP production rates, as nM d^{-1}, was made by assuming that newly formed DOP will have a specific activity equivalent to the initial specific activity of the P_i pool (i.e., Bq ^{32}P mol^{-1} [$^{32}P_i$ + $^{31}P_i$] = Bq $DO^{32}P$ mol^{-1} [$DO^{32}P$ + $DO^{31}P_{new}$]). T of the ambient DOP pool was then calculated as:

$$T = [DOP]/PR_{DOP} \quad (4)$$

where PR_{DOP} is the production rate of DOP in nM d^{-1}, and [DOP] is the concentration of the DOP in the supernatant of the analyzed sample.

RESULTS

Dissolved and particulate material and microbial community composition

Ambient inorganic nutrient concentrations were low at all stations, typical for the oligotrophic NPSG (Table 1). SRP concentrations averaged < 50 nM, with a minimum of 14 ± 1 nM at Stn Climax in July 1997. P_i averaged 63 ± 10% (range 42 to 93%) of the corresponding SRP concentrations. For all station locations and dates, DOP exceeded P_i, sometimes by 1 order of magnitude (Table 1).

For all station locations and dates, DON exceeded [NO_3^- + NO_2^-] concentrations by 3 to 4 orders of magnitude (Table 1). The TDN:TDP ratios were consistently enriched in N relative to P and ranged from 17:1 to 39:1 (median 22:1, mean 23.6:1, n = 7), as were the ratios of DON:DOP, compared to the classical Redfield molar ratio of 16N:1P (Redfield et al. 1963).

The mean particulate matter concentration in surface waters at Stns ALOHA and Climax was 2.31 ± 0.24 µM PC (n = 6), 0.30 ± 0.08 µM PN (n = 7), and 14.8 ± 1.8 nM PP (n = 7; Table 2). The N:P ratios in the particulate matter during summer ranged from 18:1 to 24:1 (mean 20:1, median 22:1, n = 6). The estimated total living biomass by particulate ATP ranged from 6.7 to 15.4 µg C l^{-1} (mean 9.1 ± 2.9 µg C l^{-1}, median 8.0 µg C l^{-1}, n = 7).

Table 1. Dissolved phosphorus and nitrogen concentrations and concentration ratios for water samples collected at 5 m depth in the North Pacific subtropical gyre (NPSG) at Stn ALOHA (22.75° N, 158.00° W) and Stn Climax (28.11° N, 155.36° W). SRP = soluble reactive phosphorus, P_i = inorganic phosphate, DOP = dissolved organic phosphorus (TDP–SRP), TDP = total dissolved phosphorus, BAP = biologically available phosphorus, N+N = [NO_3^- + NO_2^-], TDN = total dissolved nitrogen. – = not determined, BDL = below detection limit

Stn	Cruise date	SRP (nM)	P_i (nM)	DOP (nM)	TDP (nM)	BAP (nM)	N+N (nM)	TDN (nM)	DON:DOP (mol:mol)	TDN:TDP (mol:mol)
ALOHA	Jul 1996	119 ± 2	76 ± 2	231 ± 2	350	–	1.0	5870	25:1	17:1
Climax	Jul 1996	45 ± 3	19 ± 3	140 ± 3	185	–	BDL	4600	33:1	24:1
ALOHA (HOT 78)	Dec 1996	25 ± 0	–	245 ± 0	270	–	0.5	5250	21:1	19:1
ALOHA (HOT 84)	Jun 1997	55 ± 2	–	285 ± 2	340	111 ± 16	0.3	5730	20:1	17:1
ALOHA	Jul 1997	43 ± 1	31 ± 3	195 ± 1	238	56 ± 6	0.4	5210	27:1	22:1
Climax	Jul 1997	14 ± 1	13 ± 2	141 ± 1	155	13 ± 1	0.8	6120	43:1	39:1
ALOHA (HOT 86)	Aug 1997	22 ± 1	–	238 ± 1	260	50 ± 3	0.2	6940	29:1	27:1

Flow cytometry data revealed a remarkably similar composition of the microbial community between stations and years (Table 3). The microbial community was dominated by prokaryotic organisms. *Prochlorococcus* spp. dominated the phototrophic component (>97% by numbers) and constituted between 20 and 30% of the total prokaryotes. In biomass terms *Prochlorococcus* spp. contributed 6.6 ± 1.2 µg C l^{-1} (range 5.1 to 8.6 µg C l^{-1}, n = 7), or 50.1 ± 10.7% of the total microbial biomass (Table 3).

