Interactive comment on "Vertical partitioning of phosphate uptake among picoplankton groups in the P-depleted Mediterranean Sea" by A. Talarmin et al. *bg-2014-464*

Each point is structured as: *Referee's comments*

Author's response

Author's changes to manuscript

1. General response of the authors to Handling Editor, Editors, and Reviewers with major changes operated in the revised manuscript

2. Point-by-point reply to reviewer #1

3. Point-by-point reply to reviewer #2

4. Point-by-point reply to reviewer #3

5. Marked-up revised manuscript

January 6th, 2014

Dear BG Editors,

Our manuscript has been revised based on suggestions and clarifications asked by the Associated Handling Editors and 3 Anonymous Reviewers, who we hereby greatly acknowledge.

Among the main changes, you will find the updated title "Vertical partitioning of phosphate uptake among picoplankton groups in the **low-P** Mediterranean Sea" instead of "Vertical partitioning of phosphate uptake among picoplankton groups in the **P-deficient** Mediterranean Sea", a completely re-written abstract including a larger portion of data, a completely re-written Conclusion section and the heavily changed Discussion to include more references supporting our hypotheses, and to put some of the kinetic results into perspective. We are aware that our small data set does not allow much projections, however, by comparing data obtained at St. A and St. C, we still have the possibility to infer potential Pi uptake strategies for the different groups. Those 2 stations were selected because comparable SRP concentrations but very different Pi turnover times were measured. All reviewer's comments have been carefully considered and they have allowed to develop an extended discussion around which factors can impact Pi uptake in oligotrophic environments.

Captions for Fig. 2 and Fig. 4 have been changed, based on comments. A supplementary figure S1 was added in our response to Reviewers 1 and 3, showing size fractionated Pi uptake contributions. It will remain a supplementary figure.

An additional figure (panels of Pi uptake rates vs SRP concentrations) was requested by Reviewer #2 who inquired more details about SRP concentrations. We do agree that the standard deviations are valuable, however, the SRP concentrations are indicative and the turnover time measurements are what the authors wish to highlight to depict the conditions of Pi bioavailability in the cruise. Fig. S2 can therefore be found within the reply to Reviewer 2's comments. The authors actually debated before submission as to adding such a plot, and we eventually decided that it did not add to what should be discussed. If split into several panels per station, each panel would have only a few points and that would add to the difficulty of interpretation.

Reviewer #2 also asked that data from Fig. 4 be added to the main Table 1. We have produced this table (Table S1) to address this request. To limit redundancy, we would like to submit these as a supplementary table, so that not all values are described in the Results, in Table 2 and in Figure 4. We do understand that data are easier to be grabbed from a table, however the Fig. 4 seems more powerful to display them in the main manuscript.

Those major modifications have been included in the following revised manuscript. We would be grateful if you would consider it for publication in *Biogeosciences*.

Sincerely,

Agathe Talarmin, On behalf of all authors.

Anonymous Referee #1

Received and published: 4 November 2014

This paper clarified vertical distributions of phosphate uptake rates by different planktonic groups in the Mediterranean Sea, where surface phosphate is often severely depleted. The results can contribute to the understanding of biogeochemical cycles of phosphorus in the open oceans. The methods employed are well established ones, and they are totally reliable. All the data have sufficient quality and novelty for publication in Biogeosciences. However, I think that the authors could expand the discussion more extensively and intensively. Some sections of the discussion give me the feeling that something is left unfinished. The authors should totally rearrange the discussion and abstract before publication in Biogeosciences.

These comments were valuable to improve the critical points to be discussed and highlight the most relevant data. The reviewer should know that conclusions and abstract were entirely re-written, the discussion deeply re-structured and reinforced with comparisons and references. Thank you for these helpful insights.

P3L7. As widely known, these are reports on utilization of organic phosphorus by marine plankton after enzymatic hydrolytic actions, not direct uptake of organic phosphorus. And the utilization of phosphomonoesters after hydrolysis catalyzed by alkaline phosphatase had been known earlier. These precedent reports should be included in references.

I agree, alkaline phosphatase activities have been reported in Pi-limited environments and in cultures a long time ago. Here we actually cite references with actual uptake of DOP compounds, apart from Glibert et al 2004 who measured uptake of DON compounds but monitored DOP utilization y measuring AP activities.

With reports of high alkaline phosphatase activities linked to other origins than strict Pi limitation [*Hoppe*, 2003; *Karl and Björkman*, 2002] and the presence of this enzyme being subjected to various interpretations [*Duhamel et al.*, 2011], we wanted to avoid focusing this point onto enzymatic activity.

Uptake of ATP: Bjorkman et al 2012, Casey et al 2009, Duhamel et al 2012, Lomas et al 2010, Sebastian et al 2012, Fu et al 2006

Uptake of ATP and numerous other compounds: Bjorkman & Karl 1994

P3L20. “realize a significant fraction of their P” I was not able to understand what this phrase means.

Indeed, this was changed for clarity.

“Some eukaryotes possess mixotrophic capabilities and grazing on P-richer prokaryotes can fill most of their requirements in P”.

P4L14. ”significantly higher” Is this true for all the 6 experiments conducted ever? This description gives us the impression that it is an established fact.

Thank you for pointing this out, I will rephrase it with a little more flexibility. In the few studies providing measurements in both non-pigmented and pigmented prokaryotes, per cell uptake rates of heterotrophs are systematically lower than Syn's, and comparable to or lower than those measured for Proc cells. Text was changed into:

"When looking into the contribution of picoplanktonic groups to total Pi uptake, prokaryotes are better competitors than eukaryotes. Among prokaryotes, taxon-specific uptake rates (per cell) of Pi higher for Syn compared to Hprok and Proc in the Sargasso Sea [*Michelou et al.*, 2011], and higher per cell rates were measured for Proc than Hprok in the North Pacific Subtropical Gyre [*Björkman et al.*, 2012], especially during light incubation [*Duhamel et al.*, 2012]."

P5L5. In this section, it is not clear where and from which depths samples were collected for some parameters (FCM, Pi uptake and nutrient concentrations).

Further details are added to the text to improve clarity.

"Pi turnover times were measured at each of the 30 stations while group-specific processes were assessed at 3 long duration stations (St. A, B, C) and at 4 short duration stations (St. 5, 9, 21, 25). Vertical Pi uptake profiles in sorted groups at stations 9, 21, A and 25 are presented here, and concentration bioassay experiments conducted at stations C, 5, B and A."

P6L15. What is the material of the bottles?

Polycarbonate

"Clean 30-mL polycarbonate Nalgene bottles were filled with 10 mL of seawater samples."

P7L10. Why did the authors choose the depth of 15 m above the DCM?

Due to the decreasing hot/cold isotopic ratio inherent to concentration kinetic experiments, they often require an increased 33P spike and a higher amount of sorted cells compared to regular ^{33}P uptake experiments. This could only be achieved (and as you can see not 100% with the below detection data) a lot deeper than the surface layer with non-preconcentrated samples.

"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, knowing that the depth of the DCM and nutriclines was expected to vary considerably along the transect. Concentration kinetics experiments were conducted at stations 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)."

P9L6. Do these observations emphasize the strong P-deficiency?

Indeed it does not. Changed to:

"emphasizing the strong oligotrophic state of the eastern waters."

P9L10. "possibly due to mesoscale variability" It does not explain the reason or mechanism for the high values in the western basin. Why or how was the mesoscale variability formed?

A companion paper from the BOUM cruise indicated that the mesoscale eddies encountered along the transect and located by the long-duration stations A, B and C could have been formed the previous winter [*Moutin and Prieur*, 2012]. Following this comment, we deem it was not relevant to develop this detail in our study, as we do not have sufficient data to compare to physical measurements. Therefore it has been re-stated as:

"possibly due to incomplete stratification of the water column and the proximity to the Rhone River."

P9L19. "below" should be replaced by "over"?

Done.

P10L17. I did not understand how the authors obtained kinetic parameters from Fig. 5. In Fig. 5, the fitting to Michaelis-Menten curve seems to be unsuccessful for the bulk community at St. C. However, the kinetic parameters are described in Table 2. How were these values obtained?

