

We would like to thank Anonymous Referee #1 for his/her comments and suggestions on our manuscript. Please find below our responses (**in bold blue**) as well as the associated modifications in the revised version of the manuscript (*in italics*).

Anonymous Referee #1

General comments:

The authors report phosphorus (P) dynamics on the zonal transect in the Mediterranean Sea using a sensitive analytical technique for determining nanomolar P. The technique enabled to represent not only spatial variations in nanomolar P but also their upward flux in the oligotrophic upper water. The authors found a west-east gradient of P dynamics from data on nanomolar P distribution/flux and DOP hydrolysis. Based on these data as well as atmospheric P deposition data, they analyzed P budgets in the mixed layer and found a large external supply of atmospheric deposition relative to upward supply and an importance of internal DOP hydrolysis for sustaining primary production. The budgetary analysis includes some assumptions, but this approach using nanomolar P data is new and is necessary for understating P dynamics in oligotrophic oceans. Overall, the manuscript is well structured and is scientifically implicated. Thus, I consider that this study is suitable for publishing in *Biogeosciences* after addressing specific comments below.

Specific comments:

L24, 25, 27: “L-1 or kg-1”

“-1” should be superscript.

This will be corrected in the revised manuscript.

L45: “physical structuration of the water column”

What is “physical structuration”? Is it physical phenomenon such as mixing and/or diffusion? Please clarify.

We apologize for the awkward phrasing. It will be modified as follows:

Concentration of dissolved nutrients are kept low by microorganisms within the euphotic zone and their replenishment is partly driven by physical structure of the water column which constrains vertical nutrient transport (Lewis et al., 1986).

L72: “North Pacific (Duhamel et al., 2011)”

In addition to Duhamel et al., Sato et al. (2013 BG, doi:10.5194/bg-10-7677-2013) is citable as a representative AP study in the North and South Pacific Ocean.

The reference will be added.

L119: “4.5% (n=10)”

What is the concentration level of sample for determining the precision? Please specify the concentration with 4.5%.

The concentration of the sample used to assess the precision was 11 nmol L⁻¹. This information will be added in the revised manuscript.

“The analytical precision, estimated through repeated measurements of a surface seawater sample with phosphate concentration of 11 nmol L⁻¹, was 4.5% (n= 10).”

L123: “two 100 nM solutions of glycerol-phosphate and glucose-phosphate”

These compounds are analogs of labile phosphate ester. Did you try to examine the digestion efficiency using a refractory P analog such as phosphonate with C-P bond?

No, we didn't, although previous studies have reported satisfactory recovery efficiencies of UV-digestion on phosphonate analogs (Armstrong et al. 1966). However, it is true that UV-digestion generally returns lower recoveries than chemistry-based oxidation methods (Karl, 2014 and references therein) and, therefore, DOP estimated through UV-digestion should be considered as a lower limit of ambient DOP concentration. We will add a sentence on this issue in the revised manuscript.

“The efficiency of digestion on organic P compounds other than phosphoesters was not assessed in this study, although UV-digestion has also proven to return complete hydrolysis of phosphonate compounds (Armstrong et al. 1966). However, it is true that UV-digestion generally returns lower recoveries than chemistry-based oxidation methods (Karl and Björkman, 2015 and references therein) and, therefore, DOP estimated in this study through UV-digestion should be considered as a lower limit of ambient DOP concentration.”

Karl, D. M. and Björkman, K. M.: Dynamics of Dissolved Organic Phosphorus, in Biogeochemistry of Marine Dissolved Organic Matter, pp. 233–334, Elsevier., 2015.

L167-168: “The mixed layer depth.....the profile (1 m)”

Could you please cite the reference for this statement?

A reference will be added.

The mixed layer depth (MLD) was determined at every CTD cast as the depth where the residual mass content (i.e., the vertical integral of the density anomaly relative to surface) was equal to 1 kg m⁻² (Prieur et al., 2020), with an error of estimation of 0.5 m relative to the vertical resolution of the profile (1 m).

Prieur, L., D'Ortenzio, F., Taillandier, V. and Testor, P. (2020), Physical oceanography of the Ligurian sea. In: Migon C., Sciandra A. and Nival P. (eds.), the Mediterranean sea in the era of global change (volume 1), evidence from 30 years of multidisciplinary study of the Ligurian sea. ISTE Sci. Publ. LTD, 49–78. doi:10.1002/9781119706960.ch3

L198: “unpublished measurements in the study area”

This measurement for determining the fraction of AP-DOP to total DOP is very important, because the result of the AP-DOP fraction significantly influences on in site AP hydrolysis rate. Could you please describe how to measure the AP-DOP here?

