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Vertical partitioning of phosphate uptake among picoplankton groups in the low Pi 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 phosphate (Pi) uptake fluxes in the euphotic zone of the low-Pi Mediterranean Sea and estimated Pi uptake kinetic characteristics. Surface soluble reactive phosphorus (SRP) concentrations were in the range of 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, four vertical profiles of Pi uptake rates were established for heterotrophic prokaryotes (Hprok), phototrophic picoeukaryotes (Pic) and Prochlorococcus (Proc) and Synechococcus (Syn) cyanobacteria. Hprok cells contributed up to 82% of total Pi uptake fluxes in the superficial euphotic zone, through constantly high abundances (2.7–10.2 × 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 station (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 Mediterranean seas (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; 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 diffu-
sion at higher environmental Pi concentrations (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 phototrophic eukaryotes (> 3 µm: Currie and Kalff, 1984; Currie et al., 1986). Some eukaryotes possess mixotrophic capabilities: grazing on P-richer prokaryotes (Christaki et al., 1999; Hartmann et al., 2011) as well as DOP hydrolysis induced by ectoenzymes (ATPases, alkaline phosphatas, e.g., Webb, 1992) can fill most of their requirements of P. Eukaryotic phytoplankton may compensate for 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, 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 for studying Pi uptake strategies in heterotrophic and phototrophic microbes (e.g., Björkman et al., 2012; Casey et al., 2009; Duhamel et al., 2012; 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 Synechococcus (Syn) compared to Hprok and Prochlorococcus (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, 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 Pi uptake flux in the low Pi stratified upper water column of the Mediterranean Sea (down to 200 m). An estimation of kinetic constants (maximum Pi uptake rate- $V_{max}$, and the sum of the half-saturation constant plus Pi natural concentration $K + S_n$) of $^{33}$Pi-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 the understanding of 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 undertaken 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 and 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 125 m. 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

Tripled 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 $-80^\circ$ C until further processing onboard 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 distinguished 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 determined 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 Pi 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, taxon-specific Pi uptake rates were based on the protocol by Talarmin et al. (2011b). In brief, a radioactive working solution 15 min later, with an activity of 0.34 MBq per sample, i.e., a final radioactive Pi concentration of 60 pmol L\(^{-1}\), and incubated for 45 min. Bulk Pi uptake rates, bulk Pi turnover times, bulk and taxon-specific kinetic constants \(V^{\text{max}}\) (maximum velocity of Pi uptake) and \(K + S_0\) (\(K\) being the half-saturation constant and \(S_0\) the natural Pi concentration, their sum being determined as the intersection of the plotted line with the \(x\) axis) were based on the work of Thingstad et al. (1993). Such estimations were obtained using the linear regression of Pi turnover time vs. added concentrations.

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 taxon-specific Pi uptake under ambient concentrations of Pi. Samples were spiked with a final concentration of 20 pmol L\(^{-1}\) of the \(^{33}\)P-orthophosphate working solution and incubated for 15 min.

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 phototrophic picoeukaryotes (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 nuetrilines 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\(_2\)PO\(_4\) solution (0, 4, 8, 10, 15, 20, 40, 60, 80, 100 pmol L\(^{-1}\) added concentration). Samples were spiked with the radioactive working solution 15 min later, with an activity of 0.34 MBq per sample, i.e., a final radioactive Pi concentration of 60 pmol L\(^{-1}\), and incubated for 45 min.

Bulk Pi uptake rates, bulk Pi turnover times, bulk and taxon-specific kinetic constants \(V^{\text{max}}\) (maximum velocity of Pi uptake) and \(K + S_0\) (\(K\) being the half-saturation constant and \(S_0\) the natural Pi concentration, their sum being determined as the intersection of the plotted line with the \(x\) axis) were based on the work of Thingstad et al. (1993). Such estimations were obtained using the linear regression of Pi turnover time vs. added concentrations.

2.3.3 Bulk Pi uptake measurements

The 5 mL aliquots were gently filtered on a 0.2 \(\mu\)m polycarbonate membrane superimposing a GF/D filter soaked with a cold Pi solution without rinsing. A 5 s 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 photo- and heterotrophic cells. The sheath fluid was 0.2 \mu m 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 ± 1 standard deviation (SD). The Michaelis–Menten equation was used with estimated \( V^\text{max} \) and \( K + S_0 \) to fit a Monod curve to the Pi uptake rates measured. They were only shown when a significant correlation between the model and the data was found (\( p < 0.05 \)). Using the turnover time approach to determine kinetic parameters, \( V^\text{max} \) and \( K + S_0 \) can always be determined, but only \( V^\text{max} \) is relevant in samples where \( V = V^\text{max} \).