The kinetic parameters were obtained as described in [*Thingstad et al.*, 1993], which is an approach detailed by [*Wright and Hobbie*, 1966] for glucose and acetate uptake when Sn was unknown. In our case, this was used due to uncertainties related to the reliability of Sn determination. Sn was low, but certainly not negligible compared to the maximum Sa of 100 nM, which is one requirement of application of the Michaelis-Menten equation extrapolated to uptake kinetics. Also, the application of M-M is only valid if Km is large. [*Björkman et al.*, 2012] for this reason chose not to discuss the Km parameter, and it is partly why we chose to use an alternative option with Kt+Sn. The other reason is that Kt is supposedly a good proxy of the affinity for the substrate.

In the reference paper by [*Wright and Hobbie*, 1966], they develop the Michaelis-Menten equation in order to calculate kinetic parameters independently from the ambient substrate concentration Sn:

$$\frac{K + Sn}{Vmax} + \frac{Sa}{Vmax} = Tt$$

The turnover time of Pi in low-Pi environment always increased linearly with the addition of Pi in our experiments. Therefore, when plotting the concentration of added substrate Sa versus the Turnover time at a given concentration (independent from the in situ concentration), the linear regression gives: Ttx = α × S + β. intercept with the Y axis gives an estimated turnover time in the sample at ambient concentrations (h), the inverse of the slope is the estimated Vmax and K + Sn is the intercept of the regression with the X axis, i.e. when the turnover time tends to zero, i.e. K+Sn = - β / α. As explained in

the legend of Figure 5, the added lines are the estimated K+Sn and Vmax. Sn was those parameters in the Michaelis-Menten equation, we drew the fit curves

Kinetic experiments from St. 5 and St. B are now removed. From Fig. 5, Table 2 and the text, as well as the plotted estimated Vmax and K+Sn values for Proc and Hprok at St. C.

Results and discussion were modified accordingly.

P12L6. Does higher taxon-specific Pi uptake rate by Synechococcus just reflect their higher biomass, or higher affinity to Pi or both, compared to picoeukaryotes?

I understand this refers to volumetric rates at Station 25 compared to the small contribution of Syn to the bulk Pi uptake at other stations. This has been further developed in the text as follows:

Paragraph 4.1. ends with

"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."

P12L7. Figs. 4 and 5 seem to show me that Hprok-specific rates of Pi uptake was not always lower compared to cyanobacteria.

That is correct when looking at what we defined as volumetric rates (in nmol P L^{-1} h^{-1}) and contributions. The text has been modified to specify per cell rates vs volumetric rates. Per cell rates of Pi uptake for Hprok are consistently lower than cyanobacteria's per cell rates, with the exception of one depth at St. B out of 24 Hprok rates measured.

P12L26. "found no difference" sounds too strong. "found no clear difference" or "found no significant difference" may be more appropriate.

Done.

P13L12. What do the authors think caused the difference found among the areas?

Based on our small data set and the limited amount of environmental variables to compare them to, we did not have a chance to run multivariate analyses to point out one or more variables explaining most of the differences in Pi uptake along our transect. Therefore, we can only speculate about differences across regions, and this was added to the discussion in section 4.1.

End of paragraph 4.1.

We suggest 4 main reasons to explain differences across regions: i) different composition of the cyanobacterial community between the Sargasso Sea and the Mediterranean, ii) the very low proportion of *Prochlorococcus* cells (<10%) able to oxidize Pi in the subsurface layers of the Sargasso Sea [*Martínez et al.*, 2012], iii) different proportions of live versus dead cyanobacterial cells across oceanic regions and depths [*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.*, 2011].

P13L14.Theoretically, the community maximum uptake rate should be the sum of each population, thus this description seems no wonder.

Indeed. This formulation was not adequate, the point was to mention that the missing Vmax to add up to the sorted groups could be Vmax from larger organisms that are not considered in our study.

Summed volumetric Vmax of sorted groups added up to the community Vmax or below. This missing fraction might belong to unsorted larger protists which do not contribute highly to bulk Pi uptake fluxes (> 2 µm, data not shown), but may have the ability to store large amounts of Pi in case of upwelled or deposited inputs. Our kinetic experiment results 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.

P13L20. Is there any evidence or reference to support the low uptake by larger populations?

During the BOUM cruise, we also measured size-fractionated Pi uptake rates. Those data will not be presented in the paper because the point was to bring more details using cytometric groups rather than size fractions, and also because those measurements were often below detection limit and therefore no entire profile of size fractionated rates could be obtained. Nevertheless, data from stations 1 (5m), 5 (50m), 9 (5 and 100 m), C (40 and 100 m), 3 disrupted profiles at St. B (75, 100, 120, 140, 160 m), 14 (125 m) and A (25, 100, 130 m) gathered in supplementary figure S1 showed that the >2µm size fraction contributed the least to total Pi uptake fluxes:

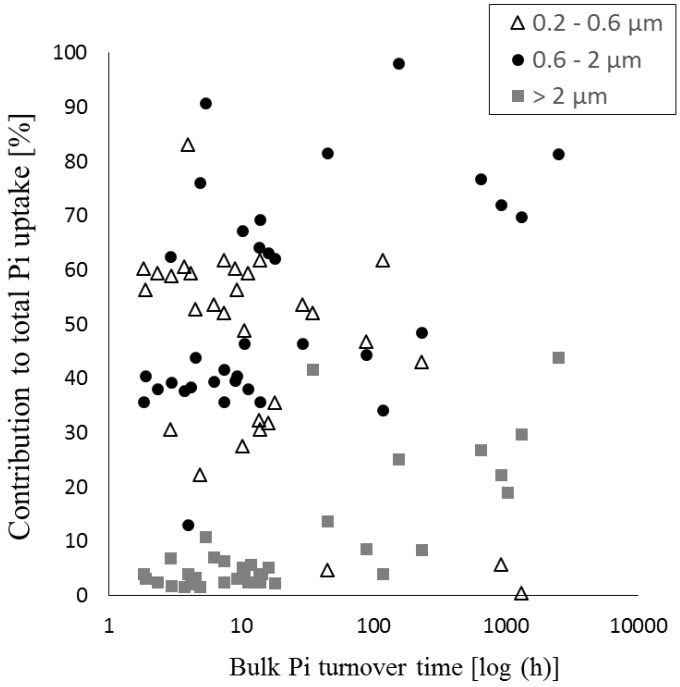


Fig. S1. Contribution of size fractions to total Pi uptake as a function of bulk Pi turnover time at various stations and depths

It does however show that, in the > 2 µm size fraction, contribution to bulk Pi uptake increases linearly with increasing turnover time (r2=0.6).

A study in the North Atlantic showed that under SRP concentrations below 10 nmol P L^{-1} h^{-1}, per cell rates of Pi uptake in pico and nanoeukaryotes are higher than for cyanobacteria [*J. R. Casey et al.*, 2009], but the total contribution to Pi uptake fluxes is lower for larger cells.

P14L1. The authors can estimate cell volume of phytoplankton measured by a flow cytometer, if they obtained scatter (FSC or SSC) data. The data can be calibrated against standard beads, and converted to cell diameter.

This could indeed have been done if calibration had been conducted to process all cruise samples using different sets of beads. However, the counts presented here were acquired using only 1 µm fluorescent beads and no size calibration at the time was conducted. Plus, counts were conducted on PFA-fixed cells, which volume likely shrunk compared to live cells [*Sherr and Sherr*, 1993]. We are aware that this is a major missing information in our data set, we discuss the issue in more details and do suggest that more size/biovolume analyses are conducted along with uptake rate measurements as recommended by [*John R. Casey et al.*, 2013].

P14L9. This discussion seems to contain some leaps in logic. I was not able to understand why the vertical partitioning of Pi uptake in the present study may show that Pi concentration was a major factor

explaining the distribution of osmotrophs. Additionally, does “distribution” in this sentence mean vertical distribution or horizontal distribution?

This paragraph and the entire discussion have been largely remodeled.

“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.”

P15L10. “the spatial distribution . . . was partly attributable to their respective capabilities to take up Pi” I do not fully agree to this idea. As mentioned in the comment above,this is not sufficiently supported by observations. The spatial distribution of plankton taxa seems to just reflect their Pi uptake traits.

Indeed, thank you for this comment. The idea was not expressed as it was meant.

The Conclusion was re-written and starts as:

“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). 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.”

P15L18. “While a few taxon-specific. . .” This sentence should appear on the top of this paragraph.