AP-DOP in the mentioned studies (Djaoudi et al. 2017 and unpublished measurements) was measured based on Hashihama et al (2013). Briefly, AP-DOP is calculated as the increase in phosphate concentration after incubation of the seawater sample with a purified alkaline phosphatase. The basis of the measurement will be described in the revised manuscript.

In situ alkaline phosphatase hydrolysis rates. In situ hydrolysis rates for alkaline phosphatase (AP) were computed using estimated in situ concentration of DOP hydrolysable by AP (AP-DOP) as substrate concentration (S) in Michaelis-Menten kinetic equation as following:

$$V_{insitu} = \frac{(V_{max} \cdot AP - DOP)}{(K_m + AP - DOP)}$$

AP-DOP concentration is usually measured as the increase in phosphate concentration after incubation of a seawater sample with a purified alkaline phosphatase (Hashihama et al. 2013). Therefore it is used as an estimate of phosphate monoesters (Yamaguchi et al. 2019). In the absence of AP-DOP measurements in this study, we applied a fraction of AP-DOP to DOP of $31 \pm 18\%$ ($n=36$) an average value previously measured in the study region (Djaoudi et al., 2018b, and unpublished data) following the Hashihama et al. (2013) method.

L247-251: “Using measured density gradient..... $14.4 \mu\text{mol m}^{-2} \text{d}^{-1}$.”

While this paragraph for phosphate upward flux is informative, I am thinking that the authors can also calculate upward flux of DOP as well as phosphate. If DOP upward supply is significant, it may contribute new production in the mixed layer as well as phosphate upward supply. I am glad the authors consider this point.

We thank the reviewer for this suggestion. However, DOP profiles inside the phosphate depleted layer did not reveal any positive gradient of concentration with depth suggesting the lack of upward fluxes of DOP. In addition, a previous work on the study region showed a significant decrease of DOP concentration with depth (Djaoudi et al. 2018) inside the phosphate depleted layer suggesting downward rather than upward DOP fluxes.

L291: “PO4”

“4” should be subscript.

This will be corrected in the revised manuscript.

L306: “ nM m^{-1} ”

In the Results section, vertical gradient of phosphate is expressed as a unit of $\mu\text{mol kg}^{-1}$. Why do the authors use “ nM m^{-1} ” here? I think it is better to use same unit throughout the manuscript.

We have used nM m^{-1} instead of $\mu\text{mol kg}^{-1}$ in this part of the discussion in order to compare our results to phosphate vertical gradients reported by Djaoudi et al (2018a) who expressed them in nM m^{-1} . We will slightly rephrased this sentence to avoid confusion between units.

Yet, our nanomolar phosphate data revealed the presence of phosphate vertical gradients inside the PDL (except in stations 6 and ION) ranging between 1.8 and 10.6 $\mu\text{mol kg}^{-1}$ and decreasing from west to east. In a recent study in the western Mediterranean Sea, Djaoudi et al. (2018a) reported phosphate gradients over depth above the phosphocline between 0.04 and 3.2 nM m^{-1} , higher than observed in the current study (0.01-0.3 nM m^{-1} if expressed as gradient of phosphate concentration over depth).

L309-313: “Similar vertical gradients.....South China Sea”

The authors need to cite the reference here.

Following the suggestion below, we will remove this sentence.

L309-313: “Similar vertical gradients.....(Van Wambeke et al. 2020)”

Why do the authors describe vertical gradient of nitrate here? Since this subsection is for vertical variations in phosphate as indicated by its title, the description of nitrate is likely off-topic.

We will remove these sentences on the vertical gradients of nitrate, as suggested.

L339: “analytical locks”

What is it?

We will replace it by “technical limitations”.

L345: “oligotrophic South China Sea (0.21-0.44 $\mu\text{mol P m}^{-2} \text{d}^{-1}$)”

Hydrographic condition is likely different between the Mediterranean Sea and South China Sea. Can you simply compare vertical P fluxes between these different areas?

This comparison was intended to frame the order of magnitude of our estimated fluxes since we did not have *in situ* measurements of ϵ . We will slightly modify the sentence to precise that fluxes might indeed be different as a consequence of different hydrological conditions between the two regions. Moreover, we will add a reference to very recent publication (Hashihama et al. 2021) which reports nanomolar phosphate fluxes to the mixed layer of the subtropical North Pacific of the same order of magnitude than our estimates.

