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Dissolved inorganic and organic phosphorus uptake in *Trichodesmium* and the microbial community: The importance of phosphorus ester in the Sargasso Sea

Elizabeth D. Orchard,[a] James W. Ammerman,[b,1] Michael W. Lomas,[c] and Sonya T. Dyhrman[d,*]

[a] Massachusetts Institute of Technology–Woods Hole Oceanographic Institution Joint Program in Oceanography/Applied Ocean Science and Engineering, Woods Hole, Massachusetts

[b] Rutgers University, Institute of Marine and Coastal Sciences, New Brunswick, New Jersey

[c] Bermuda Institute of Ocean Sciences, Ferry Reach, St. Georges, Bermuda

[d] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Abstract

The dynamics and relative importance of inorganic phosphate (P*i*) and dissolved organic phosphorus (DOP) uptake were examined in *Trichodesmium* and the microbial community. *Trichodesmium* DOP and P*i* uptake rates were also compared to literature values from several other taxonomically important groups in the Sargasso Sea. Uptake rates and uptake kinetics of ^{33}P*i* and DOP (using alpha-labeled adenosine-5′-triphosphate ^{33}P-ATP as a model P ester compound) were assayed during two cruises. The in situ uptake rates suggest that the contribution of P ester to total P uptake can be greater than 25% for *Trichodesmium*, the microbial community, and key phytoplankton groups (*Prochlorococcus*, *Synechococcus*, picoeukaryotes, and nanoeukaryotes), depending on the location. Based on the kinetics of P*i* uptake and P ester uptake, *Trichodesmium* is a poor competitor for P*i* but a much better competitor for P esters as compared to the microbial community. *Trichodesmium* growth rates calculated from the P*i* and P ester uptake parameters suggest that only a small fraction of the P ester pool needs to be bioavailable to allow reasonable growth rates. These data underscore the importance of P esters in supporting production by *Trichodesmium* in the Sargasso Sea, and provide the first in situ measurements of P ester uptake and uptake kinetics in this important N_2-fixing genus.

Oceanic gyres represent the earth's largest ecosystem, and have a significant effect on global biogeochemical cycles and climate (Karl 2002). Phosphorus (P) has increasingly been identified as a limiting nutrient in the ocean gyres, particularly in the Sargasso Sea region of the North Atlantic (Wu et al. 2000; Maranon et al. 2003). Furthermore, it is P rather than nitrogen (N) that is thought to be the limiting nutrient over geological time scales because of inputs of N from N_2 fixation (Longhurst 1991). Inorganic phosphate (P*i*) concentrations in gyres, such as the Sargasso Sea, are extremely low, ranging from sub-nanomolar to 10 nmol L^{-1} (Cavendar-Bares et al. 2001; Lomas et al. 2010), and numerous approaches have identified evidence of P deficiency in phytoplankton from this region (Wu et al. 2000; Ammerman et al. 2003; Lomas et al. 2004).

The major forms of P in the upper water column are dissolved P*i* and dissolved organic P (DOP). P*i* is considered the preferable P source for microbes because it is directly available for growth (Vershinina and Znamenskaia 2002), but in the upper water column of oligotrophic regimes DOP often comprises a significant portion of the total P (Karl and Bjorkman 2001). It is difficult to chemically characterize the composition of the DOP pool, and therefore, assaying the bioavailability of DOP to a given microbial group remains an ongoing challenge. Within the high-molecular-weight DOP pool that is amenable to characterization (approximately 20–40% of the DOP), roughly 25% has a phosphonate bond and roughly 75% has an ester bond (Clark et al. 1998). As such, P esters represent a larger potential P source than phosphonates, and are the focus of this study.

One enzyme known to hydrolyze P esters, alkaline phosphatase, has received a great deal of attention because of its importance to microbial DOP hydrolysis in the ocean gyres, and because there are commercially available substrates to measure its activity (Ammerman and Glover 2000; Dyhrman et al. 2002; Lomas et al. 2004). Using community alkaline phosphatase activity (APA) as a proxy for DOP hydrolysis, a recent study by Mather et al. (2008) suggested that DOP could fuel up to 30% of the primary productivity in the North Atlantic. However, both phytoplankton and heterotrophic bacteria contribute to community hydrolysis rates, and cell-specific assays of APA have shown that there can be a great deal of taxonomic heterogeneity in the activity of this enzyme (Dyhrman et al. 2002; Lomas et al. 2004; Webb et al. 2007). Both of these factors would influence production estimates based on community hydrolysis rates.

Although studies measuring APA have provided insight into the importance and scale of P ester hydrolysis in the ocean, APA assays are problematic in that they typically involve adding excess substrate, and therefore measure a maximal rate of hydrolysis, not the in situ rate. Another approach for measuring nutrient utilization involves short-term incubations with tracer levels of radiolabeled compounds to measure the uptake rate of these isotopes into cells (Fu et al. 2005*a*; Moutin et al. 2005; Zubkov et al. 2007). This method has the added advantage of allowing

* Corresponding author: sdyhrman@whoi.edu

[1] Present address: School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, New York

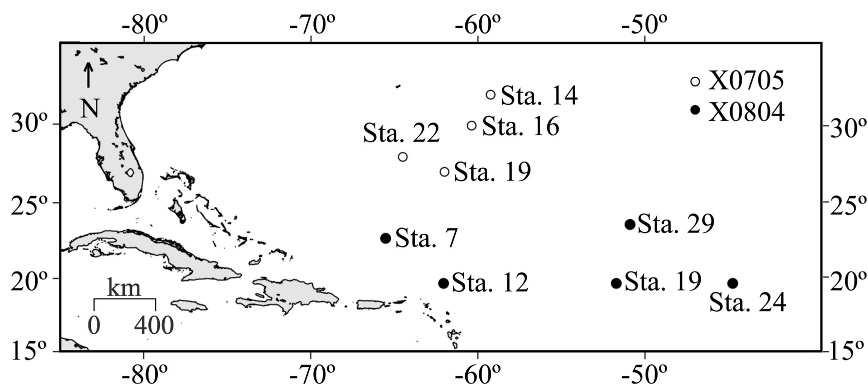

Fig. 1. Station locations for two cruises to the Sargasso Sea: X0705 in June 2007 and X0804 in May 2008.

direct comparisons of the uptake of both P*i* and DOP, and has been previously employed in a culture study of *Synechococcus* (Fu et al. 2006).