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 m were up to twice as high in the western basin (e.g., station (St.) 25) compared to the Levantine Basin (e.g., St. 9; Table 1), emphasizing the strong oligotrophic state of the eastern waters.
Table 1. Environmental context of the experiments: sampled depth (m), depth of the deep chlorophyll maximum (DCM), 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.

<table>
<thead>
<tr>
<th>Station</th>
<th>Depth (m)</th>
<th>DCM$_d$ (m)</th>
<th>Chl $a$ int (mg C m$^{-2}$)</th>
<th>[SRP] (nmol L$^{-1}$)</th>
<th>Cellular Pi uptake rate (amol P cell$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Syn</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>108</td>
<td>25.10</td>
<td>12.8 ± 6.7</td>
<td>49.8</td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>6</td>
<td>88</td>
<td>24.70</td>
<td>10.6 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25</td>
<td>51</td>
<td>38.50</td>
<td>17.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25</td>
<td>40</td>
<td>18.00</td>
<td>18.00</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>18.67</td>
<td>49.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>25</td>
<td></td>
<td>19.10</td>
<td>NA</td>
</tr>
</tbody>
</table>

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), which were mostly Proc cells in open sea samples (Fig. 3a, c). Syn cells constituted 53 % of bulk signal at the coastal station (Table S1), where they reached the highest abundance measured during the cruise, while their average contribution over the cruise was 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 PL$^{-1}$, whereas it was in the range of 15.7–34.4 nmol PL$^{-1}$ along the profile at St. 25. These were 300 and 25–55 times higher than the biomass of Syn at the respective stations. Proc estimated biomass was of the same order of magnitude as Syn, around 0.3 nmol PL$^{-1}$, and Hprok biomass was twice lower than Pic. In 22 out of 23 sorts, Hprok specific rates were consistently lower compared to cyanobacteria. Over all experiments, cellular uptake rates were in the range of 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). Over all sampled depths, Pi uptake was dominated by the smallest organisms up to 99 % (< 2 µm, Fig. S1).

3.5 Kinetics of the Pi uptake

Data from St. C and A could only be used to explore kinetic characteristics, while no response to Pi addition was observed at St. 5 and B. Cellular ($V_{c}^{\text{max}}$) and volumetric ($V_{V}^{\text{max}}$) 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 $V_{V}^{\text{max}}$ was obtained for cyanobacterial sorts (Table 2, Fig. 5). Cellular maximum uptake rates ($V_{c}^{\text{max}}$) were found in different rel-
suggest four main reasons to explain differences across re-
sured in the Sargasso Sea (< 1 amol P cell
\(n=\) cells over of 10-fold higher (16.1 ±
Syn in Michelou et al., 2011 and Syn and Pic in Zubkov
from the North Atlantic (see, e.g., Pic in Lomas et al., 2014,
ing higher cellular Pi uptake rates, like there were in samples
cessfully sorted, there was not a single group clearly show-
most samples. When all three phototrophic groups were suc-
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 three phototrophic groups were suc-
successfully sorted, there was not a single group clearly show-
ing higher cellular Pi uptake rates, like there were in samples
from the North Atlantic (see, e.g., Pic in 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 of 10-fold higher (16.1 ± 13.6 amol P cell\(^{-1}\) h\(^{-1}\),
\(n=15\) in the Mediterranean Sea compared to rates mea-
sured 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 four main reasons to explain differences across re-
gions: (i) different composition of the cyanobacterial com-
munity between the Sargasso Sea and the Mediterranean, no-
tably clade HL II which is underrepresented in the Mediterr-
anean (Mella-Flores et al., 2011), (ii) the low proportion of
Proc cells (< 10%) able to utilize Pi in the subsurface layer-
ers of the Sargasso Sea (Martínez et al., 2012), (iii) different
proportions of live vs. dead cyanobacterial cells in the Sar-
gasso and Mediterranean seas (Agustí, 2004) and (iv) mea-
surements conducted on fixed samples in the mentioned stud-
ies from the Sargasso Sea, possibly involving a significant
leakage of intracellular Pi (Talarmin et al., 2011b). The dom-
inance 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 \(V^\text{max}\) of sorted groups added up to the
community \(V^\text{max}\) 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 should be interpreted very cautiously due to
their scarcity, and they should serve as a starting point for
inferring Pi uptake strategies with regard to environmental
conditions. At station C, the groups expected to have a higher
affinity for low concentrations did not show a response to Pi
additions because they were taking up Pi at maximum veloc-
ity. *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 C and that they did show a response to Pi additions at
both stations. Our data also suggest that microbial commu-
nities have the potential to take up more Pi when Pi turnover
times are short. Per cell Pi uptake rates converted into per
volume Pi uptake rates show opposite trends, with cyanobac-
teria harvesting Pi more efficiently than larger phytoplankton
(Casey et al., 2009). With their high surface-to-volume ra-
tio (Azam et al., 1983), prokaryotes with a biovolume below
40 µm\(^3\) may not be constrained by the theoretical surface-
limited growth rate, due to a poor cellular machinery com-
pared to the absorbing capability (Dao, 2013), hence their
higher cellular uptake rates under low concentrations com-
pared to picoeukaryotes.

