Vertical partitioning of phosphate uptake among picoplankton groups in the low-P Mediterranean Sea

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Abstract

Microbial transformations are key processes in marine phosphorus cycling. In this study, we investigated the contribution of phototrophic and heterotrophic groups to Pi uptake fluxes in the euphotic zone of the low-phosphate (Pi) Mediterranean Sea and estimated Pi uptake kinetic characteristics. Surface SRP concentrations ranged 6 – 80 nmol L^{-1} across the transect, and the community Pi turnover times, assessed using radiolabeled orthophosphate incubations, were longer in the western basin, where the highest bulk and cellular rates were measured. Using live cell sorting, 4 vertical profiles of Pi uptake rates were established for heterotrophic prokaryotes (Hprok), phototrophic picoeukaryotes (Pic) and *Prochlorococcus* (Proc), *Synechococcus* (Syn) cyanobacteria. Hprok cells contributed to up to 82 % of total Pi uptake fluxes in the superficial euphotic zone, through constantly high abundances ($2.7 – 10.2 \times 10^5$ cells mL^{-1}) but variable cellular rates (6.6 ± 9.3 amol P $cell^{-1}$ h^{-1}). Cyanobacteria achieved most of the Pi uptake (up to 62 %) around the deep chlorophyll maximum depth, through high abundances (up to 1.4×10^5 Proc cells mL^{-1}) and high cellular uptake rates (up to 40 and 402 amol P $cell^{-1}$ h^{-1}, respectively for Proc and Syn cells). At saturating concentrations, maximum cellular rates up to 132 amol P $cell^{-1}$ h^{-1} were measured for Syn at St. C, which was 5 and 60 times higher than Proc and Hprok, respectively. Pi uptake capabilities of the different groups likely contribute to their vertical distribution in the low-Pi Mediterranean Sea, possibly along with other energy limitations.

1. Introduction

Understanding nutrient uptake strategies in microorganisms is a necessity to predict their biogeochemical response to environmental changes. Heterotrophic (Hprok) and phototrophic prokaryotes (cyanobacteria) dominate the planktonic biomass in oligotrophic areas of the surface ocean and account for most of the carbon fluxes through the microbial loop (*Azam et al.*, 1983). Nanomolar concentrations of orthophosphate (Pi) and Pi turnover times as low as minutes or hours are seasonally observed in the Sargasso and the Mediterranean (e.g. *McLaughlin et al.*, 2013; *Moutin et al.*, 2002; *Sebastián et al.*, 2012; *Thingstad et al.*, 1998; *Wu et al.*, 2000). Pi is the preferred form of phosphorus for most osmotrophs, but recent studies show that dissolved organic phosphorus (DOP) can be a significant source of P as well, particularly in its most labile forms like ATP (e.g. *Björkman and Karl*, 1994; *Björkman et al.*, 2012; *Casey et al.*, 2009; *Duhamel et al.*, 2012; *Fu et al.*, 2006; *M.W. Lomas et al.*, 2010; *Sebastián et al.*, 2012). It is now well established that concentrations of Pi in the environment impact uptake processes by microbes, who rely on high affinity systems via active transport at low concentrations and high capacity systems and diffusion at higher environmental Pi (e.g. *Knauss and Porter*, 1954; *Nyholm*, 1977). Along with an increased stratification and oligotrophication of the surface ocean, a widely spread size-shift in the structure of phytoplankton communities is expected, from nano- and micro- eukaryotes to pico-sized cells, as observed in the North Pacific Subtropical Gyre (*Church et al.*, 2002; *Karl et al.*, 2001). How phytoplankton and bacteria share P resources when they are poorly available has been debated for over 30 years. The existence of different Pi acquisition systems in microorganisms was highlighted in studies where Hprok systems were found to be saturating at much lower Pi concentrations than phytoeukaryotes (> 3 µm: *Currie and Kalff*, 1984; *Currie et al.*, 1986). Some eukaryotes possess mixotrophic capabilities and

grazing on P-richer prokaryotes can fill most of their requirements in P (*Christaki et al.*, 1999; *Hartmann et al.*, 2011) as well as DOP hydrolysis induced by ectoenzymes (ATPases, alkaline phosphatases, e.g.: *Webb*, 1992). Eukaryotic phytoplankton may compensate their low affinity for the substrate at low concentrations (high Km) with high Pi storage capacity (*Cotner and Biddanda*, 2002) compared to prokaryotes and a more efficient growth mechanism, i.e. a low half-saturation constant for growth (Ks, *Rhee*, 1973). Pi uptake by microbes in natural environments has largely been assessed using size fractionation. Studies concur on the high contribution of the small size fractions (<0.8, <1, <2 or <3 µm) to Pi uptake fluxes (*Björkman and Karl*, 1994; *Currie et al.*, 1986; *Moutin et al.*, 2002; *Tanaka et al.*, 2003; *Thingstad et al.*, 1993; *Thingstad et al.*, 1998). This contribution generally increases in aquatic systems with short Pi turnover times and also in low-Pi systems after P amendments, emphasizing the idea that heterotrophic prokaryotes are high competitors in P-deficient areas (e.g.: *Björkman et al.*, 2012; *Currie et al.*, 1986; *Drakare*, 2002; *Labry et al.*, 2002). However, size fractionation offers a limited resolution of microbial processes, especially in oligotrophic environments where osmotrophs are small and where taxonomic and functional types overlap in size. The development of combined radiolabeling techniques and cell sorting by flow cytometry has improved the level of resolution to study Pi uptake strategies in heterotrophic and phototrophic microbes (e.g.: *Björkman et al.*, 2012; *Casey et al.*, 2009; *Duhamel et al.*, 2012; *Michael W. Lomas et al.*, 2014; *Talarmin et al.*, 2011b; *Zubkov et al.*, 2007)

When looking into the contribution of picoplanktonic groups to total Pi uptake, prokaryotes are better competitors than eukaryotes. Among prokaryotes, measured cellular uptake rates of Pi were higher for Syn compared to Hprok and Proc in the Sargasso Sea (*Michelou et al.*, 2011), and higher for Proc compared to Hprok in the North Pacific Subtropical Gyre (*Björkman et al.*,

2012), especially during light incubation (*Duhamel et al.*, 2012). Nevertheless, the comparison among those studies is not always possible, due to methodological differences (fixation, inducing Pi leakage) or to different targeted groups. Finally, only one study suggests the adaptation of microorganisms to P amendments (*Björkman et al.*, 2012), with concentration kinetics experiments providing useful insights into their coexistence in low-Pi environments.

The present study investigates the contribution of sorted picoplankton groups to total P_i uptake flux in the Pi-depleted stratified upper water column of the Mediterranean Sea (down to 200 m). An estimation of kinetic constants (maximum P_i uptake rate-V^{max}, and the sum of the half-saturation constant plus Pi natural concentration $K+S_n$) of $^{33}P_i$-radiolabeled orthophosphate uptake in sorted Hprok, cyanobacterial and picophytoplanktonic cells is also provided to compare competitive abilities of the groups at different depths and under different ambient Pi concentrations. In spite of the amount of phosphorus-related studies conducted in the Mediterranean Sea, such experiments have never been carried out in this system. Our results contribute to further understand how prokaryotic autotrophs and heterotrophs share scarce resources when the phytoplankton biomass is dominated by prokaryotes.