Done

Figure 5. As mentioned earlier, more than half of the fittings were insignificant. Is it appropriate to include kinetic parameters from insignificant fittings?

Indeed, based on these and another referee’s comments, we decided to take out 2 stations (5 and B) where no fitting was significant. It is reported in the results

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.

Anonymous Referee #2

Review#2

Summary comments: The authors have investigated the uptake rates of inorganic phosphate into various picoplanktonic groups in the oligotrophic upper water column by means of radio labeling techniques coupled with flow cytometric cells sorting. They present depth resolved phosphate turnover times from 33 stations along an east-west transect in the Mediterranean Sea. In addition the vertical distribution of group specific P-uptake and experiments to assess the kinetic parameters of the phosphate uptake was conducted on a few selected stations. The authors aimed to address the drivers of the vertical distribution of co-existing microorganisms in terms of competition for limiting resources, here phosphate. They find that Synechococcus cells have a higher capacity for rapid P-uptake at higher P concentrations (here ~ 100 nM-P), and also have a low half–saturation constant whereas heterotrophic bacteria and Prochlorococcus cells have low uptake capacity (i.e. low Vmax) but also low half-saturation constants and hence would do well in the typically low P environment. The authors conclude that these differences may explain the co-habitation and distribution of divers groups of picoplankton both temporally and vertically in the Mediterranean Sea.

I find that this manuscript potentially can contribute to and further our understanding of resource utilization and partitioning among picoplankton groups in the oligotrophic oceans. This is of fundamental importance to ultimately understanding the flow of carbon through these large ecosystem. However, I believe that the data has not been presented, or utilized, as well as they can be, and the manuscript is in part a little hard to follow. The material and method section can benefit from more detailed descriptions and the discussion appear to me to in part be more results than a discussion of their findings. I also believe that it should at least be mentioned that other factors than phosphate availability can shape community composition and depth distribution, for example light flux and other key nutrients.

We would like to thank Reviewer #2 for their input and for pointing out details that have allowed to improve and increase the accuracy of our report.

Detailed comments:

Abstract: ln 8 – "..these experiments were completed with.." is it meant to be "..these experiments were complemented with.."?

Indeed, this would be more appropriate. Per request of another reviewer the abstract was entirely re-written.

ln12-15 - Syn cells had the highest Vmax and the lowest K+Sn. Is that correct? If so why has Syn not outcompeted all other groups investigated?

At St. C, Syn cells had the highest per cell and volumetric Vmax, but they were present at 1.34 10^4 cells mL^{-1}, which was 4 times lower than Proc and 30 times lower than Hprok. Furthermore, they were fully using their maximum uptake capacity. We think that would outcompete all other groups in case of pulsed Pi inputs.

Ln15 – " quickly reactive to" suggest saying "react quickly to" or "quickly respond to"

The abstract was entirely re-written.

Introduction: P 14641 ln 4-5 - This sentence is a little hard to read. Would it suffice to say "Orthophosphate (Pi) is the preferred form of phosphorus for most osmotrophs"?

Yes it would suffice, thank you, this was modified as suggested.

Ln 9-10 - Is it accepted that P uptake capacity is mostly influenced by Pi limitation? And what does that mean? A reference here would be helpful.

It means that the equilibrium between extracellular and intracellular concentrations of Pi trigger one or another Pi absorption system. I am adding citations of a couple of very old but fundamental papers of the Pi uptake kinetics.

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]).

Ln 19 – "some eukaroytes.." are these flagellates or ciliates or something else?

References were made to [*Christaki et al.*, 1999] for nanoflagellates and to [*Hartmann et al.*, 2011] for their functional denomination of the smallest plastidic and aplastidic protists. Considering the size range (< 3 µm), I would assume they are not ciliates, but could be quite diverse phylogenetically, which is why we do not detail taxa here in the text.

Ln 28 - It's unclear what the list of size classes mean. Does the <0.8 µm size class take up more than >0.8 µm? Or is

this just a list over what size classes has been tested?

Different studies have used different porosity sizes, which is partly our point of showing only our FACS data and not the size classes. So this is indeed a list of the different size classes for which Pi uptake rate measurements have been conducted.

P14642 Ln 25 – change “picophytopanktonic” to “ picophytoplanktonic”

Done.

Materials and Methods:

P14643 Ln15-17 – Please add the number of depth sampled at the 33 stations. Add what stations were used for the kinetic experiments.

The total number of stations was corrected to 30 stations (there were 27 short duration stations and 3 long duration ones sampled in the BOUM cruise).

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.

Suggest changing “ along the euphotic zone” to “within the euphotic zone” or “throughout the euphotic zone”

Text was modified as shown in the previous reply, this was changed for vertical profiles.

P14644 Ln15 – of what what size and of what material were the incubation bottles?

Incubations were conducted in 30-mL translucid polycarbonate flasks. This was added to the text, while the rinsing details were removed.

Clean 30-mL polycarbonate Nalgene bottles were filled with 10 mL of seawater samples.

P14645 Ln7 – spelling “orthophoshate”

Done

Ln8 – How were the samples incubated? In the light or dark, at what temperature?

Samples were incubated on the bench of an on-deck container at room temperature (~25°C), under natural light conditions.

Ln11 – should Station 5 be included here?

It should have been, thank you. We decided to remove the kinetic experiment from St. 5 and St. B completely, as suggested by the 2 other reviewers.

Ln13 – how many concentration steps were there between 0 addition and 100 nM-P

All experiments included 2 blanks and 10 concentrations tested: +0, +4, +8, +10, +15, +20, +40, +60, +80, +100 nmol P L^{-1} (final concentration of added cold Pi). Out of 7 concentration bioassay incubations and sorts, many could not be used. Our time constraint to sort live samples within a few hours after incubation resulted in some missing points, even in the attached presented experiments. Very often, at the highest tested concentration of 100 nM, signals were below detection limit.

P14646 Ln4 – suggest changing "an embarked" to "an onboard"

Done.

Ln19 – the detection limit for SRP determinations is given as 5 nmol L-1. Is this correct? It seems quite high, but if correct, I would suggest not reporting the SRP values to two decimal points precision in Table 1.

Indeed, done.

Ln20-25 – I am not sure I understand this sentence. What was the minimum number of observations used to create the plots to be fitted to the Michaelis-Menten model? What were the criteria for removal of point?

There was no minimum number of observations used, we performed a linear regression between a M-M fitted equation based on the estimated Vmax and K+Sn between 0 and 100 nmol P L^{-1} and our actual data points available.

This was rephrased as:

The Michaelis-Menten equation was used with estimated Vmax 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 datacorrelation was found ($p < 0.05$).

Results: P14647 Ln8-9 – The range of SRP concentration presented are not found in Table 2 (or 1). Also, 2 nM-SRP would be below the detection limit if 5 nM DL is correct (see above).

Indeed, this range includes measurements conducted between 0 and 150m at all stations and should not be mentioned this way because they are not relevant to our uptake experiments. The range has been re-centered on the data presented in Table 2.

After several verifications, I can assure that of all measurements of SRP concentrations available, the smallest was 4.9, i.e. close to 5 nM. The lower value of the range given in the submitted version was wrongly typed in and should have been 5.

SRP concentrations ranged 6 – 80 nmol L^{-1}, varying with depth and location (Table 2).

Ln9-10 – This sentence does not appear to reflect what is given in Table 1 (e.g., SRP concentrations for Stations 21 and 25 are 9.6 and 17.33 respectively, station 9 is reported as 20.22). I would suggest adding another couple of figures, possibly to complement the contour plot of Pi turnover time (Fig 2).

Indeed, this description fits the turnover times better than the concentrations and the text will be changed accordingly. Fig. S2 will be added as a complementary figure.

Ln19 – think that "below" should be "above" here?

Yes indeed. Done.

Ln20 – is the Syn cell numbers 10^4 or 10^5 ml-1? (also see ln13 on P14648)

It is 77307 cells mL^{-1}, so 7.7×10^4 cells mL^{-1}, thank you for noticing. The second mention of this maximum in paragraph 3.4. was discarded.