Table 2. Living and total particulate matter pools for water samples collected in the NPSG. chl *a* = chlorophyll *a*, ATP = adenosine-5′-triphosphate, PC = particulate carbon, PN = particulate nitrogen, PP = particulate phosphorus. Values are mean ± 1 standard deviation (where shown). For PC, PN and PP samples n = 1. – = not determined

Stn	Cruise date	Chl *a* (ng l^{-1})	ATP (ng l^{-1})	PC (µM)	PN (µM)	PP (µM)	PC:PN:PP (mol)
ALOHA	Jul 1996	40 ± 1	26.8	–	0.22	0.013	– :18:1
Climax	Jul 1996	55 ± 6	34.7	2.53	0.36	0.015	163:23:1
ALOHA	Dec 1996 (HOT 78)	150 ± 0	31.9	2.13	0.16	0.013	170:12:1
ALOHA	Jun 1997 (HOT 84)	58 ± 1	30.3	2.16	0.29	0.013	163:22:1
ALOHA	Jul 1997	72 ± 2	32.8	2.00	0.36	0.017	119:22:1
Climax	Jul 1997	71 ± 2	36.6	2.57	0.33	0.017	156:20:1
ALOHA	Aug 1997 (HOT 86)	54 ± 3	61.4	2.47	0.37	0.016	156:24:1

Phosphate uptake rates and turnover times

Net phosphate uptake rates in the light at ambient P concentrations varied between 3.0 and 8.2 nM d^{-1} (median = 3.5 nM d^{-1}, n = 7; Table 4). The turnover times of the P_i pool varied from 2 to 40 d (median = 9 d, n = 7). Uptake and regeneration rates appeared to be balanced, because the concentration of SRP remained constant over the course of incubation. However, in samples with exogenous P_i additions ≥ 2-fold the ambient concentrations, an uncoupling between P_i uptake and P_i regeneration was observed. This resulted in a net decrease in SRP concentration with time at Stn ALOHA (Table 5). Uptake rates in these latter experiments increased with higher P_i loading and reached the highest net uptake rate (6.3 ± 0.3 nM d^{-1}) at a P_i concentration of 170 nM at Stn ALOHA. At Stn Climax, however, the P_i uptake rates continued to increase linearly even up to the highest P_i concentration tested (Table 5).

Light versus dark phosphate uptake

The uptake rates from day-night incubation experiments were compared to bottles kept in continuous darkness for 24 h. In 3 out of 5 experiments the particulate uptake rates in the dark were re-

Table 3. Microbial population estimates and community composition for water samples collected in the NPSG. HB = heterotrophic bacteria (i.e., non-pigmented *Bacteria* and *Archaea*), PRO = *Prochlorococcus* spp., SYN = *Synechococcus* spp., PEUK = pico-eukaryotic cells, TM = total microbial cells by flow cytometry, ATP = adenosine-5′-triphosphate. Biomass (µg C l^{-1}) were based on the following conversion factors (g/g): C/ATP = 250 (Karl 1980). For cell numbers (fg $cell^{-1}$): HB = 10 (Christian & Karl 1994), PRO = 30, SYN = 100, PEUK = 495 (Chavez et al. 1996, Zubkov et al. 1998)

Stn	Cruise date	Cell numbers (10^6 l^{-1})					Biomass (µg C l^{-1})					
		HB	PRO	SYN	PEUK	TM	ATP	HB	PRO	SYN	PEUK	TM
ALOHA	Jul 1996	596	230	1.76	0.05	828	6.7	5.96	6.90	0.18	0.02	13.06
Climax	Jul 1996	616	242	2.78	0.10	861	8.7	6.16	7.27	0.28	0.05	13.76
ALOHA	Dec 1996 (HOT 78)	450	235	1.27	1.05	687	8.0	4.50	7.05	0.13	0.51	12.19
ALOHA	Jun 1997 (HOT 84)	530	194	1.26	0.39	726	7.6	5.30	5.82	0.13	0.19	11.44
ALOHA	Jul 1997	701	184	2.05	1.00	889	8.2	7.01	5.53	0.20	0.50	13.24
Climax	Jul 1997	663	169	3.62	0.90	836	9.1	6.63	5.07	0.36	0.45	12.51
ALOHA	Aug 1997 (HOT 86)	684	287	1.92	1.01	974	15.4	6.84	8.61	0.19	0.50	16.14

duced by 40 to 50% compared to rates in light incubations. However, on 2 separate occasions at Stn ALOHA (December 1996, July 1997), the light and dark phosphate uptake rates were nearly identical (Fig. 2, Table 4).