Despite the above described uncertainties of our calculations, our estimates are in the lower range, but in the same order of magnitude, of the only reported diapycnal fluxes of P reaching the upper euphotic zone based on simultaneous turbulence microstructure and high-resolution chemical measurements in the oligotrophic South China Sea (0.21-0.44 $\mu\text{mol P m}^{-2} \text{d}^{-1}$) (Du et al., 2017) and in the sub-tropical North Pacific (Hashihama et al. 2021). Thus, despite hydrological differences among these oceanic regions, we assume our estimates to be valid enough for the purpose of the following section of this

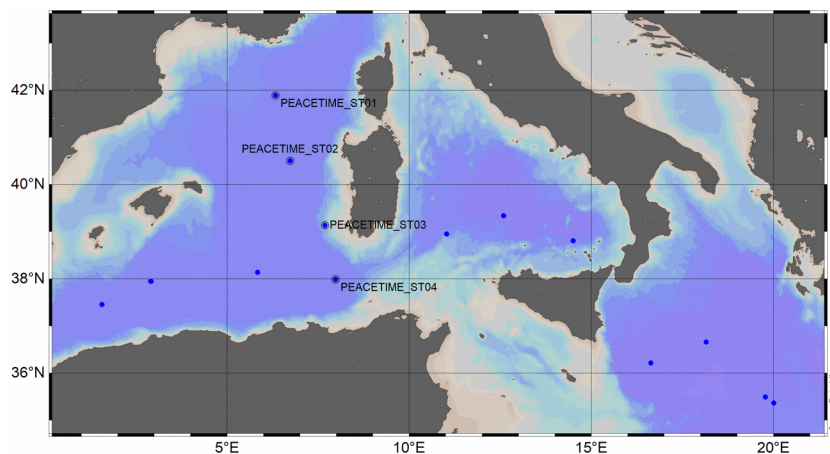
work, i.e. to assess the relative contribution of external and internal sources potentially contributing to phosphate supply to the mixed layer.

Hashihama, F., Yasuda, I., Kumabe, A., Sato, M., Sasaoka, H., Iida, Y., Shiozaki, T., Saito, H., Kanda, J., Furuya, K., Boyd, P. W. and Ishii, M.: Nanomolar phosphate supply and its recycling drive net community production in the subtropical North Pacific, *Nature Communications*, 12(1), doi:10.1038/s41467-021-23837-y, 2021.

L352-353: “However.....horizontal gradients”

The authors need to show the evidence for little horizontal gradient of surface phosphate here. In addition to the west-east gradient, the north-south gradient is likely important for lateral nutrient supply.

We agree. During the Peacetime cruise, three additional stations were sampled although they were not included in this study (cf map below). The location of these three stations, together with ST04, allows to check surface DIP variability along a north-south gradient. DIP concentration within the phosphate depleted layer in these stations was 11 ± 6 nM in ST01, 10 ± 3 nM in ST02 and 11 ± 6 nM in ST03. These values compare well with ST04 (10 ± 5 nM), at the southernmost latitude of the N-S transect and with the rest of stations across the longitudinal transect (Fig. 2 in the manuscript). This suggests little horizontal gradient of surface phosphate across a north-south gradient in the study area. This information will be added to the revised manuscript.



“Together with diapycnal fluxes, the surface mixed layer can receive new P from above, through atmospheric deposition (Pulido-Villena et al. 2010, Richon et al. 2019), and through lateral transport (Letscher et al. 2016). In this study, there was very little variability in surface phosphate concentration among the stations excluding the presence of important horizontal gradients across the longitudinal transect. Moreover, surface phosphate concentration in three stations located further north (between 42°N and 39°N along a north-south gradient) was homogeneous and similar to the study transect (11 ± 6 nM in ST01, 10 ± 3 nM in ST02 and 11 ± 6 nM in ST03, data not shown) suggesting also the absence of horizontal gradients across a north-south gradient. We thus assume that phosphate supply through

lateral transport was not dominant during the cruise and consider atmospheric deposition and diapycnal fluxes from below as the two main external sources of phosphate to the phosphate depleted layer.

L354: “lateral advection to be negligible”

This is a contradiction with the paragraph in L394-405. Please revise.