2. Material and Methods

2.1.Study sites and collection

An east-west transect across the Mediterranean Sea was realized during the BOUM cruise (Biogeochemistry from Oligotrophic to Ultraoligotrophic Mediterranean) on the French R/V L'Atalante from 16 June to 20 July 2008 (Fig. 1). Samples were collected using 12-L Niskin bottles mounted on a rosette equipped with a conductivity-temperature-density (CTD) profiler and sensors for pressure, oxygen, photosynthetically available radiation (PAR), and chlorophyll

fluorescence. Pi uptake and turnover time measurements reported in this study were fully processed onboard. Total chlorophyll-a (TChl-a) concentrations were determined as described in (*Crombet et al.*, 2011). Critical depths and integrated nutrient concentrations were calculated as described in (*Moutin and Prieur*, 2012). Pi turnover times were measured at each of the 30 stations at fixed depths of 5, 25, 50, 75, 100 and 125m. Vertical Pi uptake profiles of Pi uptake in sorted groups at stations 9, 21, A and 25 are presented here, as well as concentration bioassay experiments conducted at stations C, and A.

2.2.Enumeration of phototrophs and heterotrophs using flow cytometry

Triplicated aliquots of 1.8 ml were sampled and fixed with a 2 % (w/v) formaldehyde solution, stored for at least 30 min at room temperature, flash frozen in liquid nitrogen and then stored at −80°C until further processing onshore within 6 months. Samples were thawed an hour before analysis and 1 µm yellow-green beads (Fluoresbrite, Polysciences) were systematically added as a standard. The sheath fluid was filtered (< 0.2 µm) seawater and analyses were conducted with the software Cell Quest Pro. Microbes were enumerated using a FACScan flow cytometer (BD Biosciences) equipped with an air-cool argon laser (488 nm, 15 mW). The red fluorescence signal of the chlorophyll *a* was collected on a long-pass filter (> 650 nm) to identify phytoplankton groups including *Prochlorococcus* and pico-phytoeukaryotes, according to Marie et al. (2000). *Synechococcus* cells were discriminated from other phototrophs using their orange fluorescence signal (585/21 nm).

Samples for the enumeration of heterotrophic prokaryotes were stained with SYBRGreen I (Invitrogen–Molecular Probes) at 0.025 % (vol/vol) final concentration for 15 min at room temperature in the dark. Counts were performed using a FACSCalibur flow cytometer (BD

Biosciences) equipped with an air-cooled argon laser (488 nm, 15 mW). Stained bacterial cells, were discriminated and enumerated according to their right-angle light scatter (SSC) and green fluorescence of the nucleic acid dye (530/30 nm). Autotrophic prokaryotes were discarded from this analysis based on their red fluorescence.

2.3. Sample preparation for P_i uptake measurements

Clean 30-mL polycarbonate Nalgene bottles were filled with 10 mL of seawater samples. Preliminary measurements were conducted at the beginning of the cruise to adjust the incubation conditions so that Pi uptake was linear with time. The methodology and calculations employed for bulk and cell-normalized, group-specific P_i uptake rates were based on the protocol by (*Talarmin et al.*, 2011b). Briefly, a radioactive working solution was freshly prepared on board by diluting a carrier-free [^{33}P]-H_3PO_4 mother solution (Perkin Elmer, USA, 569.5×10^{12} Bq mmol^{-1}) in 0.2 µm-filtered milliQ water, and added to the samples. ^{33}Pi incorporation at room temperature, under natural light condition, was stopped by adding an excess amount (0.1 mM final concentration) of a cold Pi solution. Samples were split in 3 aliquots of 5 mL (for bulk-whole water- Pi uptake measurements), 3.5 mL (for sorting of phototrophic groups) and 1.5 mL (for sorting of Hprok cells), and kept in the dark at 4 °C until further processing (<1 hour for the 5 ml subsamples, 10 minutes to 6 hours for the others). A cold Pi solution was added to blank samples (final concentration of 0.1 mmol L^{-1}) 15 minutes prior to radiolabeling and processed like other samples. Blank values represented on average 5.0 to below 13 % of regular values estimated for bulk (n=11) and sorted groups (n=36), respectively. Blank values were systematically subtracted from the counts, in dpm cell^{-1}, for the sorted samples. The radioactivity was counted onboard within 5 hours after addition of the scintillation cocktail using a Packard LS 1600 liquid scintillation counter.

2.3.1. *In situ* Pi uptake rates

At stations A, 9, 21 and 25, samples were collected at several depths in order to assess the vertical distribution of the group-specific P_i uptake under ambient concentrations of Pi. Samples were spiked with a final concentration of 20 pmol L^{-1} of the ^{33}P-orthophoshate working solution and incubated for 15 minutes.

2.3.2. Concentration kinetics of Pi uptake

Surface experiments carried out between stations B and C led to unsatisfying results where signals were too weak for Pic and unstained Proc cells could not be detected. The upper deep chlorophyll maximum (DCM) depth was then chosen as a biogeochemically consistent level, considering that the depth of the DCM and nutriclines was expected to vary considerably along the transect. Concentration kinetics experiments were conducted at stations 5, A, B and C by adding increasing quantities of a cold KH_2PO_4 solution (0, 4, 8, 10, 15, 20, 40, 60, 80, 100 nmol L^{-1} added concentration, Sa). Samples were spiked with the radioactive working solution 15 minutes later, with an activity of 0.34 MBq per sample, i.e. a final radioactive P_i concentration of 60 pmol L^{-1}, and incubated for 45 minutes. Bulk P_i uptake rates, bulk Pi turnover times, bulk and taxon-specific kinetic constants V^{max} (maximum velocity of P_i uptake) and $K+S_n$ (K being the half-saturation constant and Sn the natural P_i concentration, their sum being determined as the intersection of the plotted line with the x axis), were based on the work by *Thingstad et al.* (1993). Such estimations were obtained using the linear regression of P_i turnover time versus Sa.

2.3.3. Bulk Pi uptake measurements

The 5-mL aliquots were gently filtered on a 0.2 µm polycarbonate membrane superimposing a GF/D filter soaked with a cold Pi solution without rinsing. A 5-second increase of the vacuum

pressure ended the filtration to remove non-incorporated Pi. Filters were then placed in scintillation vials with 5 mL of Phase Combining System scintillation cocktail (PCS, GE Healthcare).

2.3.4. Flow sorting of labeled picoplankton groups

Other aliquots were processed by flow sorting on an onboard FACSAria cell sorter (BD Biosciences) equipped with two lasers: a 488 nm (13 mW, Coherent, Sapphire Solid State) and a 633 nm (11 mW, JDS Uniphase air-cooled HeNe), using the same detection strategy as for enumeration of phototrophic and heterotrophic cells. The sheath fluid was 0.2-µm filtered seawater. The instrument was controlled by a computer equipped with the FACSDiva software set on the four-way sorting 0/32/0 purity mode. Re-sorting of sorted samples was conducted randomly to check for the purity of the sorts, which reached 98 %. Sorted cells were collected in 2-mL microcentrifuge tubes where PCS scintillation cocktail was added up to a final volume of 2 mL.

Experiments were replicated on one occasion, and cellular Pi uptake rates varied by less than 4% in prokaryotic sorts and by 11% in Pic (n=4). Analytical error associated with cellular uptake rates was about 10% and error on cell abundances around 5%.

2.4. Soluble reactive phosphorus (SRP)

SRP was measured at the nanomolar level according to the Rimmelin and Moutin procedure (*Rimmelin and Moutin*, 2005), derived from the initial MAGIC method proposed by *Karl and Tien* (1992), using 250-mL triplicates per depth. The detection limit of this technique was around 5 nmol L^{-1}.

2.5. Statistical analyses and data treatment

Averaged values are reported as mean ± one standard deviation (sd). The Michaelis-Menten equation was used with estimated V^{max} and K+Sn to fit a Monod curve to the Pi uptake rates measured. They were only shown when a significant between the model and the data correlation was found ($p < 0.05$). Using the turnover time approach to determine kinetic parameters, V^{max} and K+Sn can always be determined, but only V^{max} is relevant in samples were $V=V^{max}$.