There have been several studies that have focused on P*i* uptake in cultures (Fu et al. 2005*a*) and field populations (Moutin et al. 2005; Sohm and Capone 2006; Sohm et al. 2008) of *Trichodesmium*, a colony forming N_2-fixing cyanobacteria, which can dominate the phytoplankton community in oceanic gyres such as the Sargasso Sea (Capone et al. 1997; Carpenter et al. 2004). This genus has received much attention because as a diazotroph its growth is not limited by N, but may be limited by P. Additionally, *Trichodesmium* can be an important source of N to other co-occurring microbes (Orcutt et al. 2001; Mulholland et al. 2006). In previous studies of P*i* uptake it has been suggested that *Trichodesmium* might meet a majority of its P demand from DOP in the form of P esters, and that *Trichodesmium* is a poor competitor for P*i* relative to the rest of the microbial community (Mulholland et al. 2002; Sohm and Capone 2006). However, in situ rates of DOP uptake have not been measured in *Trichodesmium* field populations.

In this study, we compare the variability and relative importance of P*i* and DOP uptake in *Trichodesmium*, the community, and several taxonomically important groups. To accomplish this, uptake rates and uptake kinetics of ^{33}P*i* and DOP (using alpha-labeled adenosine-5′-triphosphate ^{33}P-ATP as a model P ester compound) were assayed during two cruises in the Sargasso Sea.

Methods

Sample collection—Samples were collected on two cruises to the Sargasso Sea in June 2007 (X0705) and May 2008 (X0804; Fig. 1). *Trichodesmium* colonies were collected from the near surface (roughly within the top 20 m) using a handheld 130-μm net. Single colonies were picked into 0.2-μm-filtered local surface seawater. Colonies were then transferred into fresh 0.2-μm-filtered water to reduce contamination of closely associated organisms, and were separated by morphotype (either "puff," with radial trichomes, or "raft," with parallel trichomes) for further analyses. Samples of the bulk microbial community, and for flow cytometric sorting, were collected from a Niskin bottle on a rosette from a depth of 5 m and processed as described below and in Casey et al. (2009).

Uptake measurements—Uptake measurements for *Trichodesmium* and the bulk microbial community were modeled after a previous *Trichodesmium* ^{33}P*i* uptake study (Sohm and Capone 2006). In brief, 10 washed *Trichodesmium* colonies were transferred into 10 mL of surface seawater. In each incubation only one colony morphotype (puff or raft) was used depending on which morphotype was most abundant at that station. To account for the possibility of free *Trichodesmium* trichomes contributing to the microbial community uptake calculations, 4 liters of surface seawater was filtered onto a 5-μm filter, and trichomes were counted. At most there were six trichomes L^{-1}, and therefore it is unlikely that free trichomes had a large effect on uptake rates that were assayed in 10 mL. Coincident with the *Trichodesmium* colony sampling, 10 mL of surface seawater was sampled, and both *Trichodesmium* and the surface seawater (referred to as the microbial community) were incubated with either 37–93 kBq of $H_3^{33}PO_4$ or 9.2–37 kBq of alpha-labeled ^{33}P-ATP for 20–40 or 40–70 min, respectively. ATP and other nucleotides are the only commercially available radiolabeled P esters that could be obtained at the time of the cruise, and as such ^{33}P-ATP was used as the model for P ester uptake. ATP has previously been used to examine P ester uptake in cultures of *Synechococcus* (Fu et al. 2006) and in field populations of phytoplankton (Casey et al. 2009). The $H_3^{33}PO_4$ and ^{33}P-ATP were sampled in sets, and the differences in incubation time reflect the time needed to sample one set. Data are referred to herein as ^{33}P*i* or ^{33}P ester uptake for clarity. Replicate samples for both *Trichodesmium* and the microbial community were killed with 1 mL of formalin (1% final concentration) to account for abiotic adsorption of P. The activity of the killed control (typically about 10% of the total) was subtracted from the total measured activity. To further explore the importance of adsorbed P, ^{33}P*i* uptake was assayed as above on an oxalate-washed *Trichodesmium* sample and a non-washed *Trichodesmium* sample following the protocol of Sañudo-Wilhelmy et al. (2004), on a subsequent cruise in a similar region of the Sargasso Sea. In all cases, incubations were ended by filtration onto 0.2-μm polycarbonate filters, and then 5 mL of scintillation cocktail (AquaSol, Perkin Elmer) was added

Table 1. Station data for each cruise, including inorganic phosphorus (P*i*), dissolved organic phosphorus (DOP), and total dissolved phosphorus (TDP) concentrations, as well as *Trichodesmium* and bulk microbial community values for ^{33}P*i* and ^{33}P ester uptake, chlorophyll *a* (Chl *a*), and alkaline phosphatase activity (APA).*

Cruise	Station	Local time of sampling	P*i* (nmol L^{-1})	DOP (nmol L^{-1})	75% DOP (nmol L^{-1})	TDP (nmol L^{-1})
X0705	14	15:00	1.7	54.8	41.1	55.3
X0705	16	10:00	≤0.5‡	51.0	38.3	51.5
X0705	19	15:30	≤0.5	12.9	9.7	13.4
X0705	22	11:00	3.2	41.8	31.4	45.0
X0804	7	18:30	≤0.5	23.3	17.5	23.8
X0804	7§	18:30	≤0.5	23.3	17.5	23.8
X0804	12	08:00	3.6	17.7	13.3	21.3
X0804	19	16:00	3.3	91.8	68.9	95.0
X0804	19§	16:00	3.3	91.8	68.9	95.0
X0804	24	04:00	10.6	87.3	65.5	97.9
X0804	29	04:00	3.8	51.9	39.0	55.7
Mean (SD)			3.0(3.1)	48.0(28.1)	36.0(21.1)	51.0(30.1)

* nd, no data.
† Range of 1 nmol L^{-1} to 75% of the DOP concentration.
‡ Detection limit was 0.5.
§ P chemistry values are listed twice here because both colony morphotypes were measured. Duplicate P chemistry values were not used for averages and standard deviation calculations.

to each sample, and the samples were shaken, and counted on a Tri-Carb 2900 liquid scintillation counter (Packard). Assays of uptake for flow-sorted populations were performed by J. Casey et al. (2009).

