Biogeosciences Discuss., 11, C7858–C7868, 2015
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Biogeosciences Discussions Open Access

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

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Received and published: 6 January 2015

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 1.34 104 cells mL-1,

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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 sufïňĄce 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 inïňĆuenced 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 ïňĆagellates 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.

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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 ($\sim$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

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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 ïňĄtted 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 wrong 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 reïňĆect 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 ïňĄgures, possibly to

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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 10ËĘ4 or 10ËĘ5 ml-1? (also see ln13 on P14648) It is 77307 cells mL-1, so 7.7 $\times$ 104 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 ïňĄgure 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 33Pi 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 ïňĄt 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,

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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.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

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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.

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.

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

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 ïňĄndings 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 μm3 may not be submitted to the theoretical surface-limited growth rate, due to a poor cellular ma-

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chinery 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 ïňĄnd it hard to actually see this).

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 deïňĄne NA here too. Chlorophyll data were obtained from a different cast than our samples, which is why we presented it 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 *.

Interactive comment on Biogeosciences Discuss., 11, 14639, 2014.

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

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

Fig. 1.

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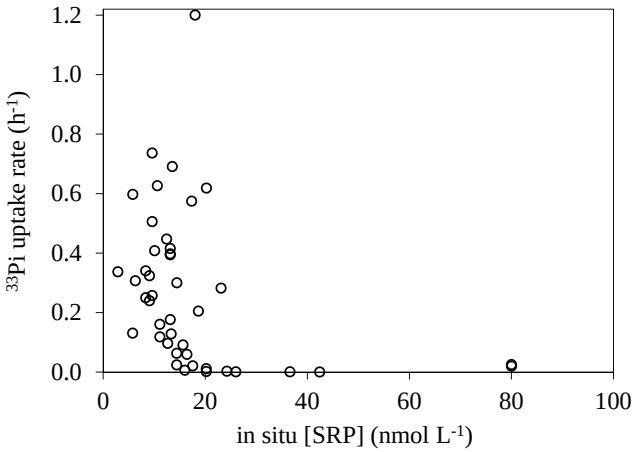

Fig. S2. Bulk Pi uptake rates (h^{-1}) plotted against SRP concentrations.

Fig. 2.

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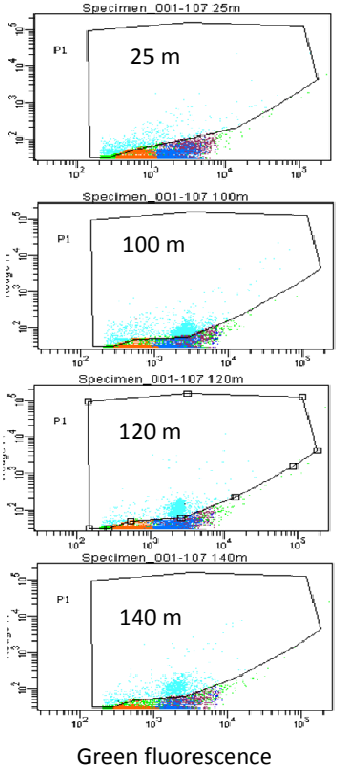

Fig. S3: Cytograms showing the green fluorescence of SYBR-Green I versus Red fluorescence from chlorophyll a at 4 depths between 25 and 140 m at a station of the BOUM transect.

Fig. 3.

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