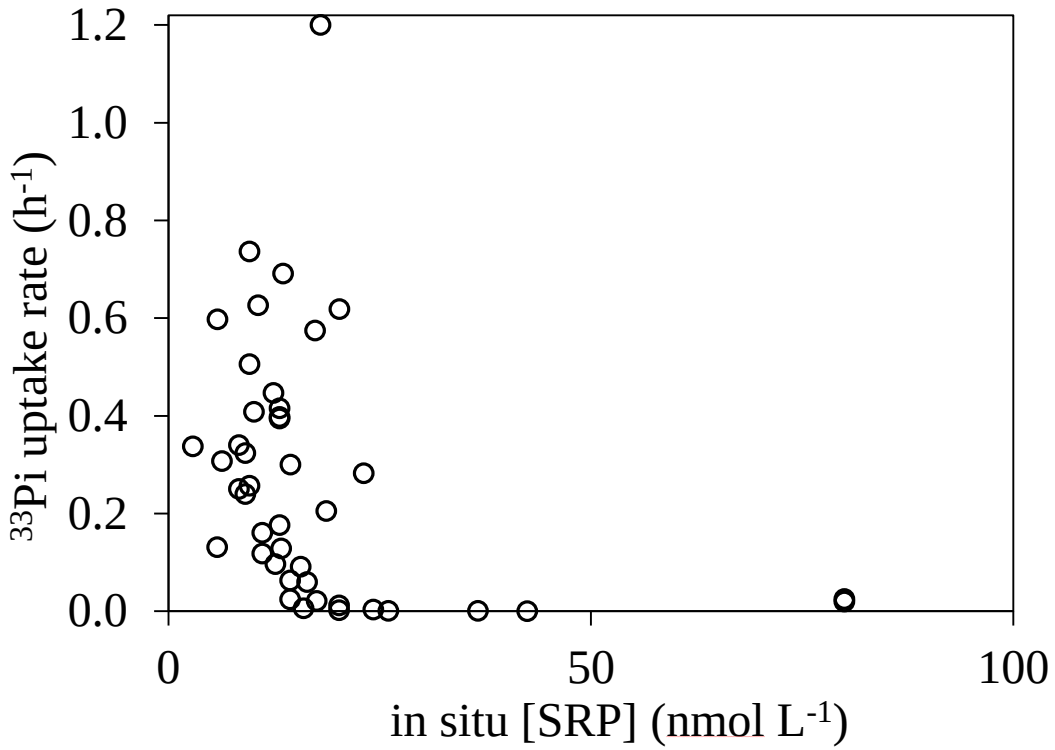
Ln24-27 – Is this the data range covering the 33 stations along the transect? Is it the horizontal or vertical range in rates? It is unclear as written. Again, I believe a figure complementing Fig 2 with SRP conc and Pi-uptake rates would be welcomed.

This is the range covering our uptake experiments, as only the turnover times were calculated over the 30 stations. Turnover times presented in Fig. 2 were measured at a higher frequency than the SRP concentrations.

Our article is meant to use turnover times as a proxy for Pi limitation of the system rather than SRP concentrations. Below you can find Fig. S2 (Supplementary figure S1 was added in the reply to the first reviewer) showing bulk ^{33}Pi uptake rates versus SRP concentrations at all depths where Pi uptake experiments were conducted (n> depths presented in the paper for sorts).

It seems that the lowest uptake rates are measured when SRP concentrations are high. SRP concentrations are indicative of the environmental conditions, but not of the bioavailability of Pi, which is better given by turnover times.

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



P14649 Ln3 – spelling “Memten”

Done.

Discussion: As mentioned above, much of what is presented in the discussion seems to fit better in the results section.

This was done. Both the Results and Discussion were massively re-structured and clarified based on all 3 reviewers’ comments.

Also, I think some of the very large span in rates needs to be discussed in more depth, especially for the kinetic study. What triggered the very long turnover time at Station A (90m). Were there large differences in community composition or other factors that may explain this? Was it consistent with the bulk rates? The ambient SRP was not very different from stations B and C for the kinetic experiments.

Only hypotheses can be made regarding these observations, as we have little data and few environmental variables to compare them to. One hypothesis is that St. A being in the center of a mesoscale eddy formation at the time of sampling, with waters possibly isolated from the last winter, Pi has already cycled several times within the microbial communities and it less bioavailable (SRP

concentrations not very different from other stations, but much higher turnover times). A second hypothesis is that organisms capable of acquiring Pi through other forms may have more sources available at this station which is offshore but could be slightly influenced by the Rhine river plume.

Here is the last paragraph of the discussion where some of those elements were included.

The vertical partitioning of Pi uptake observed in the present study suggested that different nutrient uptake strategies and capabilities may contribute to explaining the spatial distribution of osmotrophs. 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, as it was done in mesocosms from 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]).

P14649 Ln23-24 – Can part of the discrepancy in the recovery of sorted groups to bulk rates be attributed to the inability to resolve Prok in surface waters? Or would that signal be included in the Hprok?

This is a quite likely assumption that we did not mention, mostly for the reasons newly added to the paper regarding the percentage of dead Proc cells and their sensitivity to light.

However, exclusion cytograms used to look at SYBR-stained from a different profile (Fig. S3, St. B) during BOUM show that indeed autofluorescence is indistinguishable from the SYBR signal in subsurface layers.

This figure S3 is provided for the sole purpose of answering the reviewer's question. It will not be included as a supplementary figure.

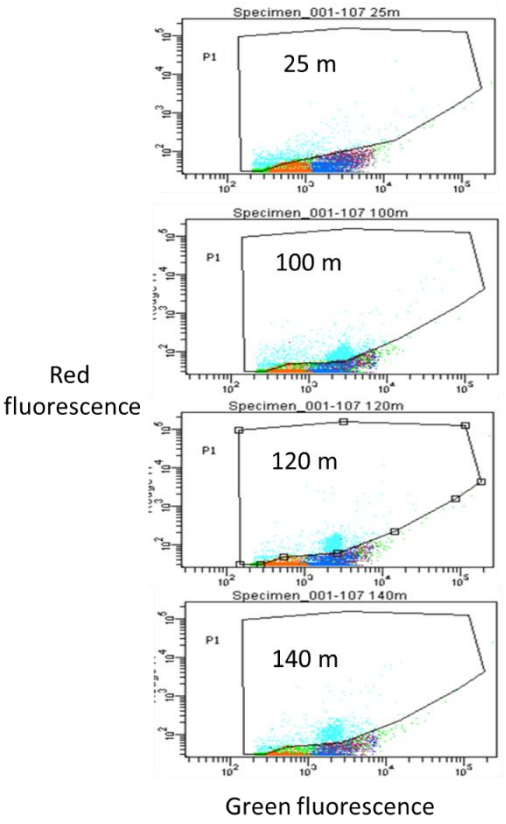


Fig. S3: Cytograms showing the green fluorescence of SYBR-Green I versus Red fluorescence from chlorophyll a at 4 depths between 25 and 140 m at a station of the BOUM transect.

P14651 Ln4-7 – this sentence is confusing to me are we talking surface to volume relationships, or just surface? Is it consistent with Casey et al.'s findings or Vadstein and Olsen's?

I think this regards P14652. [*Vadstein and Olsen*, 1989] concluded their mesocosm study with the fact that under low-Pi conditions, bacteria are superior competitors to phytoplankton regarding Pi uptake. That is consistent with the assumption that the growth of organisms smaller than 40 um3 is not 'surface-limited'. The higher surface-to-volume ratio of smaller organisms is the underlying link between those mentioned studies. We modified this section to make it clearer.

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 per cell uptake rates under low concentrations compared to picoeukaryotes.

Table 1 Could the bulk rates be added to this table, as well as the per volume rates for the picoplankton groups?

I think that would add a great deal of information (I do realize this data is presented in Fig 4, but I find it hard to actually see this).