DOP production rates and DOP pool turnover

Net DOP production rates ranged from 0.6 to 2.5 nM P d^{-1}, equivalent to approximately 10 and 40%, respectively, of the net P_i uptake rate (Table 4). At a given station this ratio was constant regardless of whether the incubations were day-night or dark treatments, with the exception of the December 1996 experiment (HOT 78), for which we also observed a significant light versus dark difference in P_i uptake rates. The turnover times of the total DOP pool ranged from 40 to 300 d.

Bioavailable phosphorus pool

Several estimates of the BAP pool were made using different techniques. Results from experiments using additions of known concentrations of P_i to seawater samples, to ascertain if the bioavailable fraction differed from the ambient P_i pools, were inconclusive. These experiments indicated either that the measured P_i concentrations were equal to the BAP pool or that the exogenous P_i caused an increase in community uptake rates (Table 5), thereby violating the primary assumption of this experimental design. When using the specific radioactivity of the intracellular ATP pool as a means of assessing the total BAP pool, results indicated that the BAP pool could range from being equal to, or up to twice that of, the measured P_i pool (Table 1).

Table 4. Phosphate uptake rates, production of DOP, and primary production measured by ^{14}C-bicarbonate incorporation for water samples collected in the NPSG. SRP uptake = soluble reactive phosphorus uptake based on the SRP or P_i concentrations, P = phosphorus, 1° production = primary production. C:P uptake ratios were calculated by converting 1° production from µg l^{-1} h^{-1} to nM h^{-1} and dividing by the light-dependent P uptake rate (nM h^{-1}). – = not determined

Stn	Cruise date	SRP uptake (nM d^{-1})		P turnover time (d)		1° production (µg C l^{-1} h^{-1})	DOP production (nM P d^{-1})		C:P uptake (mol:mol)
		Light	Dark	Light	Dark		Light	Dark	
ALOHA	Jul 1996	3.02 ± 0.25	1.40 ± 0.02	40	85	0.57	–	–	377:1
Climax	Jul 1996	3.05 ± 0.67	1.92 ± 0.12	9	15	0.49	–	–	321:1
ALOHA	Dec 1996 (HOT 78)	8.17 ± 0.73	10.67 ± 1.93	3	2.4	0.39 ± 0.09	2.32 ± 0.21	0.97 ± 1.34	95:1
ALOHA	Jun 1997 (HOT 84)	3.80 ± 0.89	–	14	–	0.63 ± 0.17	–	–	333:1
ALOHA	Jul 1997	3.47 ± 0.18	3.73 ± 0.24	9	8	–	0.72 ± 0.12	0.62 ± 0.07	–
Climax	Jul 1997	6.59 ± 0.60	3.14 ± 0.31	2	4	0.26 ± 0.01	2.46 ± 0.53	1.22 ± 0.17	74:1
ALOHA	Aug 1997 (HOT 86)	3.36 ± 0.12	–	6	–	0.25 ± 0.00	–	–	146:1

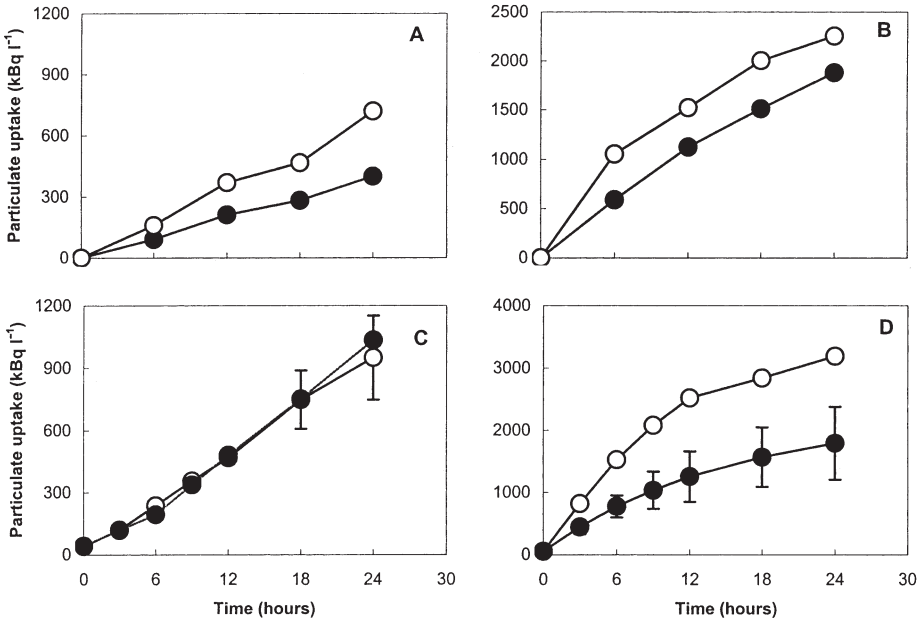

Fig. 2. Representative time-course experiment showing the uptake of $^{32}P_i$ by natural assemblages of microorganisms collected in the NPSG. Incubations were either in continuous darkness (●) or under natural light (○). (A) ALOHA July 1996, (B) Climax July 1996, (C) ALOHA July 1997, (D) Climax July 1997. Error bars are ± 1 standard deviation from the mean of duplicate samples. Error bars are sometimes smaller than the symbols used