Trichodesmium uptake rates were obtained by subtracting the microbial community rates from the *Trichodesmium* and microbial community combined uptake values. Samples were taken in triplicate, but on occasion *Trichodesmium* biomass was lost to the walls of the incubation bottle or filter tower, and one of the replicates had to be dropped. Reported uptake rates are the average of duplicate or triplicate samples. Uptake rates were calculated using the following formula: uptake rate = $(R_s - R_k) \times P \times (A \times T \times B)^{-1}$ where R_s is the radioactivity of the sample, R_k is the radioactivity of the killed control, A is the total radioactivity added to incubation, T is the time of incubation, B is the chlorophyll *a* (Chl *a*) concentration of a replicate sample or *Trichodesmium* colony number (used for growth rate calculations), and P is the ambient concentration of the compound of interest in the sample. For ^{33}P*i* uptake rates, P was the measured soluble reactive P (SRP) concentration. The ambient ATP concentration was not measured in this study, but previous bioassay measurements of ATP in the North Atlantic found concentrations around 1 nmol L^{-1} (Zubkov et al. 2007). Therefore, an ambient ATP concentration of 1 nmol L^{-1} was used to estimate a minimum ^{33}P ester uptake, as has been used in previous work (Casey et al. 2009). However, this might be a slight overestimate of this minimum value, because North Pacific ATP concentrations were found to be 0.3–0.5 nmol L^{-1} in the top 100 m of the water column using a chemical precipitation approach (Karl and Bjorkman 2001). A maximal ^{33}P ester uptake rate was calculated assuming that ATP is a model for all P ester. Because 75% of high-molecular-weight DOP has been shown to be P ester (Clark et al. 1998), the ^{33}P ester uptake rates are reported as a range using both 1 nmol L^{-1} and 75% of the DOP concentration for the constant P in the uptake equation, unless otherwise noted. To compare uptake rates, all values were normalized to Chl *a* after Sohm and Capone (2006). However, we acknowledge that there was heterotrophic uptake in both the *Trichodesmium* and microbial community samples, as has been noted in previous studies (Sohm and Capone 2006; Sohm et al. 2008).

Uptake kinetics—For each kinetic curve, 0–1.0 μmol L^{-1} cold P*i* or cold ATP was added in addition to the ^{33}P*i* or ^{33}P-ATP spike to duplicate incubations and assayed as above. Data was fit (Prism, Graphpad) using the Michaelis-Menten equation: $V = V_{max} \times S (K_m + S)^{-1}$, where V_{max} is the maximal uptake velocity, S is the substrate concentration (either the measured P*i* concentration or the estimated value of 1 nmol L^{-1} for P ester), and K_m is the nutrient concentration where the uptake is half of V_{max}.

Chl a—Ten puff or raft colonies were washed and filtered onto GF/F filters for *Trichodesmium* analyses, or 1–2 liters of seawater was filtered onto a GF/F for microbial community analyses, and all samples were frozen at −20°C. Filters were extracted overnight in 90% acetone at −20°C. Chl *a* fluorescence was measured on an Aquafluor handheld fluorometer (Turner Designs) or a TD-700 fluorometer (Turner Designs) and analyzed according to Arar and Collins (1997). In all cases, colonies were chosen to best mimic the size of colonies used for the other assays of *Trichodesmium*, and the Chl *a* colony^{-1} is reported for recalculating the normalized data as necessary.

P chemistry—SRP was measured according to the magnesium-induced co-precipitation method (Karl and

Table 1. Continued.

	Trichodesmium				Microbial community			
Colony type	^{33}P*i* (nmol P h^{-1} μg Chl *a*$^{-1}$)	^{33}P ester (nmol P h^{-1} μg Chl *a*$^{-1}$)†	Chl *a* (ng Chl *a* colony^{-1})	APA (nmol P h^{-1} μg Chl *a*$^{-1}$)	^{33}P*i* (nmol P h^{-1} μg Chl *a*$^{-1}$)	^{33}P ester (nmol P h^{-1} μg Chl *a*$^{-1}$)†	Chl *a* (ng Chl *a* L^{-1})	APA (nmol P h^{-1} μg Chl *a*$^{-1}$)
raft	0.80	nd	38	11.0	4.7	nd	38	nd
raft	0.20	0.17–6.5	38	17.8	10	0.82–31.4	33	nd
raft	nd	0.08–0.78	43	13.2		0.12–1.16	32	nd
raft	0.80	0.045–1.4	50	10.3	2.6	1.5–47.1	42	nd
puff	nd	0.018–0.31	18	17.8	nd	1.1–19.0	20	93.9
raft	nd	0.033–0.57	18	30.1	nd	1.1–19.0	20	93.9
raft	0.50	0.21–2.8	17	31.9	11.0	0.61–8.1	21	92.0
raft	1.3	0.057–3.9	11	42.0	20	1.4–95	38	20.6
puff	2.2	0.43–24	14	12.3	20	1.4–95	38	20.6
puff	2.0	0.041–2.7	28	11.9	130	2.8–190	25	34.0
raft	0.010	0.049–1.9	28	16.0	89	2.9–110	31	63.0
	0.98(0.80)	0.11–4.5(0.13–7.1)	28(13)	19.5(10.5)	38(50)	1.4–63(1.0–65)	31(7.7)	60.7(33.2)

Tien 1992), with a detection limit of 0.5 nmol L^{-1}. SRP concentrations measured here are referred to as the P*i* concentration for consistency with the reporting of the uptake data. The total dissolved P (TDP) was processed according to the persulfate oxidation method (Valderrama 1981). DOP concentrations were calculated as the difference between TDP and P*i*.