4.3 Contribution of the sorted groups to total Pi uptake

Biogeochemically, using volumetric Pi uptake rates is more
valuable than the cellular values for highlighting the over-
all contribution of one group to Pi fluxes throughout the
marine microbial P cycle. Down to about 25 m above the
DCM, at the four stations sampled for depth profiles, the
main contributors to Pi uptake fluxes were the heterotrophic
prokaryotes. Michelou et al. (2011) found dominance of bulk

Figure 2. Phosphate turnover time (h) of the bulk community
(> 0.2 µm) over the BOUM cruise transect.
Pi uptake by Hprok in the Sargasso Sea, in surface and DCM layers. These authors determined that cyanobacteria (Proc + Syn) contributed 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 cellular uptake rates than Proc and Hprok and intermediate Proc abundances, combined with (ii) lower cellular rates for Proc (0.4 amol P cell$^{-1}$ h$^{-1}$). In contrast, in the Mediterranean Sea we found 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 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 in the eastern Mediterranean Sea (Banyuls-sur-Mer, France), Syn contributed the most to the total Pi uptake (35%) among three 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
Table 2. Pi uptake kinetic characteristics of the bulk community and sorted groups: \( V_{\text{max}} \) is the theoretical volumetric maximum uptake rate of a group, \( V_{\text{max}}^c \) is the cellular maximum uptake rate, and \( K + S_n \) is the theoretical sum of the half saturation constant for uptake and the natural [Pi]. LD: below the limit of detection (< 3 blank values). NA: data not available.

<table>
<thead>
<tr>
<th>St.</th>
<th>z (m)</th>
<th>( V_{\text{max}} ) (amol P cell(^{-1}) h(^{-1}))</th>
<th>( V_{\text{max}}^c ) (nmol P L(^{-1}) h(^{-1}))</th>
<th>( K + S_n ) (nmol P L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>100</td>
<td>132</td>
<td>21</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LD 2</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>12</td>
<td>99</td>
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</tr>
<tr>
<td>C</td>
<td></td>
<td>NA 0.03</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>LD ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>370.9</td>
<td>1.1</td>
<td>LD 0.6</td>
<td>15.3</td>
</tr>
<tr>
<td>A</td>
<td>22.7</td>
<td>27.7</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Bulk and taxon-specific Pi uptake rates (\( V \)) at increasing Pi concentrations (in situ + added) in sorted Synechococcus (Syn), Prochlorococcus (Proc), picophytoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) at St. A and C. Straight horizontal and vertical lines represent computed \( V_{\text{max}} \) 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 occurred, the uptake curve was fitted to the data.

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 the explanation of the vertical structure of microbial communities throughout the water column. In the surface where SRP concentrations are the lowest, only organisms with the lowest \( K + S_n \) 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. Eighty-nine percent of dust deposition was found to be 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 with 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).
5 Conclusions

While a few taxon-specific Pi uptake rates from various areas have been published in the past 7 years, our study was the first to focus 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 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 dominated 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 dominated 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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