Bioavailability of selected DOP compounds

When specific combined P compounds were added to seawater samples, nucleotides and RuBP appeared to be more bioavailable than monophosphate esters. However, P_i was always the most readily available, and presumably the 'preferred' substrate for the growth of marine microorganisms in the NPSG. The PRP varied among the tested compounds and among cruises, ranging from 0 nM d^{-1} for PEP to over 200 nM d^{-1} for ATP. The proportion of the exogenous DOP converted to SRP over a 24 h period could be >65% for selected compounds; the highest values were observed for nucleotides. The PRP was generally higher than the calculated net uptake rates of SRP which ranged from 2.9 to 9.6 nM d^{-1} in the control incubations, and the P release rates from exogenous nucleotides could exceed the rate of P uptake by as much as 50-fold (Fig. 3, Table 4).

DISCUSSION

At the time of the establishment of the HOT program and its deep water hydrographic Stn ALOHA in October 1988, the NPSG was largely considered a relatively homogenous and static environment (Karl & Lukas 1996). However, over the past decade a new view has emerged, indicating that this ecosystem is more dynamic than previously assumed (Karl 1999). Historical data on nutrient dynamics in the oligotrophic NPSG, to a large extent, stem from studies conducted within the Climax region, which was intermittently sampled between 1968 and 1985 (Hayward 1987). Observations made in the Climax region over this nearly 2-decade-long investigation period revealed several significant changes in fundamental biological parameters. Venrick et al. (1987), for example, observed a nearly 2-fold increase in chl *a* concentration (integrated 0 to 200 m, 12.7 ± 3.4 mg m^{-2} prior to 1973, 22.0 ± 2.2 mg m^{-2} following 1980) over the 17 yr period. At Stn ALOHA the chl *a* concentrations have remained at a level consistent with the higher chl *a* reported by Venrick et al. (1987) at 22.5 ± 4.6 mg m^{-2} (integrated 0 to 200 m) since the beginning of the HOT program in 1988 (Karl et al.

Table 5. Effects of P_i addition on rates of phosphate uptake for samples collected in the NSPG. ΔP_i = difference between initial and endpoint P_i concentration, P_i turnover = phosphate pool turnover time. Data are mean ± 1 standard deviation (where shown)

Sample and treatment	P_i (nM)	ΔP_i (nM)	P_i uptake (nM d^{-1})	P_i turnover (d)
Stn ALOHA				
Control	31 ± 2	–	3.3 ± 0.1	10.3
1	54 (n = 1)	1 ± 2	3.3 ± 0.1	16.2
2	99 (n = 1)	−30 ± 11	5.1 ± 1.2	19.6
3	171 ± 2	−42 ± 11	6.3 ± 0.3	26.9
4	326 ± 4	−88 ± 4	5.9 ± 0.6	55.6
Stn Climax				
Control	13 ± 1	–	6.6 ± 0.6	2.0
1	36 ± 2	−4 ± 2	6.2 ± 0.9	5.8
2	49 ± 0	0 ± 2	5.8 ± 0.6	8.5
3	72 ± 8	2 ± 8	6.5 ± 0.9	11.1
4	138 ± 4	7 ± 4	7.5 ± 1.4	18.4
5	249 ± 0	24 ± 1	9.2 ± 1.5	27.0

2000a). Furthermore, recent evidence suggests that the ecosystem has undergone dramatic changes in the past decade on several levels, including a change from a predominantly N-controlled to a potentially P-controlled environment, with profound effects on food web structure and nutrient cycling pathways (Karl 1999). These ecosystem changes are hypothesized to be governed by predominantly climatological factors, such as an intensification of the occurrence and duration of El Niño Southern Oscillation (ENSO) events, which have given rise to changing circulation and mixing patterns, and the Pacific Decadal Oscillation (PDO). These conditions are believed to have selected for N_2-fixing organisms, such as *Trichodesmium* spp., and their activities have resulted in a replenishment of the fixed N inventory in the upper ocean driving the NPSG ecosystem towards P limitation (Karl et al. 1995, 1997, Cullen et al. 2000).