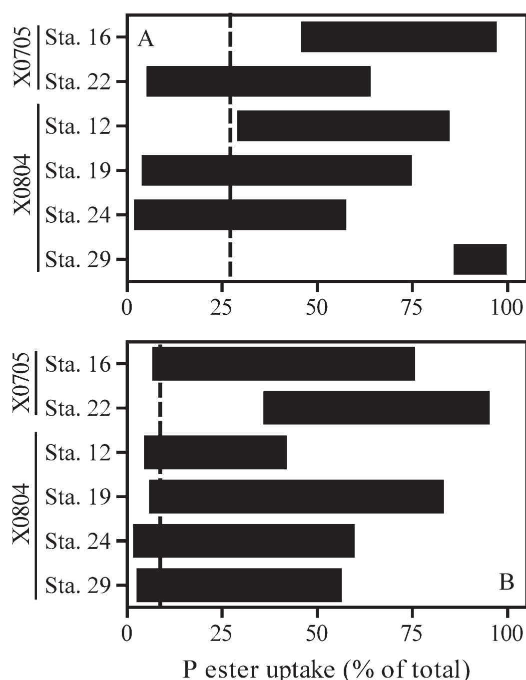

Fig. 2. The estimated range of P ester uptake relative to total P uptake for (A) *Trichodesmium* and (B) the microbial community. The black bars span the upper and lower limits of P ester uptake, and were calculated using a lower limit of 1 nmol L^{-1} (the predicted ATP concentration) and an upper limit of 75% of the ambient DOP concentration (representing the potential P ester concentration). The dashed line indicates the average lower limit of P ester uptake relative to total P uptake.

APA—For *Trichodesmium* APA assays, 3–5 colonies were washed and placed on a 5-μm polycarbonate filter. For microbial community APA, 300 mL of seawater was collected from 5 m and filtered onto a 0.2-μm filter, and in both cases the filters were stored frozen at -20°C. Samples were processed as described elsewhere (Dyhrman and Ruttenberg 2006). Briefly, 10 μmol L^{-1} 6,8-difluoro-4-methylumbelliferyl phosphate (Invitrogen) was added to each sample in a petri dish with artificial seawater containing no added P, and fluorescence was measured on a Fluostar Optima plate reader (BMG Labtech) every 5–20 min for five time points, within the linear range of the assay. Previous kinetics experiments found the 10 μmol L^{-1} substrate concentration to be saturating (data not shown). Standard curves were generated for each assay using 6,8-difluoro-7-hydroxy-4-methylcoumarin as a standard.

Statistics—Two-tailed paired *t*-tests were performed to compare data sets. Two data sets were determined to be significantly different from each other if they had a *p* value less than 0.05.

Results

The P*i* concentrations were low for both transects, averaging 3.0 $\pm$ 3.1 nmol L^{-1} (mean $\pm$ SD; Table 1). The DOP concentration was more than 15 times higher than the P*i* concentration, with an average of 48.0 $\pm$ 28.1 nmol L^{-1} ($p < 0.001$; Table 1). The Chl *a* content per colony was similar between stations, with an average of 28 $\pm$ 13 ng Chl *a* colony^{-1}, as was the microbial community Chl *a* concentration, which averaged 31 $\pm$ 7.7 ng Chl *a* L^{-1} (Table 1). The microbial community APA averaged 60.7 $\pm$ 33.2 nmol P h^{-1} μg Chl *a*$^{-1}$, whereas *Trichodesmium* APA had an average of 19.5 $\pm$ 10.5 nmol P h^{-1} μg Chl *a*$^{-1}$ (Table 1).

The average *Trichodesmium* ^{33}P*i* uptake for both cruises was 0.98 $\pm$ 0.80 nmol h^{-1} μg Chl *a*$^{-1}$ (Table 1). Although not statistically different, the average microbial community

^{33}P*i* uptake was higher than that of the *Trichodesmium*, and at each station ^{33}P*i* uptake was higher for the microbial community than for *Trichodesmium* (Table 1). Furthermore, the average proportion of ^{33}P*i* uptake by *Trichodesmium* (8%) was significantly lower ($p < 0.001$) than the average proportion of ^{33}P*i* uptake by the microbial community (92%) relative to the total ^{33}P*i* uptake at each station. P*i* can adsorb to *Trichodesmium* colonies and other constituents of the microbial community (Sañudo-Wilhelmy et al. 2004). To account for this, the activity assayed in killed controls (approximately 10% of the total), was subtracted from the total activity to calculate the reported uptake rates. Oxalate washing (Sañudo-Wilhelmy et al. 2004) is a more stringent way to remove adsorbed P. A comparison of ^{33}P*i* uptake on an oxalate-washed *Trichodesmium* sample vs. a non-washed *Trichodesmium* sample suggests that this more stringent approach results in 26% of the uptake being the result of P*i* adsorption. Therefore, the values presented here (calculated with 10%) might be a roughly 16% overestimate of the *Trichodsemium* ^{33}P*i* uptake as they include some P*i* adsorption.