For your information, here is the entire table, that will be split in two

Station	depth (m)	DCMd (m)	Chl *a* int (mg C m^{-2})	[SRP] (nM) mean ± sd	Cell Pi uptake rate (amol P cell^{-1} h^{-1})				Bulk Pi uptake rate (nmol L^{-1} h^{-1})	Contribution to total Pi uptake (%)				Abundances (10^3 cells mL^{-1})			
					Syn	Proc	Pic	Hprok		Syn	Proc	Pic	Hprok	Syn	Proc	Pic	Hprok
C	100	108	25.10	12.8 ± 6.7	49.8	17.5	NA	1.2						13.42	52.08	NA	398.82
9	5	128	16.20	20.2 ± 2.3	58.7	NA	NA	27.7	12.51	1.33	NA	NA	59.64	2.82	NA	NA	268.89
9	50			17.6 ± 1.6	14.0	NA	NA	0.7	5.56	1.31	NA	NA	52.83	2.52	NA	NA	339.53
9	75			12.4 ± 3.7	17.6	NA	NA	7.6	6.53	5.73	NA	NA	42.56	4.13	NA	NA	384.29
9	105			23.1 ± 3.7	41.7	35.5	NA	7.1	9.37	4.17	5.60	NA	48.54	8.98	14.90	NA	389.77
9	120			13.6 ± 1.4	46.5	39.9	NA	13.5	1.22	14.02	51.62	NA	12.08	8.39	13.13	NA	336.04
5	50	114	24.70	12.7 ± 6.7	43.0	13.0	NA	0.4						3.98	48.33	NA	338.84
B	100	141	21.20	8.6 ± 0.5	24.3	20.3	NA	36.1						1.64	NA	0.23	376.75
21	5	87	22.40	9.6 ± 1.9	401.6	NA	NA	3.6	5.96	2.05	NA	NA	33.74	7.17	NA	NA	564.26
21	50			13.1 ± 3.6	9.6	12.7	NA	2.6	5.29	0.76	3.26	NA	34.42	4.34	13.80	NA	652.59
21	70			14.4 ± 2.0	9.0	2.2	8.4	0.2	0.91	7.11	8.26	0.44	9.85	5.45	34.19	0.48	506.12
21	85			80.0 ± 17.6	23.5	5.8	49.4	0.7	1.79	2.77	3.64	1.45	21.72	1.68	11.52	0.49	523.86
A	6	88	24.70	10.6 ± 2.8	24.1	NA	8.6	11.5	6.64	2.40	NA	0.11	64.29	6.61	NA	0.86	372.30
A	13			5.8 ± 2.0	10.7	NA	4.0	5.8	3.47	1.32	NA	0.10	74.71	4.29	NA	0.89	446.45
A	25			6.3 ± 1.0	5.8	NA	3.3	3.2	1.94	0.63	NA	0.17	82.54	2.12	NA	1.01	504.92
A	75			8.3 ± 3.9	8.6	11.7	11.7	1.8	2.84	4.62	56.40	0.45	36.80	10.01	136.15	1.09	574.86
A	90			8.3 ± 0.8	13.1	9.5	33.7	0.4	0.98	8.98	62.87	10.19	21.42	3.50	65.04	2.96	526.89
A	100			16.4 ± 1.4	25.1	1.6	7.9	0.1	0.08	14.15	34.70	12.63	48.98	2.16	16.76	1.22	345.85
A	110			24.2 ± 3.4	5.0	1.5	7.3	NA	0.04	23.61	25.49	7.77	NA	1.73	7.26	0.44	306.67
A	130			20.2 ± 1.3	5.7	3.6	3.8	0.1	0.03	16.97	21.84	3.04	66.37	0.64	1.72	0.23	280.82
25	5	51	38.50	17.3 ± 2.3	68.7	NA	44.9	8.7	9.97	13.70	NA	0.38	57.87	19.89	NA	0.84	665.83
25	25			18.00	48.7	37.2	55.5	16.2	21.61	11.50	5.86	0.34	65.79	51.01	34.00	1.31	877.74
25	40			18.00	NA	30.2	67.0	1.3	10.01	21.37	9.00	1.17	13.59	77.31	29.81	1.75	1016.47
25	50			18.67	49.0	NA	134.8	0.8	3.83	53.41	NA	6.46	20.00	41.78	NA	1.84	1004.72
25	60			19.10	NA	NA	NA	NA	0.15	19.22	NA	13.53	19.23	11.42	NA	0.51	571.65

Table S1 (attached) was produced, including the bulk Pi uptake rates and the contribution of the groups to the bulk uptake, as in Fig. 4.

This request allowed me to detect an error in Fig. 4 where the bulk rate at St. 25, 40m was obtained after multiplication by 0 instead of the actual SRP concentration. Fig. 4 has therefore been updated.

Also, the SRP measurements were made in triplicate (in materials and methods), please add the standard deviation here. Is there chlorophyll data per each depth? That seem to me to be the more relevant in this table. Please define NA here too.

Chlorophyll data were obtained from a different cast than our samples, which is why we presented it only as integrated values. Caption was corrected and mean +/- sd were added as asked. We included contributions instead of group-specific volumetric rates in order to vary the data presented. Table S1 (attached with this comment) will be presented for publication as a supplement. Data description in the text, Table 1 and Fig. 4 seems too redundant.

Added to the legend: NA= no available data

Table 2 Should station 5 data be included here, it is in Fig 5?

St. 5 was removed from the displayed data.

Figure 2. As mentioned above I think additional panels with SRP and P-uptake rates would be a valuable addition.

Our data set is too small to display several panel, and even combined, as in Fig. S2, this does actually not add to anything of our findings.

Figure 4. What does the * mean?

Added to the caption: Missing groups are specified with a *.

Anonymous Referee #3

The authors present an interesting manuscript assessing the relative contribution of groups of picoplankton to Pi uptake in the Mediterranean. They also measured Pi uptake kinetic constants per picoplankton group.

In general, the manuscript is well written except for a few sentences that need to be rewritten. However, the discussion would benefit from some additional work. My main concern is that this paper is very descriptive and that there are only a few data to support the author's conclusions, especially for the Pi uptake kinetic constants. Yet the data presented are unique and will be useful to the scientific community. I have a few comments, which the authors might wish to consider in revising the manuscript.

The discussion has been modified significantly in order to add more comparisons and support to our hypotheses. Our limited data set does require cautious though regarding conclusions and interpretation, which is what we have mostly worked on, and these comments were very helpful in doing so.

General comments:

1. You state that the Mediterranean Sea is P-deficient, implying that cells were P-limited while in fact the data presented in Figure 5 clearly show that most groups were taking up Pi at saturating concentration. Instead, I suggest using the term low-Pi, which only refers to Pi concentrations and not to the physiological state of microorganisms. Please consider this aspects in the discussion and conclusion.

I agree that part of the results do not support a Pi-limitation like it is currently thought and this is now further discussed in the last parts of the article. The system itself, though, the stratified Mediterranean Sea, is clearly Pi-depleted. The short Pi turnover times and low levels of TChl a, as well as the possible multiple nutrient limitation suggested by on board microcosm experiments, do suggest a Pi-limitation.

The statements P-limited and P-deficient were modified for more clarity into low-Pi, or explicitly developed.

2. Concentration bioassays: how did you calculate K+Sn for HProk and Proc since on Fig. 5 it appears to be impossible (i.e. no dose response)? I think that this is a critical aspect of this paper and the authors need clarify their approach and results.

The kinetic parameters were obtained as described in [*Thingstad et al.*, 1993], which is an approach detailed by [*Wright and Hobbie*, 1966] for glucose and acetate uptake when Sn was unknown. In our case, this was used due to uncertainties related to the reliability of Sn determination. Sn was low, but certainly not negligible compared to the maximum Sa of 100 nM, which is one requirement of application of the Michaelis-Menten equation extrapolated to uptake kinetics. Also, the application of M-M is only valid if Km is large. [*Björkman et al.*, 2012] for this reason chose not to discuss the Km parameter, and it is partly why we chose to use an alternative option with Kt+Sn. The other reason is that Kt is supposedly a good proxy of the affinity for the substrate.

In the reference paper by [*Wright and Hobbie*, 1966], they develop the Michaelis-Menten equation in order to calculate kinetic parameters independently from the ambient substrate concentration Sn:

$$\frac{K + Sn}{Vmax} + \frac{Sa}{Vmax} = Tt$$

The turnover time of Pi in low-Pi environment always increased linearly with the addition of Pi in our experiments. Therefore, when plotting the concentration of added substrate Sa versus the Turnover time at a given concentration (independent from the in situ concentration), the linear regression gives: Ttx = α × S + β. intercept with the Y axis gives an estimated turnover time in the sample at ambient concentrations (h), the inverse of the slope is the estimated Vmax and K + Sn is the intercept of the regression with the X axis, i.e. when the turnover time tends to zero, i.e. K+Sn = - β / α. As explained in the legend of Figure 5, the added lines are the estimated K+Sn and Vmax. Sn was those parameters in the Michaelis-Menten equation, we drew the fit curves

Kinetic experiments from St. 5 and St. B are now removed. From Fig. 5, Table 2 and the text, as well as the plotted estimated Vmax and K+Sn values for Proc and Hprok at St. C.

