Interactive discussion on "Vertical partitioning of phosphate uptake among picoplankton groups in the low-P Mediterranean Sea" bg-2014-464

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We would like to thank once again the anonymous reviewers for their detailed comments which have helped this manuscript to reach a richer and clearer content.

All comments are addressed below, along with the appropriate changes made to the text. We are grateful to the reviewers and the handling editor for considering our manuscript for publication in Biogeosciences.

Sincerely,

Agathe Talarmin, on behalf of all authors

Reviewer #1

This revised manuscript shows clear improvement compared with the first manuscript, and the discussion has become much clearer. However, there are some faults probably caused in the process of revision. And I found the abstract did not sufficiently reflect the revision in discussion and conclusion. I proposed that these should be corrected before publication in Biogeosciences.

P3L11 “cells.mL-1” should be “cells mL-1”.

Done

P3L16 “Hprok,” should be “Hprok.”

Done

P3L18 In this abstract, the total revision in discussion is not fully included. I found some inconsistency between the abstract and conclusion.

Inconsistencies were revised and the abstract was modified to better reflect points further discussed in the paper.

P4L18 “by Church et al. (2002) and Karl et al. (2001)” should be “(Church et al., 2002; Karl et al., 2001)”.

Done

P8L16 Remove “ml”.

Done

P8L16 “+4°C” should be “4 °C”.

Done

P10L14 and hereafter “per cell uptake” is inappropriate. “cellular uptake” or “cell-specific” uptake is more appropriate.

The taxon-specific uptake was designated as cellular throughout the manuscript.

P10L21 “and data treatment” should be bold.

Done

P11L11 Citation is inappropriate.

Citation removed.

P13L1 This should appear in Materials and Methods?

Indeed. It was moved to section 2.3.4.

P13L13 µg P should be converted into µgmol P.

Data converted in the text in nmol P.

“The biomass of Pic in the bioassay of St. A reached 1.2 nmol P L^{-1}, and ranged 15.7 – 34.4 nmol P L^{-1} along the profile at St. 25, which was 300, and 25 – 55 higher than the biomass of Syn, respectively. Proc estimated

biomass was in the same order of magnitude as Syn, around 0.3 nmol P L^{-1}, and Hprok biomass was twice lower than Pic."

P13L20 "10^-18 mol" means "amol"?

Indeed, this was modified.

P14L7 "compared" should be "comparable"? Anyway, I could not catch the meaning of this sentence.

Sentence re-phrased: "Estimations of the $K+S_n$ constant Proc and Pic cells at St. A and Syn cells at St. C were comparable and about 5 times lower than the value measured for Syn cells at St. A (128.4 nmol P L^{-1})"

P14L13 "low" should be "short"?

Done

P14L21 I could not understand this sentence. What is Taxon 1?

This sentence was rephrased:

"When all 3 phototrophic groups were successfully sorted, there was not a single group clearly showing higher cellular Pi uptake rates, like there were in samples from the North Atlantic (e.g. Pic *Lomas et al.*, 2014, Syn in *Michelou et al.*, 2011 and Syn and Pic in *Zubkov et al.*, 2007)."

P15L7 Phosphorus is not oxidized when assimilated into organisms.

Replaced oxidized with utilized

P17L17 "who" should be "which".

Done

P17L21 I could not understand this sentence. Why does it end with "such"?

Re-written as:" Finally, we also considered the possibility that Proc cells from surface layers had too low chlorophyll a content to be discriminated from Hprok in stained samples, resulting in an overestimation of the Hprok contribution to bulk Pi uptake in the surface samples where Proc cells were not detected."

Tables 1 and 2 "NA" is an abbreviation for "(data) not available".

Done.

- Reviewer #2

The manuscript is much improved. However, there are remaining minor issues in the new text, listed below in order of appearance, that will need to be addressed prior to publication considerations. There also seems that text may be missing (p17).

P5, ln 22. Insert “were” between ‘Pi’ and ‘higher’

Done.