Biomass estimations were used to discuss our results, using our cell abundances and cellular P quotas by *Bertilsson et al.* (2003) and *Ho et al.* (2003).

3. Results

3.1. Environmental and biological conditions

A westward shoaling of the DCM (Table 1) and nutricline depths (120 to 50 m and 200 to 50 m, respectively) was observed along the transect. Total chlorophyll-a concentrations integrated over 150 meters were up to twice as high in the western basin (e.g. St. 25) compared to the Levantine basin (e.g. St. 9; Table 1), emphasizing the strong oligotrophic state of the eastern waters.

SRP concentrations ranged 6 – 80 nmol L^{-1}, varying with depth and location (Table 1). The highest maximum surface values were measured in the Western Basin (St. 21 and 25), possibly due to incomplete stratification of the water column and the proximity to the Rhone River compared to other stations. However, short Pi turnover times (0.8 to 10 h) were estimated (Fig. 2), revealing a high turnover of Pi in microbial communities along the whole transect at surface depths. It is important to point out that the detection limit for SRP measurements was close to the ambient concentrations, hence a potential overestimation of the latter and of Pi uptake rates

calculated using Sn. This caveat would not affect the main results about the relative capabilities of sorted groups in a given sample to take up Pi, as the same Pi concentration was multiplied by the turnover rates (h^{-1}) of each sorted group or bulk community for a given depth.

3.2. Enumeration of microbial groups

Hprok cells were the most abundant group, and varied little over sampled stations and depths, with an average of $3.9 \pm 0.3 \times 10^5$ cells mL^{-1} (n=24), and ranging 2.7×10^5 - 10.2×10^5 cells mL^{-1} (Fig. 3). Abundances of Proc cells varied between undetected levels to 1.4×10^5 cells mL^{-1}. Their pigment signature was too weak to be detected in surface waters without staining, therefore many experiments are missing data for Proc cells above 50 m. Syn cells were the most abundant at the coastal station 25 (7.7×10^4 cells mL^{-1} at 40 m) and the least abundant in the deep euphotic zone (130 m St. A, 0.6×10^3 cells mL^{-1}; Fig. 3). Finally, Pic cells were the most abundant in the Western Basin, ranging 230 cells mL^{-1} up to 0.3×10^5 cells mL^{-1}.

3.3. Bulk Pi uptake

P_i uptake rates ranged between 0.03 and 21.6 nmol P L^{-1} h^{-1} and varied by a factor of 10 along the water column at stations with the lower turnover times (9 and 21), while rates could be multiplied by 200 from one depth to the other at St. 25 and A (Fig. 4). No significant correlation was found between Pi uptake rates and SRP concentrations. In the Western basin, SRP concentrations were close to or no more than twice as high as in the Eastern basins (17.2 ± 14.0 nmol P L^{-1} h^{-1}, n=15, across the sampled depths), but Pi turnover times however could be 300 higher in the Western basin.

3.4. Group-specific P_i uptake

When all available, summed contributions of the 4 sorted groups to Pi uptake under ambient Pi concentrations represented 83.3 ± 30 % of the bulk signal (n=8). At the 4 stations, a higher contribution (up to 82.5% at St. A; Fig. 4) of Hprok cells was observed, with cellular uptake rates between 0.74 and 27.75 amol P $cell^{-1}$ h^{-1} (Table 1) between surface and 25 or 50 m. In the vicinity of the DCM, cyanobacterial cells were the major contributors to bulk signal (up to 72% at St. A; Fig. 4), mostly Proc cells in open sea samples (Fig. 3 a, c). Syn cells contributed to bulk signal by 53 % at the coastal station, where they reached the highest abundance measured during the cruise, while their average contribution over the cruise was of 16.3 ± 14 % (n=18). Differences between cellular mean rates were not significant (p=0.06), due to a high variability across samples for a single group (CV ≥ 84%). However, in half of the samples for which both Syn and Pic were sorted, cellular Pi uptake rates were higher in Syn than in Pic cells (Table 1). The biomass of Pic in the bioassay of St. A reached 1.2 nmol P L^{-1}, and ranged 15.7 – 34.4 nmol P L^{-1} along the profile at St. 25, which was 300, and 25 – 55 higher than the biomass of Syn, respectively. Proc estimated biomass was in the same order of magnitude as Syn, around 0.3 nmol P L^{-1}, and Hprok biomass was twice lower than Pic. In 22 out of 23 sorts, Hprok specific rates were consistently lower compared to cyanobacteria. At station A, volumetric Pi uptake rates were the highest for picoeukaryotes, and consistently high for all sorted groups (Table 1). Over all experiments, cellular uptake rates ranged 2.6 – 402, 3.6 – 40.0, 8.4 – 134.8, and 0.05 – 36.1 amol P $cell^{-1}$ h^{-1} for Syn, Proc, Pic, and Hprok, respectively (Table 1).

3.5. Kinetics of the P_i uptake

Data from St. C and A only could be used to explore kinetic characteristics, while no response to Pi addition was observed at St. 5 and B. Cellular (V_c^{max}) and volumetric (V^{max}) rates are reported in Table 2. Signals measured at St. B and C in sorted Pic cells during the isotope dilution

experiments did not show any dose response, and therefore no kinetic parameters were estimated. At St. A and C, the highest V^{max} was obtained for cyanobacterial sorts (Table 2). Cellular maximum uptake rates (V_c^{max}) were found in different relative order at both stations, with V_c^{max} Syn > Proc > Hprok at St. C and Pic > Syn > Proc at St. A (Table 2). Estimations of the $K+S_n$ constant Proc and Pic cells at St. A and Syn cells at St. C were comparable and about 5 times lower than the value measured for Syn cells at St. A (128.4 nmol P L^{-1}). At St. A, Syn, Proc and Pic exhibited a subsaturated Pi uptake under natural conditions (rates measured from profiles compared to V^{max} at this depth) with 13, 44 and 86 %, respectively. Both cellular and volumetric maximum rates were drastically lower at St. A compared to St. C, while K+Sn values in the bulk and Syn sorts were higher at St. A.

4. Discussion

A recent review on phosphorus marine biogeochemistry pointed out marine microbial cycling of phosphorus as the most dynamic and complex cycle of all (*Karl*, 2014). With consistently short Pi turnover times (< 10 h) measured in surface samples from the eastern basin, congruent with previous studies (*Flaten et al.*, 2005; *Moutin et al.*, 2002), our study emphasizes the short time scale and dynamics of Pi cycling in the Mediterranean. Low Pi turnover times deepening towards the East in the while SRP concentrations did not show a clear longitudinal and suggest a higher limitation of microbial communities in the Eastern basin.

4.1. Taxon-specific Pi uptake

Cellular Pi uptake rates suggest that there was variability across sorted groups, with Hprok having the lowest rates in most samples. When all 3 phototrophic groups were successfully sorted, there was not a single group clearly showing higher cellular Pi uptake rates, like there

were in samples from the North Atlantic (e.g. Pic *Lomas et al.*, 2014, Syn in *Michelou et al.*, 2011 and Syn and Pic in *Zubkov et al.*, 2007). We measured cellular Pi uptake rates for Proc cells over 10 fold higher (16.1 ± 13.6 amol P $cell^{-1}$ h^{-1}, n=15) in the Mediterranean Sea compared to rates measured in the Sargasso Sea (< 1 amol P $cell^{-1}$ h^{-1}: *Casey et al.*, 2009; *Michelou et al.*, 2011; *Zubkov et al.*, 2007]). We suggest 4 main reasons to explain differences across regions: i) different composition of the cyanobacterial community between the Sargasso Sea and the Mediterranean, notably clade HL II which is underrepresented in the Mediterranean (*Mella-Flores et al.*, 2011) ii) the low proportion of Proc cells (<10%) able to utilize Pi in the subsurface layers of the Sargasso Sea (*Martínez et al.*, 2012), iii) different proportions of live versus dead cyanobacterial cells in the Sargasso and Mediterranean Sea (*Agusti*, 2004), and iv) measurements conducted on fixed samples in the mentioned studies from the Sargasso Sea, possibly involving a significant leakage of intracellular Pi (*Talarmin et al.*, 2011b). The dominance of Syn in Pi uptake fluxes above the DCM at St. 25 was likely due to their higher affinity for Pi compared to Pic cells, because their biomass was 50 times lower than Pic.