We agree that this part of the discussion could appear contradictory since it mixed up mechanisms and processes occurring at different spatio-temporal scales. We have rephrased this section considering two different scales of lateral fluxes: large scale (basin/year) and mesoscale. We will make the distinction in the revised paper between the basin scale (where homogeneity in surface phosphate precludes horizontal gradients) and the particular case of ST10 where lateral phosphate supply may be more important due to local transport specific to the presence of a mesoscale gyre (Guieu et al. 2020 for details).

“The imbalance between estimated external sources of P and new production was particularly marked in ST10, the westernmost station of the transect, where external P supply may explain up to 10% of new production in the best case. This contrasts with the rest of stations in the Algerian basin where external P supply explained between 30 and 90 %. As said before, it was hypothesized that, at the basin scale, lateral transport was not a dominant P source mainly based on the homogeneity of surface phosphate concentration excluding the presence of important horizontal gradients. Nevertheless, ST10 might be an exception to this assumption. Using satellite derived currents, we observed the presence of a mesoscale eddy and ST10 was located on the edge of this eddy (Guieu et al. 2020). This can imply a mesoscale transport of nutrients from Atlantic water containing significant levels of phosphate (Huertas et al., 2012). It may not be excluded that in ST10 lateral transport contributed to phosphate external supply to upper waters, explaining the low contribution of both atmospheric deposition and diapycnal fluxes to new production”.

L400: “Seawater”

“S” should not be italic.

This will be corrected in the revised manuscript.

L428: “P demand”

P demand of what?

We are sorry for the imprecision. We will replace ‘P demand’ by ‘estimated P requirements of phytoplankton and heterotrophic prokaryotes’.

To scale the estimated AP_{insitu} within the P cycle, we computed the daily fraction of phosphate potentially released by AP activity and the contribution of AP_{insitu} to total P requirements by both phytoplankton and heterotrophic prokaryotes in the surface mixed layer.

L442-443: “In the eastern.....exceeded 100%.”

The >100% INT/TPR is likely due to preferential recycling of P relative to C as reported in a range of biogeochemical studies (Clark et al. 1998 Nature, doi.org/10.1038/30881; Loh and Bauer 2000 DSR-I, doi:10.1016/S0967-0637(00)00027-3; Paytan et al. 2003 Mar. Chem., doi:10.1016/S0304-4203(03)00052-5; Duhamel et al. 2007 BG, doi:10.5194/bg-4-941-2007; Letscher and Moore 2015 GBC, doi:10.1002/2014GB004904). I recommend the authors use these references to explain the results of >100% INT/TPR.

We agree with the referee that preferential recycling of P relative to C has been widely reported and that it should be particularly strong under conditions of P-limitation like those likely encountered in the study region. This will be added to the revised manuscript.

However, we are not sure that this preferential remineralization would be enough to explain the apparent over-hydrolysis of DOP found in this study. Indeed, the selective removal of phosphorus from DOM presumably reflects the nutrient demand of marine microorganisms which has been considered in this study in the estimation of total P requirements of phytoplankton and heterotrophic prokaryotes. An estimation of resulting C:P ratios will be provided, assuming that P from DOP hydrolysis is fully assimilated by phytoplankton and heterotrophic prokaryotes. C:P ratios would be lower than 20 for heterotrophic prokaryotes and 40 for phytoplankton in all the stations located in the Tyrrhenian and Ionian basins which seems unrealistic even under extreme conditions of P-limitation.

One intriguing aspect of estimated AP_{insitu} rates concerns the anomalously high contribution of DOP hydrolysis to total phosphate requirements in the surface layer, exceeding 100% in 7 out of 10 stations (Table S2). Although preferential remineralization of P relative to C is a common feature in oceanic regions (e.g. Clark et al. 1998, Letscher and Moore, 2015), in this study DOP appears to be over-hydrolyzed compared to P requirements. Indeed, if P from DOP hydrolysis was fully assimilated by phytoplankton and heterotrophic prokaryotes, C:P ratios would be lower than 40 and 20, respectively, in all stations of the Tyrrhenian and Ionian basins, which seems unrealistic.

L458-460: “the proportion of AP-DOP to DOP.....(Hashihama et al. 2013).”

In addition to Djaoudi et al. (2018b) and Hashihama et al. (2013), Yamaguchi et al. (2021 Front. Microb., doi: 10.3389/fmicb.2020.570081) recently reported a review of the proportion of AP-DOP to DOP. Table 1 in Yamaguchi et al. is useful for this study, and the multiple comparison of AP-DOP/DOP ratios in different regions would strengthen the discussion here.

Thank you for the suggestion. We will add this reference to the revised manuscript to strengthen this part of the discussion.