Trichodesmium rates of ^{33}P ester uptake were variable, with an average minimum of 0.11 $\pm$ 0.13 and an average maximum of 4.5 $\pm$ 7.1 nmol P h^{-1} μg Chl a^{-1} using 1 nmol L^{-1} or 75% of the DOP concentration as the ambient P ester concentration respectively (Table 1). The ^{33}P ester uptake rates for the microbial community ranged from a minimum of 1.4 $\pm$ 1.0 to a maximum of 63 $\pm$ 65 nmol P h^{1} μg Chl a^{-1}, calculated with ranges as described above (Table 1). Therefore, for both *Trichodesmium* and the microbial community, the ^{33}P ester uptake ranged from lower than the ^{33}P*i* uptake rate to higher, depending on what the ambient bioavailable P ester concentration is assumed to be.

To compare the relative importance of P ester vs. P*i* uptake, the %P ester uptake of the total P uptake (P*i* plus P ester) for *Trichodesmium* and the microbial community was calculated (Fig. 2). For *Trichodesmium*, the contribution of P ester to total P uptake was variable, and spanned different ranges for each station depending on the ambient DOP concentration used to estimate the P ester concentration (Fig. 2). At three of the six stations, P ester accounted for at least 25% of the P uptake in *Trichodesmium*, and the average minimum for all stations was 29% (Fig. 2). P ester typically contributed a smaller portion to total P uptake for the microbial community (Fig. 2). In this case, P ester accounted for at least 25% of the total P uptake at only one out of six stations for the microbial community, and the average minimum contribution was 10% (Fig. 2).

Using the X0705 cruise taxon-specific uptake rates measured by Casey et al. (2009), the contribution of P ester to total P uptake was calculated for picocyanobacteria, picoeukaryotes, and nanoeukaryotes. The contribution of P ester to total P uptake in *Prochlorococcus* and *Synechococcus* was variable depending on the station; for example, at several stations, P ester contributed a minimum of 25% to the total P uptake, but not at all stations (Fig. 3). In contrast, P ester contributed a minimum of 25% of the total P uptake for every station assayed for the picoeukaryotes and the nanoeukaryotes. At X0705 Sta. 16, the only station where both *Trichodesmium* and the flow-sorted data are available, the contribution of P ester to total P uptake was 25% or more for each group (Figs. 2, 3).

Assays for *Trichodesmium* and the microbial community ^{33}P*i* uptake kinetics were performed at four stations: one station on X0705 and three stations on X0804. ^{33}P ester uptake kinetics were assayed at three stations on cruise X0804. For the ^{33}P*i* uptake kinetics, *Trichodesmium* had an average V_{max} of 210 $\pm$ 120 nmol P h^{-1} μg Chl a^{-1} and an average K_m of 0.68 $\pm$ 0.33 μmol L^{-1} (Table 2). The average K_m value is similar to those obtained in previous studies with non–oxalate-washed samples that include some adsorbed P (Table 3); however, the V_{max} calculated herein is higher than that in those previous studies (Table 3). The V_{max} for ^{33}P ester uptake had an average of 54 $\pm$ 34 nmol P h^{-1} μg Chl a^{-1}, and the K_m averaged 0.29 $\pm$ 0.18 μmol L^{-1} (Table 2). For the microbial community, the K_m and V_{max} of ^{33}P*i* uptake averaged 0.057 $\pm$ 0.081 μmol L^{-1} and 17 $\pm$ 9.1 nmol P h^{-1} μg Chl a^{-1} respectively (Table 2). The K_m and V_{max} of ^{33}P ester uptake for the microbial community averaged 0.51 $\pm$ 0.42 μmol L^{-1} and 11 $\pm$ 6.1 nmol h^{-1} μg Chl a^{-1} respectively in the microbial community samples (Table 2).

One measure of maximal P ester hydrolysis and uptake is the V_{max} of ^{33}P ester uptake as described above. An alternative measure of maximal P ester hydrolysis is APA. The ^{33}P ester V_{max} for *Trichodesmium* was similar (within the same order of magnitude) to, but always slightly higher than, the APA (Table 2). Likewise, ^{33}P ester V_{max} for the microbial community was also similar (within the same order of magnitude) to the APA. There were very few stations where both the APA and the V_{max} of ^{33}P ester uptake were measured (Table 2); therefore, none of the kinetic parameters were statistically significantly different from each other because of the limited dataset.

Discussion

The Sargasso Sea has previously been identified as a region where P*i* is limiting to phytoplankton growth (Wu et al. 2000; Mather et al. 2008). In this region, being able to utilize the DOP pool as a P source may be an important growth advantage for microbes. However, determining the bioavailability of DOP is a difficult question to address, as the bioavailability of DOP varies among, and sometimes within, microbial taxa (Lomas et al. 2004; Moore et al. 2005). Therefore, determining the importance of DOP relative to P*i* uptake remains a challenge, but is central to understanding how P controls growth in this region of the ocean. By combining measurements of ^{33}P*i* and ^{33}P ester uptake and uptake kinetics, we can gain new insights into the importance of DOP to *Trichodesmium*, and the microbial community, in the low-P Sargasso Sea.

Uptake rates—The Chl *a*–normalized ^{33}P*i* uptake rate for *Trichodesmium* and the microbial community were variable across both transects, with no clear trend with ambient P*i* or DOP concentration. However, at each individual station the ^{33}P*i* uptake rate for *Trichodesmium*