4.2. Kinetic parameters in sorted groups

Summed volumetric V^{max} of sorted groups added up to the community V^{max} or below. Non-sorted large protists may have higher maximum Pi uptake rates per cell (*Casey et al.*, 2009) or the ability to store large amounts of Pi in case of upwelled or deposited inputs. The kinetic experiment results presented here shall be interpreted very cautiously due to their scarcity, and they should serve as a starting point to infer Pi uptake strategies with regard to environmental conditions. At Station C, the groups expected to have a higher affinity for low concentration did not show a response to Pi additions because they were taking up Pi at the maximum velocity. *Synechococcus* was described as a group able to respond rapidly and significantly to pulsed Pi

events (*Moutin et al.*, 2002), which could be supported by the fact that Pi uptake under ambient SRP concentrations was not saturated at St. A or St. C and that they did show a response to Pi additions at both stations. Our data also suggest that microbial communities have the potential to take up more Pi when Pi turnover times are short. Per cell Pi uptake rates converted in per volume Pi uptake rates have shown opposite trends, with cyanobacteria harvesting Pi more efficiently than larger phytoplankton (*Casey et al.*, 2009). With their high surface-to-volume ratio (*Azam et al.*, 1983), prokaryotes with a biovolume below 40 μm^3 may not be submitted to the theoretical surface-limited growth rate, due to a poor cellular machinery compared to the absorbing capability (*Dao*, 2013), hence their higher cellular uptake rates under low concentrations compared to picoeukaryotes.

4.3.Contribution of the sorted groups to total Pi uptake

Biogeochemically, using volumetric Pi uptake rates is more valuable than the cell-specific values to highlight the overall contribution of one group to Pi fluxes through the marine microbial P cycle. Down to about 25 m above the DCM, at the 4 stations sampled for depth profiles, the main contributors to Pi uptake fluxes were the heterotrophic prokaryotes. *Michelou et al.* (2011) found dominance of bulk Pi uptake by Hprok in the Sargasso Sea, in surface and DCM layers. These authors determined that cyanobacteria (Proc + Syn) contributed to less than 10 % of the Pi uptake fluxes. These contributions were a result of i) low Syn abundances, although this group exhibited 2 to 5-fold higher taxon-specific uptake rates than Proc and Hprok and intermediate Proc abundances, combined with ii) lower cell specific rates for Proc (0.4 amol P $cell^{-1}$ h^{-1}). In contrast, we found in the Mediterranean Sea a higher contribution of cyanobacteria close to the DCM compared to upper layers, mostly due to abundances higher than those observed in the North Atlantic at the time of the cruise (*Michelou et al.*, 2011).

The companion paper of these experiments published earlier by Casey et al. (2009) found no clear difference in the contribution of Proc and Syn to Pi utilization. Proc contributed to 45% of the bulk Pi uptake in the North Atlantic (*Zubkov et al.*, 2007), which is within the range of our measurements (<LD – 62.9%). In the North Pacific Subtropical Gyre, Pro and Hprok cells did not reveal different contributions to total Pi uptake fluxes, despite taxon-specific rates 3-fold higher for Proc than for Hprok (*Björkman et al.*, 2012). At the SOLA station of the eastern Mediterranean Sea (Banyuls sur mer, France), Syn contributed the most to the total Pi uptake (35%) among 3 sorted groups, while Pic and Hprok showed low and non-significantly different contributions (*Talarmin et al.*, 2011b). For the first time, our results suggest that the uptake of Pi at an offshore oligotrophic location was not dominated by a single group of microbes in the upper euphotic zone. The present study together with a previously published survey conducted during the same cruise on leucine incorporation in sorted groups (*Talarmin et al.*, 2011a) showed the importance of *Prochlorococcus* cells in elemental fluxes in the Mediterranean Sea, as it was previously demonstrated in the Sargasso Sea (*Casey et al.*, 2007).

The unexplained fraction in the contribution profiles could be attributed to missing sorts or taxonomic groups that were not taken into account. For instance, viruses and bacterivorous mixotrophic ciliates or nanoflagellates, which by-pass nutrients to the highest trophic levels of the microbial loop by grazing (*Krom et al.*, 2005; *Paffenhöfer et al.*, 2007), or attached bacteria, could also contribute to bulk P_i uptake fluxes. In freshwater systems, they could take up Pi and amino acid at higher rates than free-living bacteria (*Paerl and Merkel*, 1982; *Simon*, 1985). Finally, we also considered the possibility that Proc cells from surface layers had too low chlorophyll a content to be discriminated from Hprok in stained samples, resulting in an

overestimation of the Hprok contribution to bulk Pi uptake in the surface samples where Proc cells were not detected.

4.4. Other limitations

The vertical partitioning of Pi uptake observed in the present study suggested that different nutrient uptake strategies and capabilities may contribute to explain the vertical structure of microbial communities throughout the water column. In the surface where SRP concentrations are the lowest, only organisms with the lowest K+Sn can utilize Pi efficiently, i.e. Proc and Hprok cells. The cyanobacterial contribution to Pi uptake possibly decreased below the DCM because of light limitation (*Duhamel et al.*, 2012). During this cruise, a mesocosm study showed that surface communities were submitted to N and P co-limitation or N limitation, but no strict P-limitation (*Tanaka et al.*, 2011), and no nutrient (N, P) limitation was found at St. A. Dust deposition were found to be 89% from anthropogenic sources at this station (*Ternon et al.*, 2011), which may provide more or different P sources than in the more eastern basins isolated from all inputs. A larger effort in measuring environmental data, combined to phylogenetic analyses of the sorted groups would help to further link the diversity of microbes to their Pi uptake performances. Such experiments have been conducted in mesocosm conditions and showed that different bacterial taxa responded to Pi additions with different strategies in the Mediterranean Sea (*Sebastián et al.*, 2012). The concept of competition among microbes for a limited resource in natural environments is challenged by the numerous potential sources of growth limitation and the high diversity of cytometric groups (e.g.: *Kashtan et al.*, 2014; *Marie et al.*, 2010).

Conclusion

While a few taxon-specific Pi uptake rates from various areas were published in the past 7 years, our study was the first focusing on the Mediterranean Sea and uncovering a vertical partition of Pi uptake fluxes among microbial groups. Each group studied in this survey seemed to have a key role in Pi cycling under given environmental conditions, whether it through high affinity for Pi at low concentrations (Hprok and Proc), or the ability to take up Pi at high rates (Syn and Pic) at some depths. The variability observed within and across sorted groups seems to reflect different kinetic abilities ranging along a continuum of Pi uptake strategies as well as phylogenetic diversity within cytometric groups.

We found that different groups were dominating bulk Pi uptake fluxes at different depths, with Hprok contributing the most in the surface, subsurface layers and likely also the bottom layers of the euphotic zone, while cyanobacteria were dominating fluxes around the DCM zone. Multiple nutrient and energy limitations need to be further investigated to better understand this vertical partition of Pi uptake in oligotrophic waters.