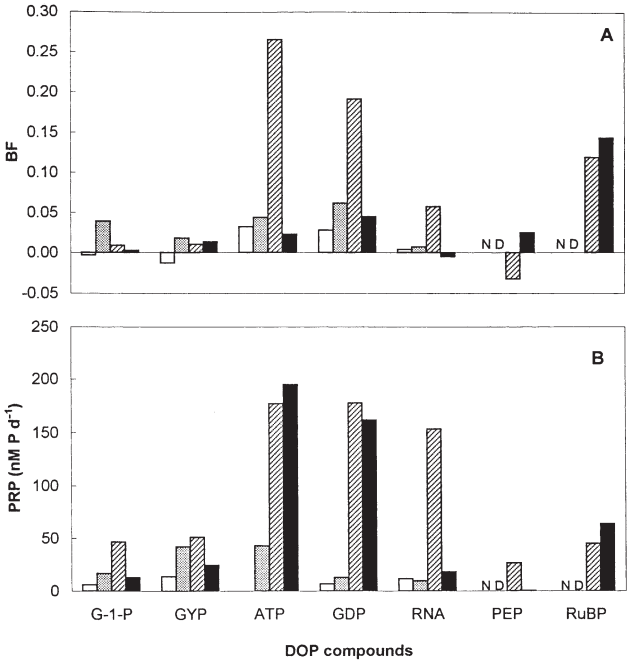

Fig. 3. Bioavailability of selected DOP compounds to natural assemblages of microorganisms in seawater samples collected in the NPSG. All exogenous compounds were added at 250 nM P. G-1-P = glucose-1-phosphate, GYP = glycerophosphate, ATP = adenosine-5′-triphosphate, GDP = guanosine-5′-diphosphate, RNA = ribonucleic acid, PEP = phosphoenol pyruvate, RuBP = ribulose-1,5-bisphosphate. (A) Bioavailability factor (BF). This is an experimentally derived estimate of the utilization of each compound tested, measured by the isotope dilution of $^{32}P_i$ relative to a positive control sample with an equivalent inorganic phosphorus (P_i) addition, the presumed preferred substrate, and a negative control with no exogenous substrate. Scaled bioavailability ranges from 0 to 1, with a score of 0 indicating an unavailable substrate and 1 indicative of a substrate with an availability equivalent to that of P_i. See text for further details. Negative BF values result from a slight enhancement in uptake rates in incubations with exogenous DOP relative to the negative controls. (B) Phosphorus regeneration potential (PRP). This is the experimentally derived estimate of P_i release from each 250 nM substrate addition following a timed incubation of 24 h. Bars are: ALOHA July 1996 = unfilled, Climax July 1996 = stippled, ALOHA July 1997 = hatched, Climax July 1997 = solid, ND = no data

P limitation has recently been demonstrated in other marine environments such as in the Sargasso Sea (Cotner et al. 1997, Wu et al. 2000) and the eastern Mediterranean Sea (Krom et al. 1991, Zohary & Robarts 1998). Both of these ecosystems exhibit extremely low P_i concentrations (<10 nM) and TDN:TDP ratios of >20:1, similar to present ratios observed in the NPSG. One proposed reason for the current P limited state of the North Atlantic region is an increased deposition of iron (Fe)-rich eolian dust. This may have relieved Fe deficiency in diazotrophic organisms and consequently allowed for increased N_2 fixation (Cotner et al. 1997). Alternatively, Fe-rich clay particles may provide a matrix onto which P is selectively adsorbed and subsequently removed from the productive layer as suggested by Krom et al. (1991) for the eastern Mediterranean.

The P uptake rate calculations presented herein are based on the specific radioactivity of a substrate (i.e., radioactivity per unit concentration). To accurately make these extrapolations, it is necessary to know the pool size into which the radiotracer dilutes. The P pools described here are operationally defined by the method of analyses. The SRP concentrations were determined by the MAGIC method (Karl & Tien 1992), P_i by the modified MAGIC procedure (Thomson-Bulldis & Karl 1998), and soluble non-reactive phosphorus (assumed to be DOP) by calculating the difference between TDP and SRP (DOP = TDP – SRP). However, SRP, often equated to orthophosphate, will contain both P_i and some portion of the base and acid labile DOP pool. The modified MAGIC method will have a smaller, if any, contribution from the DOP pool constituents. Likewise, the DOP pool may well contain non-reactive inorganic combined P compounds. Ultimately, we seek an accurate measure of the BAP pool. In field experiments, added $^{32}P_i$ will presumably dilute with the ambient $^{31}P_i$ pool; however, in terms of biological uptake, the tracer will also be diluted by all BAP compounds as well as intracellular pools of P. As such it is necessary, yet presently impossible, to determine the true size of the ecologically relevant P pool. Nevertheless, the size of the BAP pool was estimated by kinetic labeling experiments of the terminal (γ) P of particulate ATP during 4 cruises in 1997. Because γ ATP has one of the most rapid turnover times of any intracellular P pool, and ATP is ubiquitous in all living cells, the dynamics of this pool can provide invaluable information on P sources and fluxes in natural environments (Karl &