P8, ln 20. Were the blank values subtracted from the counts?

Yes, systematically. Therefore high blank values led to ‘below detection’ values which are not accounted for in this data set.

Addition:” Blank values were systematically subtracted from the counts, in dpm $cell^{-1}$, for the sorted samples.”

P11, ln 15. Change ‘Table 2’ to ‘Table 1’

Done

P12, ln 9. Syn abundance - 7700 is less than 14000, so something is incorrect in this statement.

Indeed. I apologize for this mixup in reporting values; 1.4 $\times 10^5$ was the maximum abundance for Proc cells. The minimum for Syn was 636 cells mL^{-1} at 130 at St. A.

”Syn cells were the most abundant at the coastal station 25 (7.7×10^4 cells mL^{-1} at 40 m) and the least abundant in the deep euphotic zone (130 m St. A, 0.6×10^3 cells mL^{-1}; Fig. 3).”

P14, ln5-7. This sentence is difficult to understand. Does it mean that, at Station C, K+Sn were the same for all sorted groups?

This was pointed out by Reviewer #1 also, and modified as:

“Estimations of the $K+S_n$ constant Proc and Pic cells at St. A and Syn cells at St. C were comparable and about 5 times lower than the value measured for Syn cells at St. A (128.4 nmol P L^{-1}).”

P14, ln 16. What is meant with “discrepancies’ between turnover time and SRP concentrations? What relationship was expected?

A clear gradient in SRP concentrations decreasing from West to East was expected, possibly accompanied with shorter turnover times in the eastern basin. Here, we mean to point out that the turnover times more than SRP concentrations reveal the degree of limitation of the communities.

This was re-phrased:

“Low Pi turnover times deepening towards the East in the while SRP concentrations did not show a clear longitudinal and suggest a higher limitation of microbial communities in the Eastern basin.”

P15, ln 3. These rates are quite high compared to these other studies, even when considering bulk rates measured previously in the Mediterranean (e.g. Flaten et al. 2005: ~1nmol L-1 h-1).

They are indeed higher than 1 on average, ranging 1.2 – 12.5 nmol L-1 h-1 at St. 9. Additional measurements in the Levantine and Ionian Basin would be necessary to further develop this comparison. Moreover, the mentioned article published maximum uptake rates, estimated requirements (as uptake rates) as well as Pi concentrations, and turnover

times but no bulk Pi uptake rates from the environment. Therefore the comparison will not be developed. The role of the community structure and diversity in uptake rates now better emphasized in the discussion are our main hypothesis to explain variations in bulk Pi uptake rates over time and space.

It has not been shown in the Mediterranean by lack of measurements, but a diversity shift from picoeukaryotes to mostly cyanobacteria could have happened between 2002 and 2008, like it was observed at HOT and BATS, especially with the physical characteristics of this Sea. No data support this, which is therefore not discussed either.

P15, ln 6. You present 4 possible reasons for the high per cell P uptake rates observed in Proc in this study compared to the Sargasso Sea (~ 16x higher). However, it seems to me that these points address Proc rates compared to community rates. Is that what was meant?

Yes the point was to discuss Proc rates in particular, because it is the one group for which data were collected whether they were looking at phototrophs only or picoplankton in general, allowing a better comparison across environments. We do think that multiple explanations are involved in the observed differences across environments, and a more detailed characterization of the chemical composition of our samples would have helped to tear apart some main drivers.

I also have questions with the different points.

Point i), although it is now realized that Proc group has great genetic variability, is there reason to believe that the Proc community in the Med is hugely different from that in other oligotrophic oceans?