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Table 1. Environmental context of the experiments: sampled depth (m), depth of the deep chlorophyll maximum (DCM_d), 0 – 150 m integrated chl *a* concentrations, SRP concentrations (mean ± sd), and cellular rates of Pi uptake measured in sorted groups *Synechococcus* (Syn), *Prochlorococcus* (Proc), picoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) measured under ambient concentrations. NA: data not available

Station	Depth (m)	DCMd (m)	Chl *a* int (mg C m^{-2})	[SRP] (nM)	Cellular Pi uptake rate (amol P $cell^{-1}$ h^{-1})			
					Syn	Proc	Pic	Hprok
C	100	108	25.10	12.8 ± 6.7	49.8	17.5	NA	1.2
9	5	128	16.20	20.2 ± 2.3	58.7	NA	NA	27.7
9	50			17.6 ± 1.6	14.0	NA	NA	0.7
9	75			12.4 ± 3.7	17.6	NA	NA	7.6
9	105			23.1 ± 3.7	41.7	35.5	NA	7.1
9	120			13.6 ± 1.4	46.5	39.9	NA	13.5
5	50	114	24.70	12.7 ± 6.7	43.0	13.0	NA	0.4
B	100	141	21.20	8.6 ± 0.5	24.3	20.3	NA	36.1
21	5	87	22.40	9.6 ± 1.9	401.6	NA	NA	3.6
21	50			13.1 ± 3.6	9.6	12.7	NA	2.6
21	70			14.4 ± 2.0	9.0	2.2	8.4	0.2
21	85			80.0 ± 17.6	23.5	5.8	49.4	0.7
A	6	88	24.70	10.6 ± 2.8	24.1	NA	8.6	11.5
A	13			5.8 ± 2.0	10.7	NA	4.0	5.8
A	25			6.3 ± 1.0	5.8	NA	3.3	3.2
A	75			8.3 ± 3.9	8.6	11.7	11.7	1.8
A	90			8.3 ± 0.8	13.1	9.5	33.7	0.4
A	100			16.4 ± 1.4	25.1	1.6	7.9	0.1
A	110			24.2 ± 3.4	5.0	1.5	7.3	NA
A	130			20.2 ± 1.3	5.7	3.6	3.8	0.1
25	5	51	38.50	17.3 ± 2.3	68.7	NA	44.9	8.7
25	25			18.00	48.7	37.2	55.5	16.2
25	40			18.00	NA	30.2	67.0	1.3
25	50			18.67	49.0	NA	134.8	0.8
25	60			19.10	NA	NA	NA	NA

Table 2. P_i uptake kinetic characteristics of the bulk community and sorted groups: V^{max} is the theoretical volumetric maximum uptake rate of a group, V_c^{max} is the cellular maximum uptake rate, and $K+S_n$ is the theoretical sum of the half saturation constant for uptake and the natural $[P_i]$. LD: below the limit of detection (< 3 blank values). NA: data not available

			V_c^{max} (amol P cell^{-1} h^{-1})				V^{max} (nmol P L^{-1} h^{-1})					$K+S_n$ (nmol P L^{-1})				
St.	**z (m)**	**T_{Pi} (h)**	**Syn**	**Proc**	**Pic**	**Hprok**	**Bulk**	**Syn**	**Proc**	**Pic**	**Hprok**	**Bulk**	**Syn**	**Proc**	**Pic**	**Hprok**
C	100	3.9	132	21	LD	2	4.0	1.8	1.1	LD	0.6	15.3	22.9	NA	LD	NA
A	90	370.9	12	1	99	NA	0.2	0.03	0.02	0.02	NA	62.6	128.4	22.7	27.7	NA

Table S1. Detailed values of bulk Pi uptake rates and contribution of the groups to bulk Pi uptake at St. 9, St. 21, St. A and St. 25. NA= no available data

Station	Depth (m)	Bulk Pi uptake rate (nmol L^{-1} h^{-1})	Contribution to total Pi uptake (%)			
			Syn	Proc	Pic	Hprok
9	5	12.51	1.33	NA	NA	59.64
9	50	5.56	1.31	NA	NA	52.83
9	75	6.53	5.73	NA	NA	42.56
9	105	9.37	4.17	5.60	NA	48.54
9	120	1.22	14.02	51.62	NA	12.08
21	5	5.96	2.05	NA	NA	33.74
21	50	5.29	0.76	3.26	NA	34.42
21	70	0.91	7.11	8.26	0.44	9.85
21	85	1.79	2.77	3.64	1.45	21.72
A	6	6.64	2.40	NA	0.11	64.29
A	13	3.47	1.32	NA	0.10	74.71
A	25	1.94	0.63	NA	0.17	82.54
A	75	2.84	4.62	56.40	0.45	36.80
A	90	0.98	8.98	62.87	10.19	21.42
A	100	0.08	14.15	34.70	12.63	48.98
A	110	0.04	23.61	25.49	7.77	NA
A	130	0.03	16.97	21.84	3.04	66.37
25	5	9.97	13.70	NA	0.38	57.87
25	25	21.61	11.50	5.86	0.34	65.79
25	40	10.01	21.37	9.00	1.17	13.59
25	50	3.83	53.41	NA	6.46	20.00
25	60	0.15	19.22	NA	13.53	19.23

Figure legends

Figure 1. Map of the Mediterranean Sea with locations of sampling sites during the BOUM cruise transect. Numbered and lettered stations denote locations where our experiments were conducted.

Figure 2. Phosphate turnover time (h) of the bulk community (> 0.2 µm) over the BOUM cruise transect.

Figure 3. Abundances of *Synechococcus* (Syn), *Prochlorococcus* (Proc), picophytoeukaryotes (Pic), heterotrophic prokaryotes (Hprok) at the depths of sorted experiments at stations 9, 21, A and 25.

Figure 4. Cumulated contributions of picoplankton groups (superimposed bars for: *Synechococcus Syn*, *Prochlorococcus Proc*, picophytoeukaryotes *Pic*, heterotrophic prokaryotes *Hprok*, superimposed histograms) to the bulk P_i uptake (upper scale, 100% = measured bulk P_i uptake rate) and bulk P_i uptake rates (lower scale, white triangles) along the euphotic water column at stations 9, 21, A and 25. Dotted horizontal lines mark the deep chlorophyll maximum depth. Beware of the different vertical scales. Missing groups are specified with a *.

Figure 5. Bulk and taxon-specific P_i uptake rates (V) at increasing P_i concentrations (in situ + added) in sorted *Synechococcus* (Syn), *Prochlorococcus* (Proc), picophytoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) at St A and St. C. Straight horizontal and vertical lines represent computed V^{max} and $K+S_n$, respectively. If a significant correlation ($p<0.05$) between measured Pi uptake rates and those calculated from the Michaelis-Menten model, the uptake curve was fitted to the data.

Figure S1. Contribution of size fractions to total Pi uptake as a function of bulk Pi turnover time at various depths and stations along the BOUM transect.

Figure S2. Bulk Pi uptake rates (h^{-1}) plotted against SRP concentrations.