Results and discussion were modified accordingly:

3. I find puzzling that the bulk and the group specific kinetic constants could only be measured at St. A where the turnover times of Pi were the longest: how do the authors explain that?

I understand the question as: how come that only at Station A the M-M model explains the kinetic uptake curves?

At St. A, abundances, and possibly biomass were very high for each group of interest. Longer Pi turnover time in the microbial community suggested that Pi was more available, maybe along with other sources of P, and it is likely that cells were not P-stressed with saturating uptake rates.

Specific comments:

1. P14642 L9-10: I would remove “as shown by. . ..since 2007” since there are a few missing references and this does not add to the point made.

Done. The previous list of 33P coupled with cell sorting was however updated with the very recent paper showing taxon-specific Pi uptake rates of phytoplankton groups in the Sargasso Sea by Lomas et al.

2. P14642 L21: “Pi-depleted surface waters”: do you mean euphotic layer as in the method section? To me surface is the top 5-10 m but not down to 200m. Maybe you mean upper water column?

Indeed, this was incorrect, thank you for noticing. Changed to:” 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).”

3. P14644 L27-28: this sentence is not grammatically correct

Changed to:" 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."

4. P14645 L3: I would remove "embarked" and say "The radioactivity was counted onboard. . ."

Changed to:" The radioactivity was counted onboard within 5 hours after addition of the scintillation cocktail using a Packard LS 1600 liquid scintillation counter."

5. P14645 L10-11: justify why you chose to conduct concentration kinetic experiments at 15m above the DCM: that sounds random to me.

Rewritten as: "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, knowing that the depth of the DCM and nutriclines was expected to vary considerably along the transect. Concentration kinetics experiments were conducted at stations A, B and C by adding increasing quantities of a cold KH_2PO_4 solution (0 – 100 nmol L^{-1} added concentration, Sa)."

Due to the decreasing hot/cold isotopic ratio inherent to concentration kinetic experiments, they often require an increased 33P spike and a higher amount of sorted cells compared to regular ^{33}P uptake experiments. This could only be achieved (and as you can see not 100% with the below detection data) a lot deeper than the surface layer with non-preconcentrated samples.

6. P14647 L6-7: you say that because the integrated chlorophyll concentration decreases west to east that "emphasize" the strong Pi-deficiency: I don't see why. Improve or remove.

Based on your 1st comment, this was modified to: "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."

7. P14647 L15-16: I would replace "extreme values" by ranges

Done.

8. P14647 L19: "below 50m": you mean above?

Yes, thank you. This was fixed.

9. P14648 L5 please give average +/-SD and n values

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).

9. P14648 L10: please give average +/-SD and n values

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).

10. P14648 L20: “not significant”: I would say “below the detection limit of our method”

Done.

11. P14649 L1: “higher than for other groups” but I still cannot understand how you could measure K+Sn for other groups than Syn

When using the turnover approach and extrapolating a K+Sn, values can always be obtained, unless the Vmax is extremely high, in which case the half-saturation constant is rarely relevant. Considering that our SRP concentrations are not as low as the turnover times could suggest, we could never have estimated a Km from our experiments.

When kinetic parameters are estimated based on turnover times, the dilution effect of the radio-isotope with cold Pi is strong and not corrected for like uptake rates are. This is why we could still obtain K+Sn values.

12. P14650 L9-10: this sentence needs to be rewritten.

Done. The discussion has been entirely re-structured and in large parts re-written based on the reviewers’ comments and new elements added.

13. P14651 L14: “bulk community were 2 to 40 times higher”: Does that mean that larger phytoplankton and aggregates missed in the cell sorting group-analyses present higher Vmax than the small cell groups studied here?

This should have been reported as a missing fraction rather than a ratio. Please find below the entire paragraph.

I think it is indeed a possibility that larger organisms may have a high Vmax, considering the different systems involved for algae (Pit) and bacterioplankton (high levels of Pst) in low-Pi environments.

Upregulation of RNase and de novo production of phosphatases has been shown for eukaryotic algae in low Pi conditions (Dumont et al 1993, Matagne 1976), supposedly allowing the release of phosphate within the cells, thereby increasing gradients between the outside and the inside of the cells.

We found that cells >2 um were not contributing a lot to bulk Pi uptake fluxes (cf. Fig. S1). However, we can see that the contribution of this size class to bulk Pi uptake increases linearly (when plotted on a linear scale) with increasing turnover time (r2=0.6). This last observation is consistent with a non-saturating uptake, i.e. possibly a high Vmax.

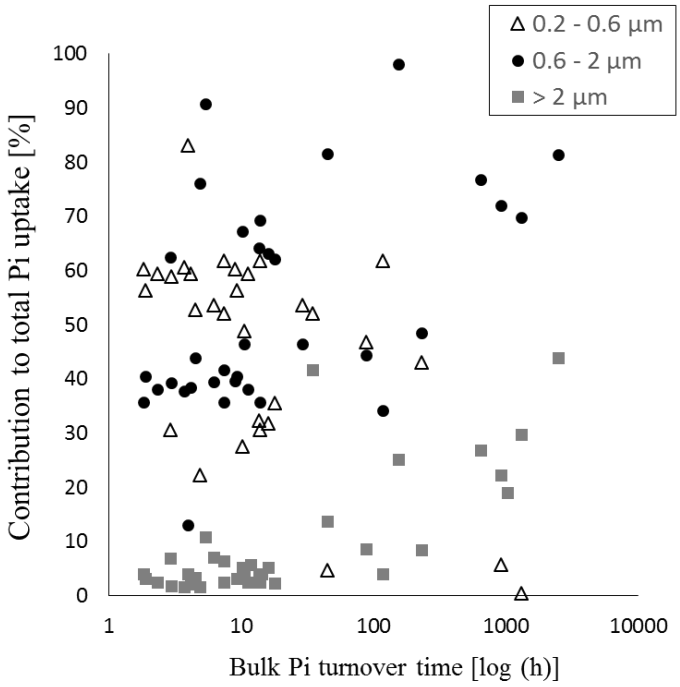


Fig. S1. Contribution of size fractions to total Pi uptake as a function of bulk Pi turnover time at various stations and depths

14. P14652 L9: I would replace “detected” by “measured”

Done.

15. The last paragraph of the discussion just throws ideas: the authors should develop those ideas or remove this paragraph which does not bring much to the paper at this stage.

Indeed, most of it was removed, or changed:

“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*Kashtan et al.*, 2014; *Marie et al.*, 2010]). "

16. Conclusion: avoid making conclusions based on the half saturation constant if this parameter could not be properly measured. I would also avoid concluding about bacteria carbon limitation unless there are any data to prove this. Finally, the term "biodiversity" is out of place in the last sentence.

"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 is for potential Pi storage capacities (Pic), possibly high affinity for Pi at low concentrations (Hprok and Proc), or the ability to take up Pi at high rates (Syn). 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 very likely bottom layer 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."

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

A. Talarmin[1,4], F. Van Wambeke[1], P. Lebaron[2,3], T. Moutin[1]

[1] Aix-Marseille Univ., Mediterranean Institute of Oceanography (MIO), 13288, Marseille, Cedex 09, France; Université du Sud Toulon-Var, MIO, 83957, La Garde cedex, France; CNRS/INSU, MIO UMR 7294; IRD, MIO UMR235

[2] Sorbonne Universités, UPMC Univ. Paris 06, USR 3579, LBBM, Observatoire Océanologique, 66650, Banyuls/mer, France

[3] CNRS, USR 3579, LBBM, Observatoire Océanologique, 66650 Banyuls/mer, France

[4] present address Red Sea Research Center, 4700 King Abdullah University of Science and Technology, Thuwal, 23955-6900, Kingdom of Saudi Arabia

Corresponding Author: Agathe Talarmin (agathe.talarmin@gmail.com)

Acknowledgments

The authors would like to thank the captain and crew of the R/V L'Atalante, Veronique Cornet for the nanomolar SRP data, Laetitia Bariat and Philippe Catala for providing part of the phytoplankton and bacterial counts, respectively, Anna Lagaria, Stella Psarra and Josephine Ras for Chl-a data, Romain Mauriac for the joined effort on turnover time measurements and all the scientists who participated in the BOUM cruise. We acknowledge the crucial contribution of Claude Courties who sorted all the samples on board. Finally, we address our gratitude to the 3 anonymous reviewers who allowed us to greatly improve this manuscript and underpinned elements that needed clarification and further detailing.