Concerning the first point, the proportion of AP-DOP to DOP has been reported to be extremely variable among oceanic regions (Yamaguchi et al. 2021). In this study, we applied a ratio of 30% to estimate AP-DOP concentration based on data reported by Djaoudi et al. 2018 for the Mediterranean Sea. However, considering a AP-DOP/DOP ratio as low as 10% would reduce the AP_{insitu} by one third.

L461-462: “Accurate determinations.....P-deficient oceanic regions”

I think the authors generally consider phosphate ester as AP-DOP. However, phosphonate is also utilized by marine diazotroph *Trichodesmium* (Dyhrman et al. 2006 Nature, doi:10.1038/nature04203) and eukaryotic phytoplankton (Whitney and Lomas 2019 Limnol. Oceanogr. Lett., doi: 10.1002/lol2.10100). Therefore, we now should consider both phosphate ester and phosphonate as bioavailable DOP. Could you please discuss phosphonate as well as phosphate ester?

We apologize for the misunderstanding. We indeed consider AP-DOP as phosphomonoesters since it is measured as the difference in phosphate concentration after hydrolysis with alkaline phosphatase which is a phosphomonoesterase. This said, we are aware that AP-DOP (i.e. phosphomonoesters) does not represent the entire pool of bioavailable DOP. To avoid misunderstanding, two new statements will be added to the revised manuscript:

- 1. In the Material and Methods section, the procedure for measuring AP-DOP will be detailed to make it clear that it correspond to the concentration of phosphomonoesters (see response to comment above)**
- 2. In the discussion section, we will now mention that our estimations of DOP hydrolysis (internal sources) might be underestimated since they consider only phosphomonoesters as internal P source while other P compounds such as phosphonates are known to provide alternative P.**

It should be noted here that DOP hydrolysis in this study was assessed solely through alkaline phosphatase activity and it was therefore restricted to phosphate monoesters. Other organic P compounds such as phosphate diesters or phosphonates are known to provide alternative sources of P (Dyhrman et al. 2006, Whitney and Lomas 2019, Thomsom et al. 2020).

*Thomson, B., Wenley, J., Lockwood, S., Twigg, I., Currie, K., Herndl, G. J., Hepburn, C. D. and Baltar, F.: Relative Importance of Phosphodiesterase vs. Phosphomonoesterase (Alkaline Phosphatase) Activities for Dissolved Organic Phosphorus Hydrolysis in Epi- and Mesopelagic Waters, *Frontiers in Earth Science*, 8, doi:10.3389/feart.2020.560893, 2020.*

L505-510: “CC and KD.....and VT”

In the Author contribution, authors’ initials KD and KDJ are vague. From the author list in L4-6, I can identify two KD but not KDJ. Please correct.

We agree although we don’t see a straightforward solution for distinguishing these two authors who share the same initials. We will ask for assistance to the editorial office.

References:

Reference style is not consistent. Please check throughout all references.

The reference style will be checked throughout.

Figure 3:

“FA, TYR and ION” in the legend should be “FA (panel A), TYR (panel B), and ION (panel C)”.

This will be corrected in the revised manuscript.

Tables:

In general, the legends of tables should be appeared just above the tables.

Table 3:

The authors should define significant level such as $p < 0.05$.

This will be corrected in the revised manuscript.

Table 4:

Please indicate how to derive the correlation matrix in the text or table legend. Did you use Spearman correlation test or Pearson correlation test?

We used Pearson correlation test. This will be specified in the table legend.

“Table 4. Pearson correlation coefficients among biogeochemical variables related to the phosphorus pool inside the phosphate depleted layer (n= 10). PD = phosphacline depth. DIP: dissolved inorganic phosphate concentration. DOP/ dissolved organic phosphorus. POP: particulate organic phosphorus. Vmax: AP maximum hydrolysis velocity. Km: AP Michaelis Menten halfsaturation constant. Vmax/POP: AP maximum hydrolysis velocity normalized to POP. TMUF: turnover time of spiked DOP substrate, MUF-P. Significant correlations at $p < 0.05$ are highlighted in bold.”

Table S1:

“Sustained PP” may be wrong. I think “Sustained NP” is correct because this is based on external P flux that corresponds to new production (NP) rather than primary production (PP). Also, “Maranon et al.” in the legend should be “Marañón et al.”.

We agree and we will replace sustained PP by sustained NP.

Table S2:

For “TPR_{moy}”, please explain the meaning of “_{moy}”.

We apologize for the mistake. This will be corrected and explained in the legend.