Fig. 1

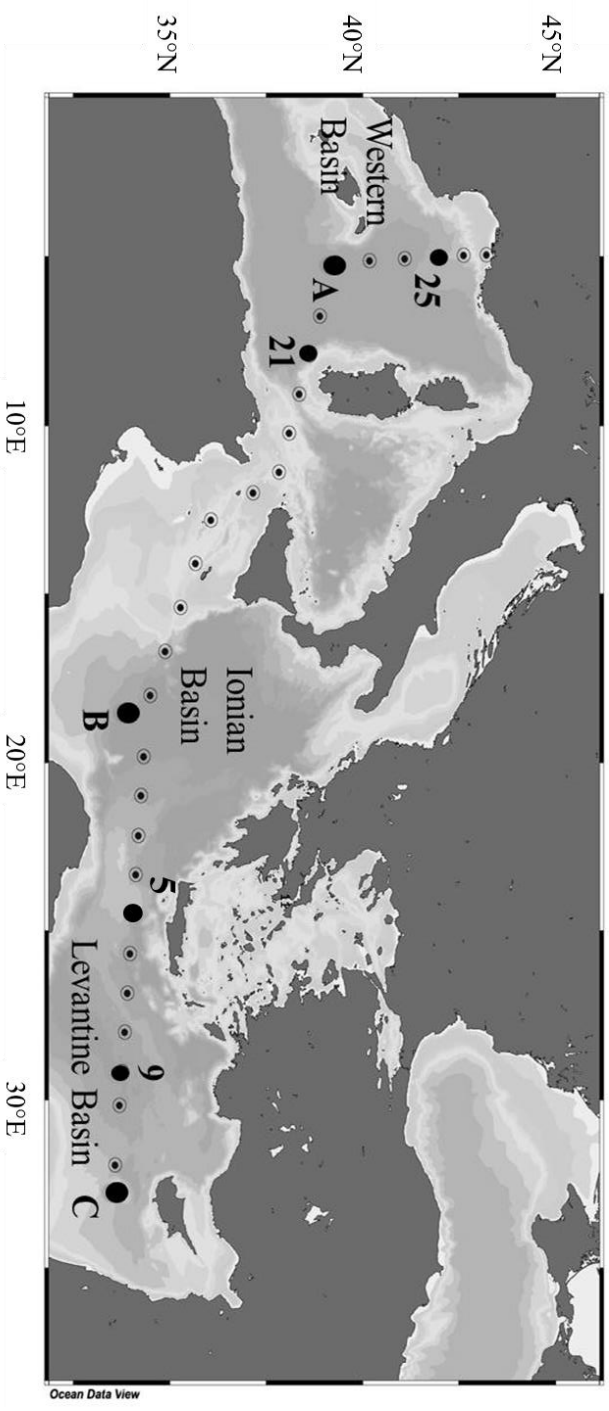

45°N
40°N
35°N
10°E
20°E
30°E
Western Basin
Ionian Basin
Levantine Basin
25
A
21
B
5
9
C
Ocean Data View

Fig. 2

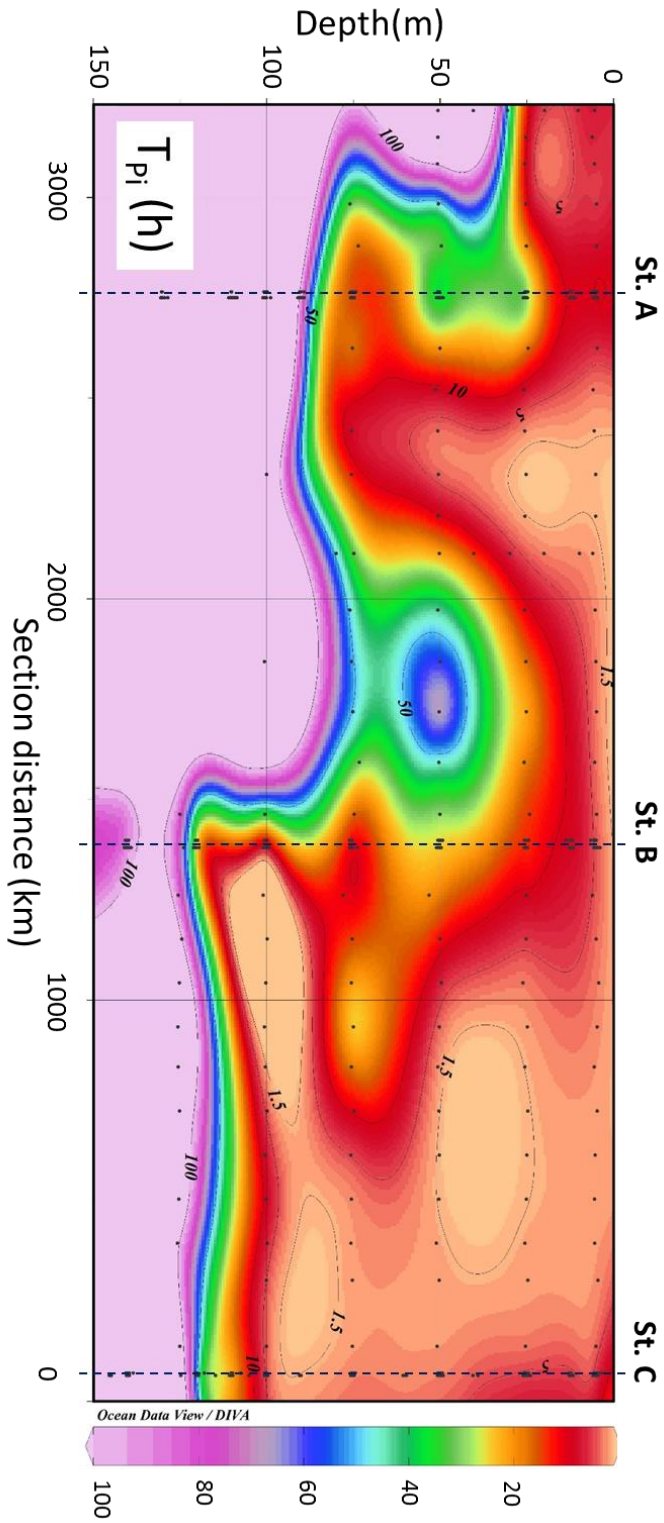

Depth(m)
0
50
100
150
T_{Pi} (h)
St. A
St. B
St. C
Section distance (km)
0
1000
2000
3000
Ocean Data View / DIVA
100
80
60
40
20

Fig. 3

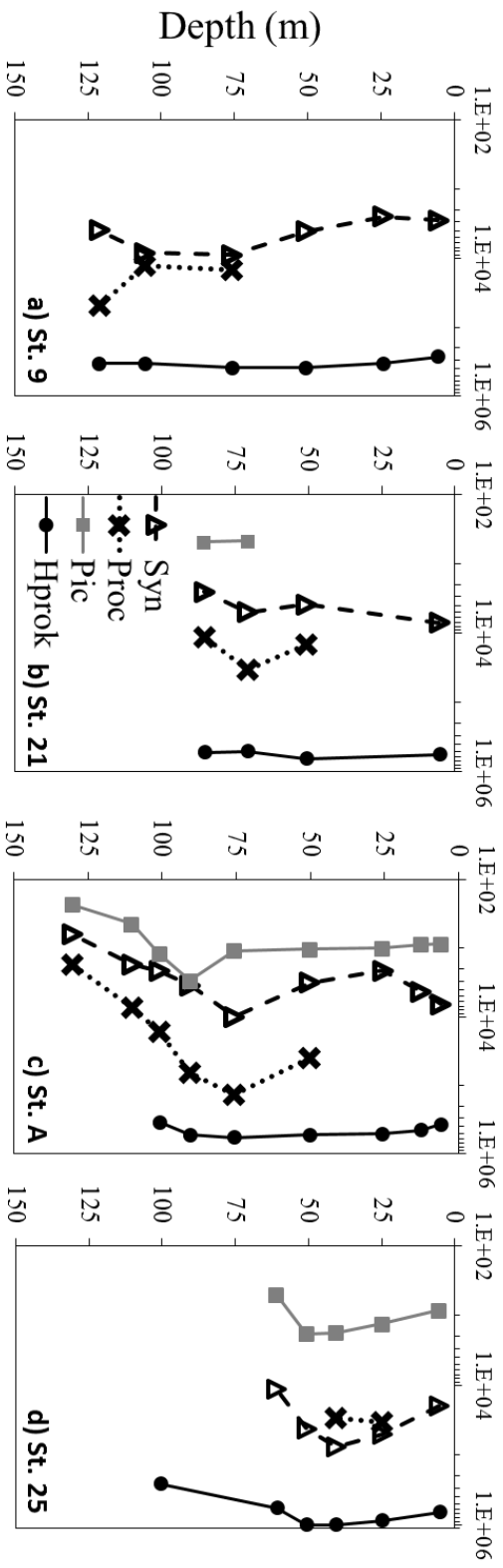

Depth (m)
Abundances (cells mL-1)
1.E+02
1.E+04
1.E+06
0
25
50
75
100
125
150
a) St. 9
b) St. 21
c) St. A
d) St. 25
Syn
Proc
Pic
Hprok