This work is a contribution of the BOUM project (Biogeochemistry from the Oligotrophic to Ultraoligotrophic Mediterranean, http://www.com.univ-mrs.fr/BOUM). It was funded by the French national CNRS-INSU (LEFE-CYBER) program and the European IP SESAME (Southern European Seas: Assessing and Modelling Ecosystem Changes), EC Contract No. GOCE-036949. We acknowledge the French Research and Education Council for the funding of Agathe Talarmin's Ph.D. training.

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. Using radiolabeled orthophosphate incubations, Pi turnover times from below 1h to over 500 days were measured within the microbial communities sampled over the first 150 meters of the Mediterranean Sea. Using live cell sorting after incubations, the vertical distribution of Pi uptake fluxes was determined 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 per cell rates (6.6 ± 9.3 amol P cell^{-1} h^{-1}). Cyanobacteria achieved most of the Pi uptake (up to 72 %) around the deep chlorophyll maximum depth, through both high abundances (up to 1.4×10^5 Proc cells mL^{-1}) and high per cell rates (up to 40 and 402 amol P cell^{-1} h^{-1}, respectively for Proc and Syn cells). At saturating concentrations, maximum per cell rates up to 132 amol P cell^{-1} h^{-1} were measured for Syn, which was 5 to 60 times higher than Proc and Hprok, Such differences in Pi uptake abilities could contribute to the distribution of the sorted groups in the Mediterranean Sea.

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 by *Church et al.* (2002) and *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, taxon-specific uptake rates (per cell) of Pi higher for Syn compared to Hprok and Proc in the Sargasso Sea (*Michelou et al.*, 2011), and higher per cell rates were measured for Proc than 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) ml 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. 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 per cell Pi uptake rates varied by less than 4% in prokaryotic sorts and by 11% in Pic (n=4).

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 Vmax 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 datacorrelation was found ($p < 0.05$).

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, *Moutin and Prieur* (2012) 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 2). 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, 1.4×10^5 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

Analytical error associated with per cell rates was about 10% and error on cell abundances around 5%. 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 per cell 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 per cell 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, per cell Pi uptake rates were higher in Syn than in Pic cells (Table 1). The biomass of Pic in the bioassay of St. A reached 0.34 µg P L^{-1}, and ranged 0.48 – 1.06 µg 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 0.01 µg 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, taxon-specific uptake rates ranged 2.6 – 402, 3.6 – 40.0, 8.4 – 134.8, and 0.05 – 36.1 × 10^{-18} mol 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. Per cell (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). Per cell 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 were over 5 times higher for Syn cells than for Proc and Pic at St. A and compared to Syn at St. C. 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 per cell 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 low 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. Discrepancies between the turnover times and SRP concentrations measured across stations and basins suggest a higher limitation of microbial communities in the Eastern basin.

4.1. Taxon-specific Pi uptake

Per cell 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 no distinct pattern explaining the highest per cell Pi uptakes (e.g. Taxon 1 ≥ Taxon 2 ≥ Taxon 3), like there were in samples from the North Atlantic (*Lomas et al.*, 2014; *Michelou et al.*, 2011; *Zubkov et al.*, 2007). We measured per cell 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, ii) the low proportion of Proc cells (<10%) able to oxidize 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. This missing fraction might belong to unsorted larger protists which do not contribute highly to bulk Pi uptake fluxes (> 2 µm, data not shown), but may have higher 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 Pi faster 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 per cell 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, who 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 consider the possibility that Proc cells from surface layers had too low chlorophyll a content to be discriminate from Hprok in stained samples: such

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).

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.

References

Agusti, S. (2004), Viability and niche segregation of Prochlorococcus and Synechococcus cells across the Central Atlantic Ocean, *Aquat. Microb. Ecol.*, *36*, 53-59.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil, and F. Thingstad (1983), The Ecological Role of Water-Column Microbes in the Sea, *Marine Ecology Progress Series*, *10*, 257-263.

Bertilsson, S., O. Berglund, D. M. Karl, and S. W. Chisholm (2003), Elemental Composition of Marine Prochlorococcus and Synechococcus: implications for the Ecological Stoichiometry of the Sea, *Limnology ans Oceanography*, *48*(5), 1721-1731.

Björkman, K., and D. M. Karl (1994), Bioavailability of inorganic and organic phosphorus compounds to natural assemblages of microorganisms in Hawaiian coastal waters, *Marine Ecology Progress Series*, *111*, 265-273.

Björkman, K., S. Duhamel, and D. M. Karl (2012), Microbial Group Specific Uptake Kinetics of Inorganic Phosphate and Adenosine-5`-Triphosphate (ATP) in the North Pacific Subtropical Gyre, *Frontiers in Microbiology*, *3*.

Casey, J. R., M. W. Lomas, J. Mandecki, and D. E. Walker (2007), *Prochlorococcus* contributes to new production in the Sargasso Sea deep chlorophyll maximum *Geophysical Research Letters*, *34*.

Casey, J. R., M. W. Lomas, V. K. Michelou, S. T. Dyhrman, E. D. Orchard, J. W. Ammerman, and J. B. Sylvan (2009), Phytoplankton taxon-specific orthophosphate (Pi) and ATP utilization in the western subtropical North Atlantic, *Aquatic Microbial Ecology*, *58*(1), 31-44.

Christaki, U., F. Van Wambeke, and J. R. Dolan (1999), Nanoflagellates (mixotrophs, heterotrophs and autotrophs) in the oligotrophic eastern Mediterranean: standing stocks, bacterivory and relationships with bacterial production, *Marine Ecology Progress Series*, *181*, 297-307.

Church, M. J., H. Ducklow, and D. M. Karl (2002), Multiyear increases in dissolved organic matter inventories at Station ALOHA in the North Pacific Subtropical Gyre, *Limnology & Oceanography*, *47*(1), 1-10.

Cotner, J. B., and B. A. Biddanda (2002), Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems, *Ecosystems*, *5*(2), 105-121.

Crombet, Y., K. Leblanc, B. Quéguigner, T. Moutin, P. Rimmelin, J. Ras, H. Claustre, N. Leblond, L. Oriol, and M. Pujo-Pay (2011), Deep silicon maxima in the stratified oligotrophic Mediterranean Sea, *Biogeosciences*, *8*, 459-475.

Currie, D. J., and J. Kalff (1984), A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus, *Limnology & Oceanography*, *29*(2), 298-310.

Currie, D. J., E. Bentzen, and J. Kalff (1986), Does algal-bacterial phosphorus partitioning vary among lakes? a comparative study of orthophosphate uptake and alkaline phosphatase activity in freshwater, *Can. J. Fish. Aquat. Sci.*, *43*(2), 311-318.

Dao, M. H. (2013), Reassessment of the cell surface area limitation to nutrient uptake in phytoplankton, *Marine Ecology Progress Series*, *489*, 87-92.

Drakare, S. (2002), Competition between Picoplanktonic Cyanobacteria and Heterotrophic Bacteria along Crossed Gradients of Glucose and Phosphate, *Microbial Ecology*, *44*(4), 327-335.

Duhamel, S., K. M. Björkman, and D. M. Karl (2012), Light dependence of phosphorus uptake by microorganisms in the subtropical North and South Pacific Ocean, *Aquatic Microbial Ecology*, *67*(3), 225-238.

Flaten, G. A. F., et al. (2005), Studies of the microbial P-cycle during a Lagrangian phosphate-addition experiment in the Eastern Mediterranean, *Deep Sea Research II*, *52*, 2928-2943.

Fu, F. X., Y. Zhang, Y. Feng, and D. A. Hutchins (2006), Phosphate and ATP uptake and growth kinetics in axenic cultures of the cyanobacterium Synechococcus CCMP 1334, *European Journal of Phycology*, *41*, 15-28.