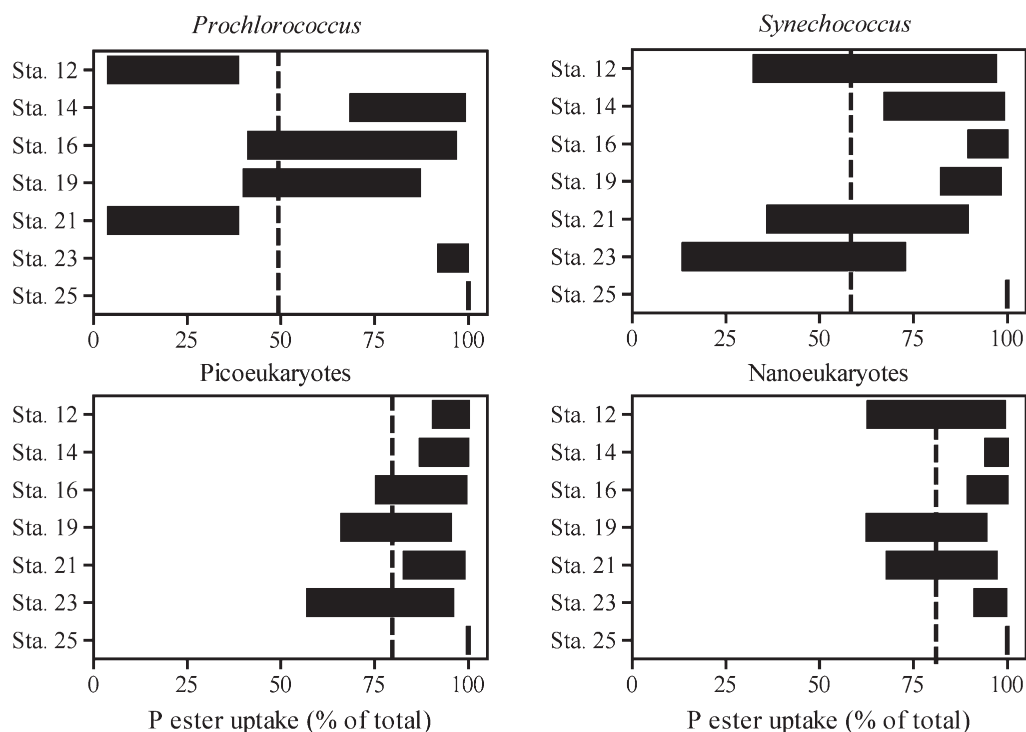

Fig. 3. The estimated range of the proportion of P ester uptake as a function of total P uptake for *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and nanoeukaryotes during X0705 recalculated from Casey et al. (2009). The black bars span the upper and lower limits of P ester uptake, and were calculated using a lower limit of 1 nmol L^{-1} (the predicted ATP concentration) and an upper limit of 75% of the ambient DOP concentration (representing the potential P ester concentration). The dashed line indicates the average lower limit of P ester uptake relative to total P uptake.

was at least an order of magnitude lower than that of the microbial community, and the proportion of total P*i* uptake for *Trichodesmium* was significantly lower than that of the community. Therefore, *Trichodesmium* is not taking up as much P*i* per unit Chl *a* as the microbial community, and this suggests that P*i* may not be as important a P source for *Trichodesmium* as it is for the microbial community. These data are consistent with previous comparisons of *Trichodesmium* and the microbial community in this region, which suggest that *Trichodesmium* is a poor competitor for P*i* (Sohm and Capone 2006). It is important to note that heterotrophic bacteria, which do not contain Chl *a*, are present in the microbial community and associated with *Trichodesmium*, and therefore likely contribute to measured uptake rates. As a result, the assay and normalization approach used here and elsewhere (Sohm and Capone 2006; Sohm et al. 2008) may overestimate the uptake rates of P*i* or P ester in the microbial community, or *Trichodesmium*. *Trichodesmium*, in particular, is known to have tightly associated epibionts. It is not clear, however, whether the P taken up or hydrolyzed by epibionts would eventually contribute to meeting the P demand of *Trichodesmium*.

Examining the percentage of P ester uptake contribution to the total P uptake allows the relative importance of P esters as a P source to be assessed for *Trichodesmium*, the microbial community, and different microbial taxa. This calculation also cancels out the Chl *a* normalization and thus eliminates this confounding factor in comparing the microbial community to *Trichodesmium*. Given the uncertainty regarding the bioavailable P ester concentration, this proportion was calculated to determine the potential minimum and maximum contribution of P ester to total P uptake. Although it is known that some of the microbial populations in this region have the genetic machinery to take up phosphonate DOP (Dyhrman et al. 2006; Ilikchyan et al. 2009), phosphonate uptake should be smaller than combined P*i* and P ester uptake, as these chemical pools are more abundant and considered more bioavailable. At half of the stations, P ester contributed a minimum of 25% of the total P uptake for *Trichodesmium*. In contrast, there was only one station where the P ester contribution was greater than 25% of the total P uptake for the microbial community. These data underestimate the importance of P ester to *Trichodesmium*, because accounting for P*i* that is adsorbed to *Trichodesmium* would reduce P*i* uptake rates by roughly 16% but should not affect P ester uptake rates (Fu et al. 2005*b*, 2006). Taken together, these results suggest that P esters are a major P source for *Trichodesmium*, but probably less important to the microbial community, although, for both *Trichodesmium* and the microbial community, the specific contribution of P ester to total P uptake is highly dependent on the actual concentration of P ester that is bioavailable.

Using flow cytometry coupled with isotope incubations, Casey et al. (2009) measured ^{33}P*i* and ^{33}P-ATP (as a proxy for P ester) uptake in several other taxa including *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and nano-

Table 2. Kinetic parameters of ^{33}P*i* and ^{33}P ester uptake assayed for *Trichodesmium* and the bulk microbial community.*

			Trichodesmium					Microbial community				
Cruise	Station	Colony morphotype	^{33}P*i* K_m (μmol L^{-1})	^{33}P*i* V_{max} (nmol P h^{-1} g Chl *a*$^{-1}$)	^{33}P ester K_m (μmol L^{-1})	^{33}P ester V_{max} (nmol P h^{-1} μg Chl *a*$^{-1}$)†	APA (nmol P h^{-1} μg Chl *a*$^{-1}$)	^{33}P*i* K_m (μmol L^{-1})	^{33}P*i* V_{max} (nmol P h^{-1} μg Chl *a*$^{-1}$)	^{33}P ester K_m (μmol L^{-1})	^{33}P ester V_{max} (nmol P h^{-1} μg Chl *a*$^{-1}$)†	APA (nmol P h^{-1} μg Chl *a*$^{-1}$)
X0705	19	puff	0.54	170	nd	nd	13	nd	nd	nd	nd	nd
X0804	12	raft	0.29	170	0.50	81	32	0.02	27	0.22	6.1	92
X0804	24	puff	0.92	390	0.18	16	12	0.15	13	1.0	18	34
X0804	29	raft	0.98	120	0.18	66	16	0.00041	9.8	0.32	9.8	63
Mean(SD)			0.68(0.33)	210(120)	0.29(0.18)	54(34)	18(9.3)	0.057(0.081)	17(9.1)	0.51(0.42)	11(6.1)	63(29)