Bossard 1985). It can be assumed that the specific activity of the γ P of ATP, when the intracellular pool reaches its isotopic equilibrium, will reflect the true specific activity of the external BAP pool. At Stn Climax the BAP pool was equal to the SRP and P_i pool at 13 nM, which is remarkable considering that the DOP pool was 141 nM. However, at Stn ALOHA the BAP pool was about 2-fold larger than the P_i pool, and the SRP pool was intermediate between the P pool estimates, suggesting that a fraction of the ambient DOP pool was being used simultaneously by the microbial assemblages (Table 1). These differences imply variability in either the microbial population structure, physiological capabilities or chemical composition of the respective DOP pools at the time of sampling, or possibly all three.

Our P uptake rates, based on SRP or P_i concentrations, are comparable to values found by Perry & Eppley (1981) and Sorokin (1985) for samples collected in the NPSG (1.2 and 1.3 to 6.5 nM P d^{-1}, respectively). Our estimates of the turnover time of the BAP pool are at the lower end of the 19 to 31 d range reported by Perry & Eppley (1981) and significantly shorter than the 40 to 100 d reported by Sorokin (1985). However, data from the hyperoligotrophic Sargasso Sea show P_i turnover times of approximately 9 h (Cotner et al. 1997), whereas turnover times in the eastern Mediterranean were highly variable (range: hours to over 35 d) even at persistently low concentrations of SRP (Zohary & Robarts 1998). When uptake rate calculations were made by applying the BAP pool concentrations, the P_i uptake rates were approximately equal between stations. Also, the difference observed in P concentrations between the two MAGIC procedures used here indicated that a variable portion of the DOP pool was contributing analytically to the determination of SRP concentrations, as previously noted by Thomson-Bulldis & Karl (1998). Clearly, differences in the BAP pool size relative to the commonly measured concentrations of SRP, or P_i pool, will have direct consequences in uptake rate estimations as exemplified here. Furthermore, the chemical characteristics of the DOP pool as well as the capacity of the extant community to process DOP is obviously of great importance and will inevitably affect the fate of any given DOP compound. These parameters may well change on temporal and spatial scales and more intensive studies will need to be conducted to elucidate the reason for these differences in BAP pool size and its turnover in different habitats and at different times.

Equally problematic as the need for accurate estimations of the BAP pool is the complete chemical characterization of the P pool constituents, especially the dominant DOP pool. Partial characterization of the DOP pool constituents at Stn ALOHA, based on relative UV photolytic stability, has shown that the DOP pool includes both monophosphate esters and nucleotides, and that the relative chemical composition varies on both temporal and spatial scales (Karl & Yanagi 1997). However, the origin of this observed variability is unresolved, as is the ecological impact of a chemically dynamic DOP pool. The bioavailability of a certain compound is clearly a time-dependent phenomenon, and is possibly also affected by changing community composition with depth (Simidi & Tsukamoto 1985), as evident from the decrease in DOP, and parallel increase of P_i concentrations, with depth. In this present study of phosphorus bioavailability of known DOP compounds, it appeared as if P_i was the most readily available form of P, a result that is similar to the pattern observed in Hawaiian coastal waters (Björkman & Karl 1994). Although the P_i uptake rates were about 1 order of magnitude lower than those observed in coastal waters (Björkman & Karl 1994), this may be a result both of lower standing stocks of microorganisms and of concentrations of BAP below the threshold for rapid uptake to take place. The very low PRP and BF at Stn ALOHA in July 1996 (Fig. 3) may be explained by the unusually high SRP concentrations in the surface waters at that time (~120 nM, Table 1). Presumably, these high SRP concentrations were sufficient to satisfy P requirements and may have inhibited/repressed phospho-hydrolytic enzymes, resulting in additional P sources not being simultaneously utilized. The high PRP rates of nucleotides imply enzymatic activities typically ascribed to 5′-nucleotidase. This ectoenzyme has been shown to regenerate P at a rate that frequently exceeds microbial P uptake rates (Siuda & Güde 1994), and has been hypothesized to provide sufficient P to fulfill microbial requirements (Tamminen 1989, Ammerman 1991). The high PRP of nucleotides may be an indirect result of microbial scavenging of purine or pyrimidine bases for cellular biosynthesis, rather than providing a primary source for P, hence, net P regeneration. Such a scavenging mechanism could result in the rapid turnover of at least a portion of the DOP pool, and may be important in P dynamics as a whole. Moreover, the production of DOP was substantial compared to net P_i uptake rates, indicating that the gross P_i uptake may be much greater than the net P_i uptake reported herein.