Fig. 4

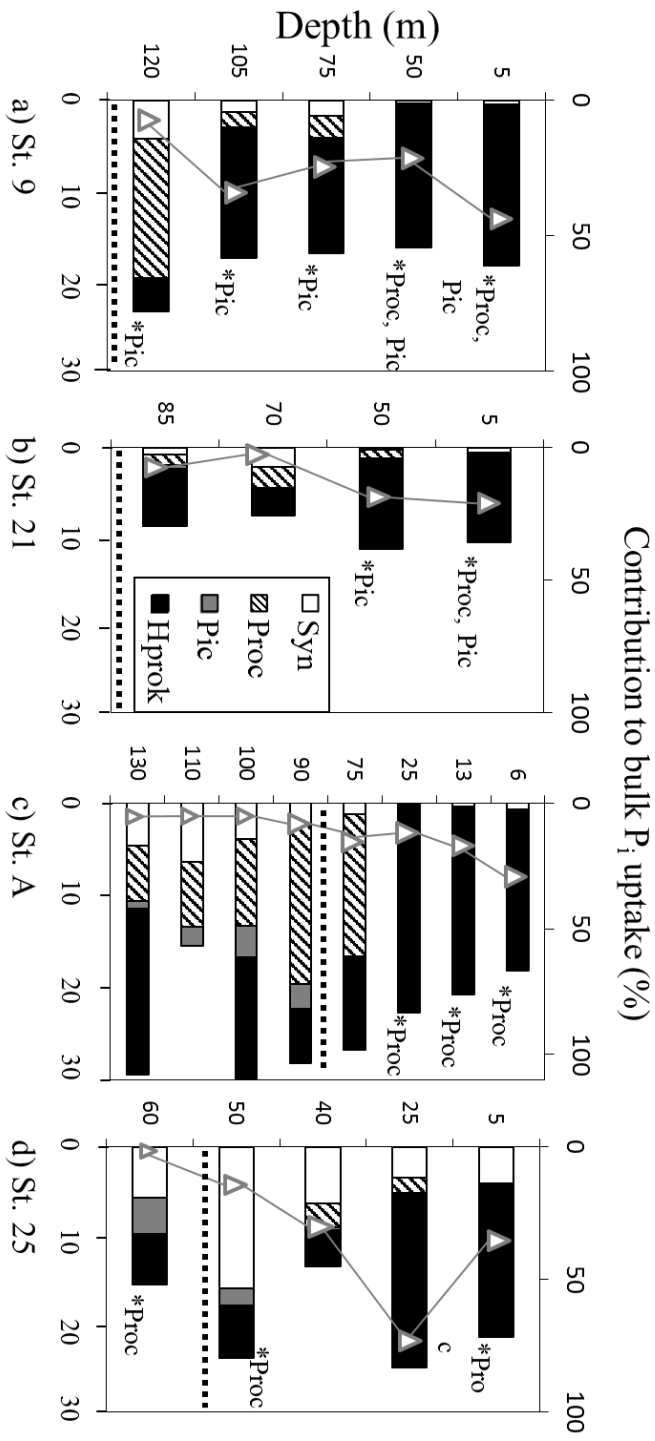

Depth (m)
Contribution to bulk P_i uptake (%)
Bulk P_i uptake rate (nmol L^{-1} h^{-1})
a) St. 9
b) St. 21
c) St. A
d) St. 25
*Proc, Pic
*Proc, Pic
*Pic
*Pic
*Pic
*Pic
*Proc, Pic
*Pic
*Proc
*Proc
*Proc
*Proc
*Proc
Syn
Proc
Pic
Hprok