* nd, no data.
† Assuming 1 nmol L^{-1} P ester.

eukaryotes. Strikingly, even at the average lower limit of P ester contribution, P ester is the major (greater than an average of 50% at all stations) P source for all four of these groups. These data suggest that, like *Trichodesmium*, these critical phytoplankton groups heavily rely on P esters to support P demand in this system. This is consistent with culture work examining ATP uptake in *Synechococcus* that suggests that, at least for some strains, P ester is important to meeting P demand (Fu et al. 2006). At Sta. 16 on X0705, there were simultaneous measurements of *Trichodesmium*, microbial community, and taxon-specific ^{33}P*i* and ^{33}P ester uptake allowing for direct comparisons. For this station, P esters were particularly important for all individual taxa, even at the lower limit of P ester contribution, although the microbial community does not reflect this trend. This observation emphasizes the fact that other groups, such as heterotrophic bacteria, are probably contributing to the microbial community uptake measurements and are good competitors for P*i*. Interestingly, Sta. 16 is one of the stations with the lowest P*i* concentrations at ≤ 0.5 nmol L^{-1}. This suggests that, as one might predict, with lower P*i* concentrations P esters become more important to P uptake.

Kinetics—The average *Trichodesmium* K_m measurements for P*i* and P ester uptake were similar. Although both affinity constants are higher than the ambient P ester and the P*i* concentrations of the upper water column in this system, the P*i* K_m is consistent with the K_m measured in previous culture and field studies, which ranges between 179 and 710 nmol L^{-1} for assays that did not remove adsorbed P (Table 3). These may be overestimates, because the K_m of P*i* uptake is reduced when surface adsorbed P is removed (Fu et al. 2005*b*). Nevertheless, the K_m values detected in the field, across a range of P*i* concentrations, are consistent with previous work, which found that *Trichodesmium* does not alter its K_m in response to P physiology or ambient P supply (Fu et al. 2005*a*). Given that the concentration of DOP (and thus likely bioavailable P ester concentration) is higher than that of P*i* in the Sargasso Sea, *Trichodesmium* is potentially better at taking up P ester than P*i*.

Unlike the K_m, *Trichodesmium* increases its ^{33}P*i* V_{max} in response to increasing P limitation (Fu et al. 2005*a*). In this study, the average V_{max} of ^{33}P*i* uptake for *Trichodesmium* was nearly an order of magnitude higher than those calculated in other studies of *Trichodesmium* from the North Atlantic (Sohm and Capone 2006; Sohm et al. 2008), which also did not remove adsorbed P. There are two additional studies that measured $V_{specific}$ of *Trichodesmium* ^{33}P*i* uptake: Fu et al. (2005*a*) in culture and Moutin et al. (2005) in the South Pacific. Approximating V_{max} from those studies (Table 3) also results in lower V_{max} values for *Trichodesmium* from the South Pacific (Moutin et al. 2005), and from P replete cultures (Fu et al. 2005*a*) relative to the V_{max} for ^{33}P*i* uptake calculated here. However, the average P*i* concentration from the stations where uptake kinetics were measured in this study was much lower than that of any previous field study (4.6 nmol L^{-1} vs. 45 nmol L^{-1} for the second lowest P*i* concentration; Table 3). Therefore,

Table 3. A comparison of *Trichodesmium* P*i* uptake kinetics and P*i* concentrations in this and other studies of *Trichodesmium* from cultures and field populations.*

Source	Location	P*i* (nmol L^{-1})	Colony morphotype	^{33}P*i* K_m (nmol L^{-1})	^{33}P*i* V_{max} (nmol h^{-1} μg Chl a^{-1})
This study	N Atlantic	4.6	puffs or rafts	680	210
Sohm and Capone (2006)	N Atlantic	65	mixed	710	10.4
Sohm et al. (2008)	N Atlantic	45	mixed	180	7.0
Moutin et al. (2005)	S Pacific	20–240	mixed	630	3.1†
Fu et al. (2005*a*)	Cultures	P-replete	NA	340	36†
		P-limited	NA	650	80

* NA, not applicable.

† These values are derived assuming a P quota of 2.6 nmol per colony from Sañudo-Wilhemy et al. (2004) and an average Chl *a* content per colony of 27 ng (Table 1).

the higher V_{max} values found in this study may reflect *Trichodesmium* populations that are more P-limited than those in previous studies. Fu et al. (2005*b*) found that when surface adsorbed P was removed this reduced the V_{max} for P*i* uptake by half for P-limited *Trichodesmium* cultures, yet a 50% reduction in the average ^{33}P*i* V_{max} calculated herein would still be higher than that found in other field studies (Table 3). This emphasizes the fact that the *Trichodesmium* in this study are in a particularly low P*i* system, and may be P-limited.

Comparisons of kinetic parameters between *Trichodesmium* and the microbial community are difficult because of the potential for differential contributions of individual taxa and heterotrophic groups. Although the K_m of ^{33}P*i* uptake for the microbial community is highly variable, the average (57 nmol L^{-1}) is an order of magnitude lower than that of *Trichodesmium*. As was discussed previously, this suggests that *Trichodesmium* is a poor competitor for P*i* as compared to the microbial community. The K_m of P ester uptake for the microbial community is similarly variable, but the average (510 nmol L^{-1}) is higher than the K_m of P ester for *Trichodesmium*. This suggests that *Trichodesmium* is a better competitor compared with the microbial community for P ester than for P*i*. This conclusion is consistent with the observations with *Trichodesmium* made above and with those of others made with assays of ^{33}P*i* alone (Sohm and Capone 2006; Sohm et al. 2008). These conclusions are also consistent with the fact that P ester represented 25% or more of the total P uptake for *Trichodesmium* at more stations than for the microbial community.