DOP is derived from several independent sources. In general DOP is believed to be a product of grazing activities, exudation from healthy cells, leaching from decaying material (Fogg 1966, Sharp 1977, Wood & van Valen 1990), and virus-induced cell lysis (Proctor & Fuhrman 1990). The experiments in this study were not designed to elucidate the sources of DOP formation, but rather to estimate the dynamics of this pool. The fraction of particulate P uptake that resulted in

DOP production was similar for both light and dark incubations in 2 experiments. This may imply that a constant fraction of the $^{32}P_i$ incorporated into organisms is regenerated as DO^{32}P, regardless of whether the P incorporated was by phototrophic or heterotrophic processes. The estimated turnover time of the DOP pool of 60 to 300 d is substantially longer than the approximately 10 d calculated by Orrett & Karl (1987) for the NPSG, but within the range for DOP turnover of approximately 200 d calculated from data provided in Jackson & Williams (1985). (Note that the estimated DOP pool turnover time of 18 d in the original paper by Jackson & Williams is due to a conversion error in their calculation.) However, depending on the chemical composition of the DOP pool, significantly faster turnover times may occur for highly bioavailable DOP compounds, and nascent DOP may well be more readily available than older DOP. Consequently, a major uncertainty in these estimates remains due to the inability to characterize the presumably diverse DOP pool. Nevertheless, these estimates of DOP pool turnover times will most likely not be underestimates (see Eq. 4) as the DOP production rate is based on the specific activity of the P_i pool. If the P_i pool is less than BAP pool the DOP pool turnover time will be overestimated.

In our experiments, light versus dark incubations showed variable results, ranging from no discernible differences to enhanced P uptake rates in the light relative to dark incubations (Fig. 2). Perry (1976), in her work in the NPSG, and Herbland (1984), in the Equatorial Atlantic Ocean, did not observe diel changes in P_i uptake rates, nor did light intensity appear to affect P_i uptake rates. However, other studies of natural populations and phytoplankton cultures have shown both increased uptake in light and circadian patterns in P_i uptake rates (Chisholm & Stross 1976, Reshkin & Knauer 1979). As the uptake of P_i is an energy-dependent process, uptake in the light may be enhanced if the cells are energy limited in the dark. In the case for phototrophs this may be the lack of light driven energy, and for heterotrophic microorganisms a possible shortage of readily available organic compounds derived from photosynthetic processes (Karl et al. 1998). As such the heterotrophic component may be more active in the light than in the dark, biasing uptake estimates for the phototrophic component. Also, uptake by the phototrophic component in the dark may occur. Furthermore, many experimental designs use only dark incubations when studying heterotrophic microorganism metabolic activities. This may confound any interpretations of the relative metabolic contribution of these 2 functional groups of microorganisms.

The microbial community composition, as determined by flow cytometry, revealed a system numerically dominated by prokaryotes, in both its heterotrophic and phototrophic components, the phototrophs consisting almost exclusively of *Prochlorococcus* spp. This community composition is similar to that previously reported for Stn ALOHA (Campbell et al. 1994, 1997). However, the total microbial biomass extrapolated from these data, using cell number to carbon conversion factors as reported above, always exceeded that estimated from particulate ATP presumed to represent the total living microbial biomass (Table 3). This may be the result of a shift in the C:ATP ratio and would be expected under P-limited conditions. It has been shown in lake studies that the C:ATP ratio may fluctuate substantially, leading to high C:ATP values during periods of P deficiency (Cavari 1976). Consequently, the conversion C:ATP would have to be adjusted upward in environments experiencing P limitation (Karl 1980). When applying the total microbial biomass estimates from flow cytometry data to obtain a C:ATP ratio for Stns ALOHA and Climax, the values were on average higher than the conventionally employed 250:1 for C:ATP (mean 380 ± 70, n = 7, range 270 to 490), but within the upper boundary of 500:1 modeled by Christian & Karl (1994).