Fig. 5

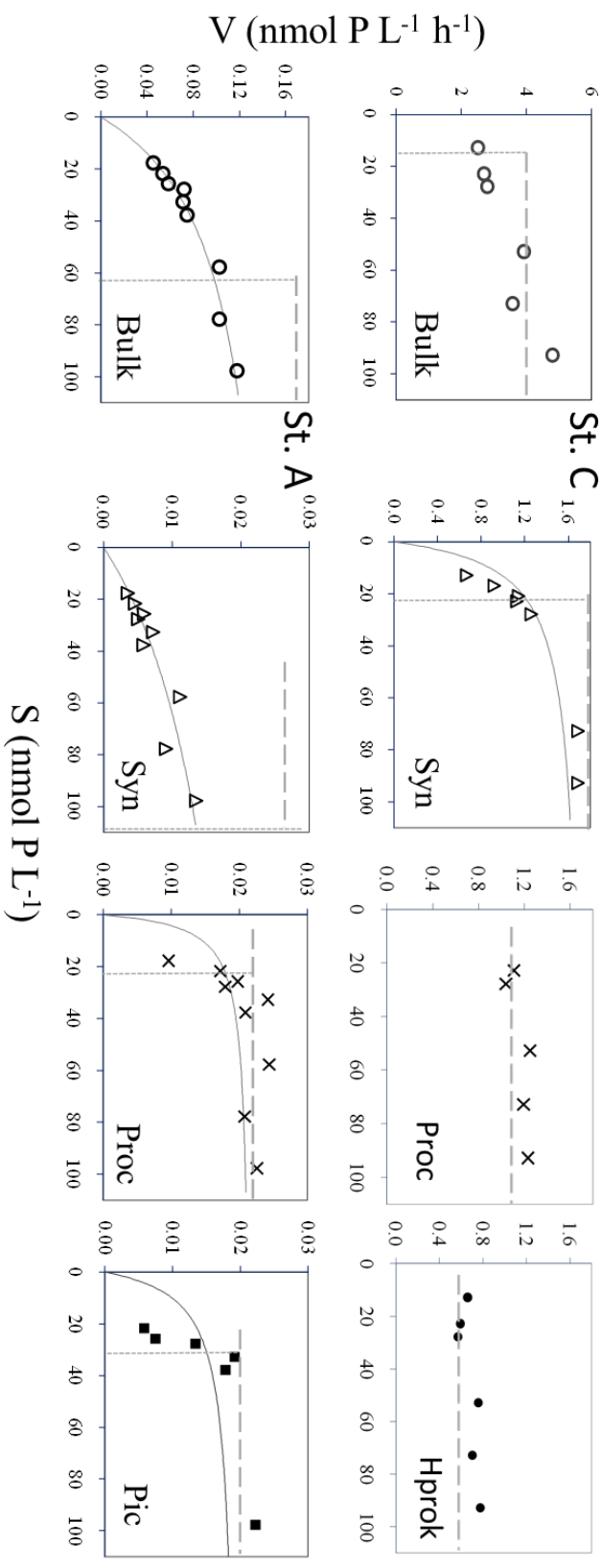


Fig. S1

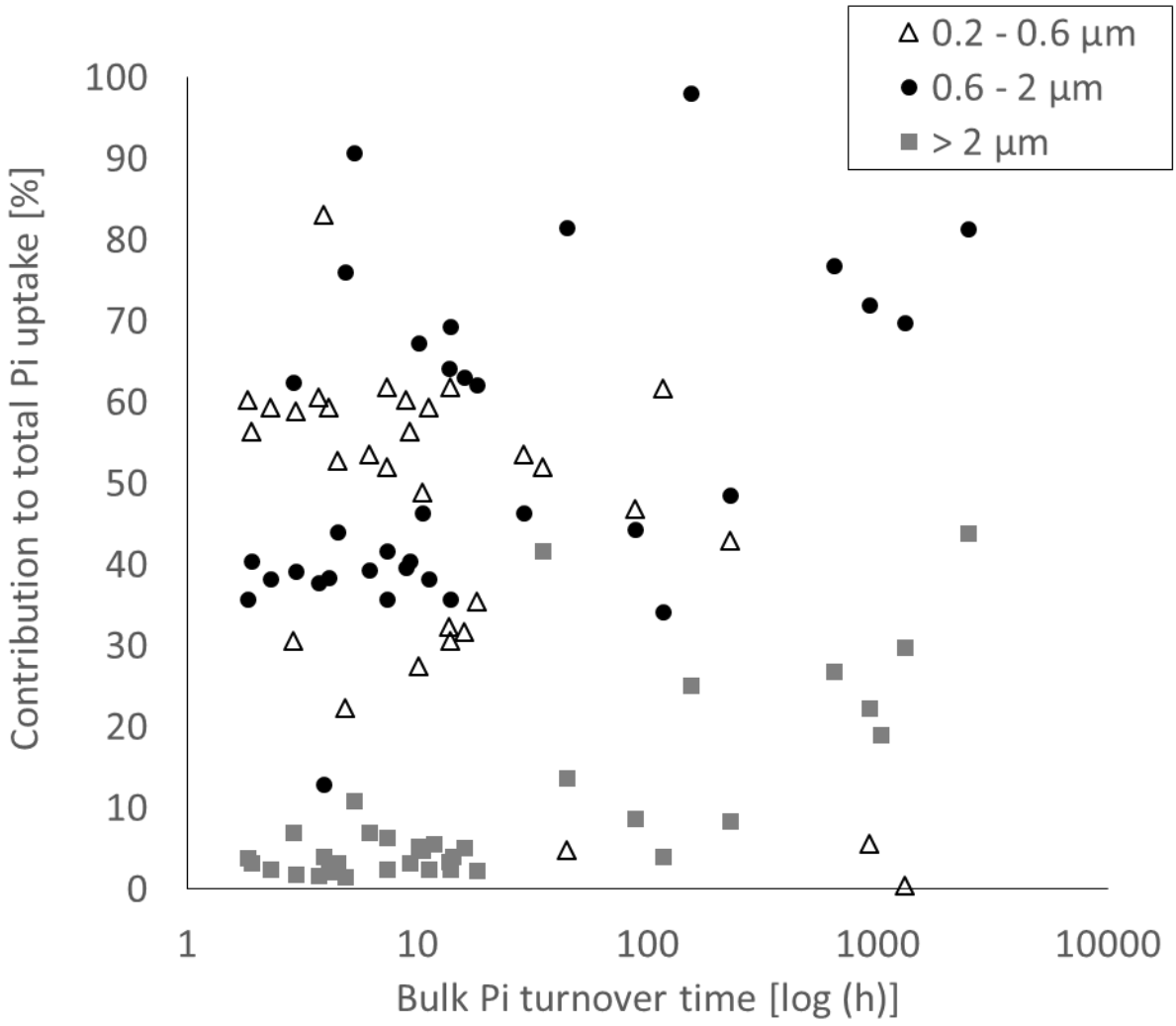
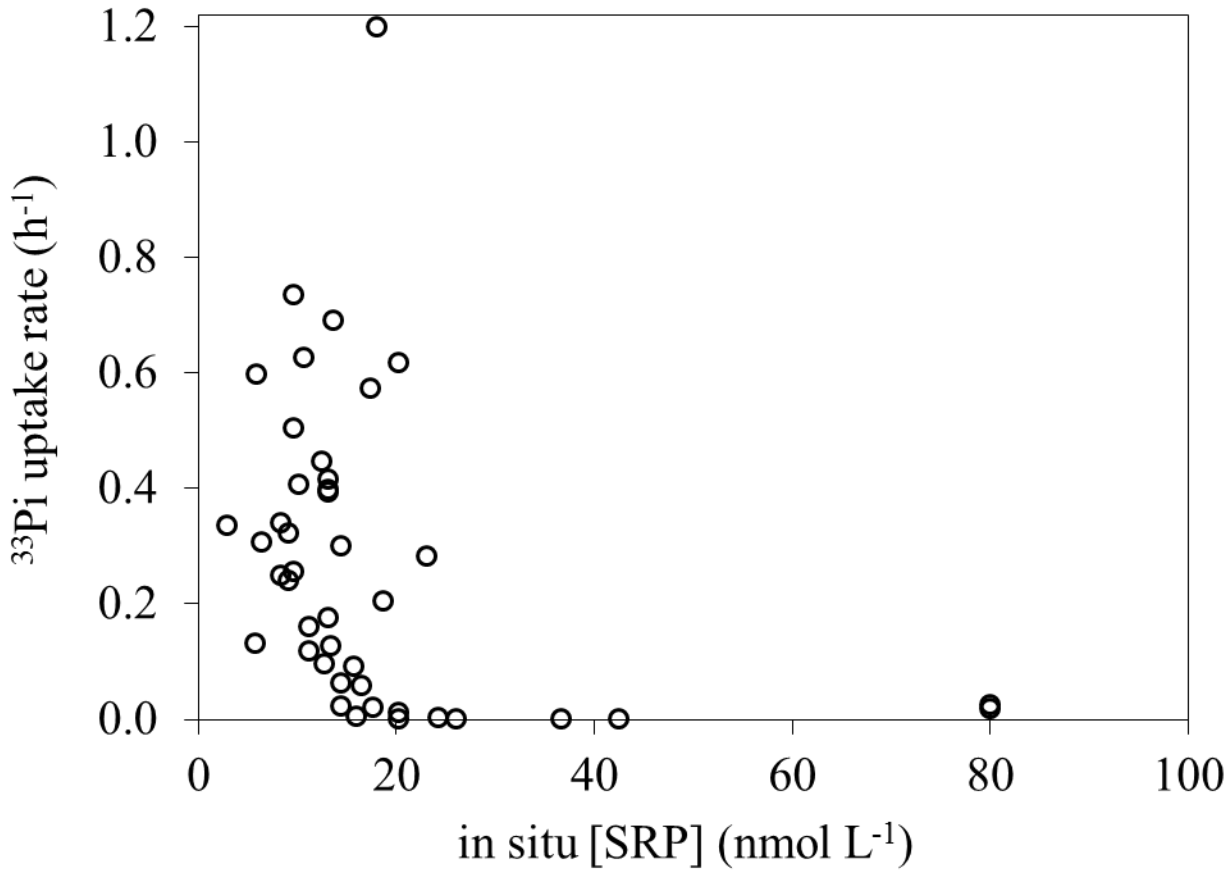

0.2 - 0.6 µm
0.6 - 2 µm
> 2 µm
Contribution to total Pi uptake [%]
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
10000
Bulk Pi turnover time [log (h)]

Fig. S2


1.2
1.0
0.8
0.6
0.4
0.2
0.0
^{33}Pi uptake rate (h^{-1})
0
20
40
60
80
100
in situ [SRP] (nmol L^{-1})