Hartmann, M., C. Grob, D. J. Scanlan, A. P. Martin, P. H. Burkill, and M. V. Zubkov (2011), Comparison of phosphate uptake rates by the smallest plastidic and aplastidic protists in the North Atlantic subtropical gyre, *FEMS Microbiology Ecology*, *78*(2), 327-335.

Ho, T.-Y., A. Quigg, Z. V. Finkel, A. J. Milligan, K. Wyman, P. G. Falkowski, and F. M. M. Morel (2003), The elemental composition of some marine phytoplankton, *Journal of Phycology*, *39*(6), 1145-1159.

Karl, D. M. (2014), Microbially Mediated Transformations of Phosphorus in the Sea: New Views of an Old Cycle, *Annual Review of Marine Science*, *6*(1), 279-337.

Karl, D. M., and G. Tien (1992), MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments, *Limnol. Oceanogr.*, *37*(1), 105-116.
Karl, D. M., R. R. Bidigare, and R. M. Letelier (2001), Long-term changes in plankton community structure and productivity in the North Pacific Subtropical Gyre: The domain shift hypothesis, *Deep Sea Research Part II: Topical Studies in Oceanography*, *48*(8–9), 1449-1470.
Kashtan, N., et al. (2014), Single-cell genomics reveals hundreds of coexisting subpopulations in wild *Prochlorococcus*, *Science*, *344*, 416-420.
Knauss, H. J., and J. W. Porter (1954), The absorption of inorganic ions by Chlorella pyrenoidosa, *Plant physiology*, *29*(3), 229-234.
Krom, M. D., et al. (2005), Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: Results from a phosphorus starved system, *Deep Sea Research Part II: Topical Studies in Oceanography*, *52*(22-23), 2879-2896.
Labry, C., A. Herbland, and D. Delmas (2002), The role of phosphorus on planktonic production of the Gironde plume waters in the Bay of Biscay, *Journal of Plankton Research*, *24*(2), 97-117.
Lomas, M. W., J. A. Bonachela, S. A. Levin, and A. C. Martiny (2014), Impact of ocean phytoplankton diversity on phosphate uptake, *Proceedings of the National Academy of Sciences*, *111*(49), 17540-17545.
Lomas, M. W., A. L. Burke, D. A. Lomas, D. B. Bell, C. Shen, S. T. Dyhrman, and J. W. Ammerman (2010), Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP), *Biogeosciences*, *7*, 695-710.
Marie, D., X. L. Shi, F. Rigaut-Jalabert, and D. Vaulot (2010), Use of flow cytometric sorting to better assess the diversity of small photosynthetic eukaryotes in the English Channel, *FEMS Microbiology Ecology*, *72*(2), 165-178.
Martínez, A., M. S. Osburne, A. K. Sharma, E. F. DeLong, and S. W. Chisholm (2012), Phosphite utilization by the marine picocyanobacterium Prochlorococcus MIT9301, *Environmental Microbiology*, *14*(6), 1363-1377.
McLaughlin, K., J. A. Sohm, G. A. Cutter, M. W. Lomas, and A. Paytan (2013), Phosphorus cycling in the Sargasso Sea: Investigation using the oxygen isotopic composition of phosphate, enzyme-labeled fluorescence, and turnover times, *Global Biogeochem. Cycles*, *27*(2), 375-387.
Michelou, V. K., M. W. Lomas, and D. L. Kirchman (2011), Phosphate and adenosine-5'-triphosphate uptake by cyanobacteria and heterotrophic bacteria in the Sargasso Sea, *Limnology & Oceanography*, *56*(1), 323-332.
Moutin, T., and L. Prieur (2012), Influence of anticyclonic eddies on the Biogeochemistry from the Oligotrophic to the Ultraoligotrophic Mediterranean (BOUM cruise), *Biogeosciences*, *9*(10), 3827-3855.
Moutin, T., T. F. Thingstad, F. Van Wambeke, D. Marie, G. Slawyk, P. Raimbault, and H. Claustre (2002), Does Competition for Nanomolar Phosphate Supply Explain the Predominance of the Cyanobacterium *Synechococcus*?, *Limnol. Oceanogr.*, *47*, 1562-1567.
Nyholm, N. (1977), Kinetics of phosphate limited algal growth, *Biotechnology and Bioengineering*, *19*(4), 467-492.
Paerl, H., and S. Merkel (1982), The effects of particles on phosphorus assimilation in attached vs. free floating microorganisms, *Arch. Hydrobiol*, *93*, 125-134.
Paffenhöfer, G. A., B. F. Sherr, and E. B. Sherr (2007), From small scales to the big picture: persistence mechanisms of planktonic grazers in the oligotrophic ocean, *Marine Ecology*, *28*(2), 243-253.
Rhee, G. Y. (1973), A continuous culture study of phosphate uptake, growth rate and polyphosphate in Scenedesmus sp., *Journal of Phycology*, *9*(4), 495-506.
Rimmelin, P., and T. Moutin (2005), Re-examination of the MAGIC method to determine low orthophosphate concentration in seawater, *Analytica Chimica Acta*, *548*(1-2), 174-182.
Sebastián, M., P. Pitta, J. M. González, T. F. Thingstad, and J. M. Gasol (2012), Bacterioplankton groups involved in the uptake of phosphate and dissolved organic phosphorus in a mesocosm experiment with P-starved Mediterranean waters, *Environmental Microbiology*, *14*(9), 2334-2347.
Simon, M. (1985), Specific uptake rates of amino acids by attached and free-living bacteria in a mesotrophic lake, *Applied and Environmental Microbiology*, *49*(5), 1254-1259.
Talarmin, A., F. Van Wambeke, P. Catala, C. Courties, and P. Lebaron (2011a), Flow cytometric assessment of specific leucine incorporation in the open Mediterranean, *Biogeosciences*, *8*, 253-265.
Talarmin, A., F. Van Wambeke, S. Duhamel, P. Catala, T. Moutin, and P. Lebaron (2011b), Improved methodology to measure taxon-specific phosphate uptake in live and unfiltered samples, *Limnology & Oceanography: Methods*, *9*, 443-453.

Tanaka, T., F. Rassoulzadegan, and T. F. Thingstad (2003), Measurements of phosphate affinity constants and phosphorus release rates from the microbial food web in Villefranche Bay, northwestern Mediterranean, *Limnology & Oceanography*, *48*(3), 1150-1160.
Tanaka, T., et al. (2011), Lack of P-limitation of phytoplankton and heterotrophic prokaryotes in surface waters of three anticyclonic eddies in the stratified Mediterranean Sea, *Biogeosciences*, *8*, 525-538.
Ternon, E., C. Guieu, C. Ridame, S. L'Helguen, and P. Catala (2011), Longitudinal variability of the biogeochemical role of mediterranean aerosols, *Biogeosciences*, *8*, 1067-1080.
Thingstad, T. F., E. F. Skjoldal, and R. A. Bohne (1993), Phosphorus cycling and algal-bacterial competition in Sandsfjord, western Norway, *Marine Ecology Progress Series*, *99*, 239-259.
Thingstad, T. F., U. L. Zweifel, and F. Rassoulzadegan (1998), P limitation of heterotrophic bacteria and phytoplankton in the northwest Mediterranean, *Limnol. Oceanogr.*, *43*(1), 88-94.
Webb, M. R. (1992), A continuous spectrophotometric assay for inorganic phosphate and for measuring phosphate release kinetics in biological systems, *Proceedings of the National Academy of Sciences of the United States of America*, *89*(11), 4884-4887.
Wu, J., W. Sunda, E. A. Boyle, and D. M. Karl (2000), Phosphate Depletion in the Western North Atlantic Ocean, *Science*, *289*(5480), 759-762.
Zubkov, M. V., I. Mary, E. M. S. Woodward, P. E. Warwick, B. M. Fuchs, D. J. Scanlan, and P. H. Burkill (2007), Microbial control of phosphate in the nutrient-depleted North Atlantic subtropical gyre, *Environmental Microbiology*, *9*(8), 2079-2089.

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 per cell rates of Pi uptake measured in sorted groups *Synechococcus* (Syn), *Prochlorococcus* (Proc), picoeukaryotes (Pic) and heterotrophic prokaryotes (Hprok) measured under ambient concentrations. NA: non available data

Station	Depth (m)	DCMd (m)	Chl *a* int (mg C m^{-2})	[SRP] (nM)	Per cell 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 per cell 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 unavailable

			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

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