The biomass conversion factors used herein are lower than those previously applied by Campbell et al. (1994) for *Prochlorococcus* spp. (30 vs 53 fg cell^{-1}), *Synechococcus* spp. (100 vs 175 to 250 fg cell^{-1}) and pico-eukaryotic cells (495 vs 2100 fg cell^{-1}). These conversion factors are based on recent revisions of biovolume: carbon estimates for these 2 groups of cyanobacteria and pico-eukaryotic cells (Chavez et al. 1996, Zubkov et al. 1998). Nevertheless, the estimated carbon biomass for *Prochlorococcus* spp. alone exceeded that calculated from chl *a* concentrations if using a C:chl *a* ratio of 50:1 (Christian & Karl 1994). This may be explained by chl *a* not being a good predictor of *Prochlorococcus* biomass (Karl et al. 2000a). Furthermore, biomass estimates based on Campbell et al. (1994) g C cell^{-1} values would exceed the total particulate carbon pool, the upper constraint on biomass C, at these locations. Although beyond the scope of this study, there still appear to be discrepancies among the different approaches to estimate carbon biomass in the oligotrophic ocean.

In P_i addition experiments performed in 1997, there were apparent increases in P_i uptake rates when external P_i concentrations exceeded 55 nM P, and at Stn ALOHA there was also a draw down of SRP at the higher concentrations (Table 5). This net P_i uptake may have been due to increased P_i uptake rates, and possibly luxury uptake, at higher P_i concentrations, generating a large enough offset between uptake and regeneration, and a sufficiently large overall P_i consumption to be analytically detected after a 24 h incubation period. The increased uptake rates may indi-

cate that the microbial community as a whole at these 2 stations was limited by available P, and that the ambient concentrations may be below the threshold levels required for optimal growth and production. In P starvation experiments with *Prochlococcus* spp., it was suggested that the growth of these organisms was limited by P_i when concentrations were in the range of 10 to 100 nM (Parpais et al. 1996). Also, it appeared as if the tested strains were unable to remove P_i at concentrations below 30 nM (Parpais et al. 1996). Although the P_i addition experiments were designed to elucidate the size of the BAP pool, the apparent rate change in P_i uptake made such an estimate impossible. Instead these data were used to estimate effects of substrate concentrations on P_i uptake kinetics.

A Lineweaver-Burke transformation was employed to estimate the values of V_{max} and the half-saturation constant K_m. At Stn ALOHA, K_m was 80 ± 5 nM and V_{max} was 8.8 ± 0.5 nM d^{-1}. For Stn Climax, K_m was about half of that observed at Stn ALOHA at 39 ± 1 nM, but V_{max} was nearly identical at 10.1 ± 0.1 nM d^{-1}. Perry & Eppley (1981) reported K_m values of 180 nM for summertime samples collected in the NPSG. The lower K_m we observed here may be in response to the depletion of SRP in the upper water column over the last decade (Karl & Tien 1997, Karl et al. 2000a). However, in the Sargasso Sea, which appears to be under severe P stress, K_m has been estimated to be as low as 5 to 10 nM and V_{max} 2-fold higher than what we observed here (Cotner et al. 1997). Although caution should be taken in using these P uptake kinetics as direct indications of P limitation, they may, when combined with other observations, add to the understanding of nutrient dynamics in the NPSG. Nevertheless, the immediate increase in uptake rates in supplemented incubations implies a microbial community pre-adapted to utilizing any available P above a certain concentration, indirectly indicating that ephemeral patches with higher concentrations of P, or stochastic mixing events, may be exploited efficiently. These findings are further corroborated by the observed excess uptake of P_i relative to $[NO_3^- + NO_2^-]$ with episodic mixing events at Stn ALOHA and a resetting of the elemental ratio to that of Redfield stoichiometry for both particulate and dissolved matter pools (Karl 1999). The anomalously high net C:P incorporation rates (Table 4) and the particulate and dissolved matter C:P and N:P stoichiometries (Tables 1 & 2) relative to Redfield ratios are all indicative of P-limited communities. Considering that the C:P uptake rates were based on the incorporation of ^{14}C-bicarbonate (i.e., primary production) and ^{32}P incorporation by both phototrophic and heterotrophic microorganisms, this ratio must be regarded as a minimum estimation of the net uptake of C:P in this environment.

Additionally, Cotner et al. (1997) interpreted the accumulation of dissolved organic carbon (DOC) in the Sargasso Sea as evidence of P rather than C limitation of heterotrophic marine bacteria. A similar trend of increasing DOC inventories in the upper water column has been observed during the last several years at Stn ALOHA. The mechanism driving this accumulation of DOC in the NPSG may well be similar to that of the Sargasso Sea and thus connected to microbial nutrient limitation such as P.

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