In this study, the maximal rate of P ester utilization was examined in two ways: through ^{33}P ester uptake kinetics and through APA assays. APA measurements here represent the maximal rate of P ester hydrolysis using a model P ester, whereas the V_{max} of ^{33}P ester uptake measures the maximal rate of P ester hydrolysis plus incorporation into the cell, assuming that the P ester is not directly taken up by the cell. Each of these assays uses very different model P esters. Given the inherent differences between these approaches, the resulting values were strikingly similar for both *Trichodesmium* and the microbial community. Together, these data are suggestive of a population that can take advantage of fluctuations in the DOP pool. But because maximal hydrolysis rates are always much higher than in situ rates, these data underscore the fact that tracer studies are excellent complements to uptake and hydrolysis rates calculated based on APA (non-tracer) alone.

Table 4. *Trichodesmium* doubling times calculated using the average ^{33}P*i* and ^{33}P ester kinetic parameters from Table 2, and a range of potentially bioavailable P concentrations.

P*i* (nmol L^{-1})	P ester (nmol L^{-1})	Doubling time (d)
6	—	1.4
3.3	—	2.5
0.42	—	20
—	36	0.36
—	10	1.4
—	1	14

Growth rate—Determining growth rates, or doubling times, for phytoplankton provides insights into how an organism is adapted to its environment, and into its nutrient-scavenging strategies. Previous studies have used C and N uptake in the field to estimate a *Trichodesmium* doubling time (Carpenter and Romans 1991; Orcutt et al. 2001). These doubling times are variable; literature values range from 0.7 to 2500 d (Orcutt et al. 2001; Moutin et al. 2005). An estimate for a rapid doubling time has been made using C uptake of roughly 2 d for *Trichodesmium* in the Sargasso Sea (Orcutt et al. 2001). This is similar to doubling times for *Trichodesmium* in culture of 2–4 d (Capone et al. 1997; Krauk et al. 2006). Using the average kinetic parameters from our study, different P-specific doubling times can be determined at a range of P*i* and P ester concentrations by following the equation after Moutin et al. (2005): $(t_d) = \ln(2)(K_m + S)(24 \times V_{sp} \times S)^{-1}$, where t_d is the doubling time, V_{sp} is the V_{max} divided by the P quota per colony; here the P quota was assumed to be 2.6 nmol P colony^{-1} (Sañudo-Wilhelmy et al. 2004), and S is the substrate concentration (Table 4). It is important to note that the P quotas are from colonies without adsorbed P and are therefore a conservative estimate (Sañudo-Wilhelmy et al. 2004). Three different P*i* concentrations were used: 6 nmol L^{-1} (the average P*i* concentration for the stations where kinetic parameters were measured), 3.3 nmol L^{-1}, and 0.42 nmol L^{-1} (based on Zubkov et al. (2007), which suggested that only 7–55% of the P*i* concentration is bioavailable). For both the 6 and 3.3 nmol L^{-1} P*i*

concentration, the doubling times are reasonable for the Sargasso Sea and roughly equivalent to the *Trichodesmium* doubling times calculated using C (Orcutt et al. 2001). For the P ester calculations, concentrations of 1, 10, and 36 nmol L^{-1} were used based on the possible range of bioavailable P ester. If only 10 nmol L^{-1} P ester is bioavailable, then *Trichodesmium* can reach the same doubling time as on 6 nmol L^{-1} of P*i*. Therefore, only a small portion of the DOP pool needs to be bioavailable P ester to fully support *Trichodesmium* P uptake, based on these data. This is true even if the P quota is increased, and using a longer doubling time in this analysis only reinforces the relative importance of P ester compared to P*i* in this system.

Here we address the role of P*i* and P ester in supporting the growth of *Trichodesmium* and the microbial community, and draw comparisons with different phytoplankton groups. The in situ uptake rates suggest that the contribution of P ester to total P uptake can be greater than 25% for *Trichodesmium*, the microbial community, and key phytoplankton groups, depending on the station. The P ester contribution to *Trichodesmium* was over 25% at half of the stations examined, and further work in other systems with variable P chemistry would confirm if this frequency is similar in higher P systems, or to what extent the P ester uptake rate changes with ambient DOP over a larger dataset. Several studies have suggested that *Trichodesmium* is P-limited in the Sargasso Sea (Sañudo-Wilhelmy et al. 2001; Dyhrman et al. 2002; Mulholland et al. 2002) and the high ^{33}P*i* V_{max} values found here support that conclusion. A comparison of the K_m values for both $^{33}P_i$ and ^{33}P ester uptake suggests that *Trichodesmium* is a better competitor for P ester than for P*i* relative to the microbial community. This is consistent with previous work (Sohm and Capone 2006), which focused solely on P*i* uptake kinetics. Future work focused on the uptake kinetics of other individual phytoplankton taxa would help evaluate the consistency of this relationship. Additionally, this work focused on enzyme activity and uptake assays highlights the importance of pairing tracer level experiments (which measure in situ uptake rates) with experiments where saturating concentrations of substrate are added (which measure V_{max} of enzymatic hydrolysis). *Trichodesmium* growth rates calculated from the ^{33}P*i* and ^{33}P ester uptake parameters indicate that only a small fraction of the P ester pool needs to be bioavailable to meet P demand, which further supports the importance of P ester to this group in the Sargasso Sea. Taken together, these data underscore the importance of P esters to supporting production by *Trichodesmium*, picocyanobacteria, and small eukaryotes in the Sargasso Sea.

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