Comparing 2 phylogenetic analyses of picocyanobacterial communities in the Atlantic [*Zwirglmaier et al.*, 2007] and the Mediterranean Sea [*Mella-Flores et al.*, 2011], there are major differences. The text was completed as:

"i) different composition of the cyanobacterial community between the Sargasso Sea and the Mediterranean, notably clade HL II which is underrepresented in the Mediterranean (*Mella-Flores et al.*, 2011)"

Point ii) the proportion of Proc to the cyanobacterial community should not affect per cell rates, but bulk rates. Also, Pi (which here equates to orthophosphate) does not readily undergo redox changes, so 'oxidation of Pi' is probably not what you intended.

Indeed, the term oxidized was misused here. However, we do not discuss the relative abundance of Proc in the picoplankton but rather the proportion of Proc cells that are actually capable of using Pi directly as a source of P.

"the low proportion of Proc cells (<10%) able to utilize Pi in the subsurface layers of the Sargasso Sea (*Martínez et al.*, 2012)"

Point iii) this would require that if 100% of Proc is live in the Mediterranean then >90% will be dead in the Sargasso Sea (15/16).

Each point does not aim at explaining alone the differences. A higher proportion of dying or freshly dead Proc cells (still exhibiting enough red fluorescence to be sorted as Proc) in the Sargasso Sea would partly account for lower averaged cellular rates.

Point iv) is valid but again requires that >90% of the label is lost. Is there such a large discrepancy between sorted rates compared to bulk rates from the cited studies?

The cited study did not involve Proc sorts but reported signal losses up to 50% (on average) of the signal for other picoplankton groups. Even if that 50% was applicable to Proc cells, this methodological aspect would not suffice to account for the 10 fold lower per cell rates in the Sargasso Sea.

P15, ln 16. If the missing fraction doesn't contribute much to the bulk P-uptake, then even with higher per cell uptake they will not make up for the missing portion, as the comparison is between bulk and sorted cells, and bulk>sum of sorted groups.

This was re-phrased for clarity, because it is not exactly a missing fraction like it is when we compare contribution of groups to total Pi uptake. Also, this comment regards Vmax values, not uptake rates in environmental conditions, therefore we remove the note about the >2um size class contribution which does not add to the point.

"Non-sorted large protists may have higher maximum Pi uptake rates per cell (*Casey et al.*, 2009) or the ability to store large amounts of Pi in case of upwelled or deposited inputs."

P16, ln 3. "Our data suggests that microbial communities have the potential to take up Pi faster when Pi turnover is short." I am not sure what is meant here. Maybe the inverse (faster rates makes shorter turnover)? However, turnover time is a function of biomass, available P-pool size and community (or group) uptake rate. Your data from the top 50 m shows rates to increase with increasing SRP concentration, which is what would be expected where the Pi pool often is below concentrations where Vmax can be reached. So it may well be that at higher SRP concentrations the highest rates are achieved and the turnover times get shorter as a result.

I very much agree with your statement and rectified the text, because it seemed unclear indeed whether this was related to Vmax or regular uptake rates. Vmax are the focus in this paragraph, and since Vmax is an upper boundary, the rates can not be discussed as faster per se, but higher Vmax indicates a higher amount of P potentially absorbed.

"Our data also suggest that microbial communities have the potential to take up more Pi when Pi turnover times are short."

P17, ln 22. Is there missing text here? "..in stained samples: such.."

Yes, I apologize, this has been rectified:

"Finally, we also considered the possibility that Proc cells from surface layers had too low chlorophyll a content to be discriminated from Hprok in stained samples, resulting in an overestimation of the Hprok contribution to bulk Pi uptake in the surface samples where Proc cells were not detected."

References cited:

Mella-Flores, D., et al. (2011), Is the distribution of Prochlorococcus and Synechococcus ecotypes in the Mediterranean Sea affected by global warming?, *Biogeosciences*, *8*, 2785-2804.
Zwirglmaier, K., J. L. Heywood, K. Chamberlain, E. M. S. Woodward, M. V. Zubkov, and D. J. Scanlan (2007), Basin-scale distribution patterns of picocyanobacterial lineages in the Atlantic Ocean, *Environmental Microbiology*, *9*(5), 1278-1290.