RC2: 'Comment on egusphere-2023-2578', Anonymous Referee #2, 30 Jan 2024 reply

General comments

The authors investigate phosphomonoesterase (PME) versus phosphodiesterase (PDE) activities in a well-known P-depleted oligotrophic environment, the Eastern Mediterranean Sea, at two contrasted seasons. They characterize maximum hydrolysis rates (Vm) and half-saturation constants (Km) of both PME and PDE activities in relation to dissolved stocks of Phosphorus : DIP, DOP and the enzymatically hydrolysable fraction of DOP. Although phosphomonoesterase, also known as alkaline phosphatase, activities have been extensively studied in P deplete oligotrophic and coastal environments during past decades, the measurements of both PME and PDE have been achieved only recently. The authors have paid particular attention on the methodology for the measurement of these activities. The results of this paper confirm the results found elsewhere that PDE activities (Vm) could be in the same order of magnitude than PME activities. PDE seem to be regulated as PME, by the availability of DIP. However the regulation of PDE by NOx:DIP ratio is also discussed as well as the occurrence of different microbial communities having different PDE expression pattern. This paper has a significant contribution to the understanding of the Phosphorus fluxes through the microbial food web, participating to the biogeochemical cycle of Phosphorus. Obviously, it is within the scope of EGUsphere.

Response: We appreciate that the reviewer has found the work interesting and worthwhile to consider for publication. We truly thank him/her for providing detailed and useful comments, and we have addressed carefully him/her feedback.

Specific responses to the reviewers' comments are provided below in blue, modified sentences included in the revised version in blue + italics and line numbers referring to the new revised version with track changes are highlighted in yellow.

Specific comments

The scientific approach and applied methods to the studies of PME and PDE activities in relation to the dissolved phosphorus pools are particularly well adapted. The measurements of nanomolar concentrations of DIP and labile DOP in such oligotrophic environments as the Mediterranean Sea are achieved with the LWCC method having a very low detection limit (1 nM). The measurements of kinetic parameters of enzymatic activities need a particular attention since methodological biases can lead to misestimated Vm and Km. The most notable divergences in existing methodologies on enzymatic activity assays in natural environment is the substrate concentrations used for assays. The range of substrate concentration significantly affect kinetic parameters estimation and it is generally recommended to use a large substrate concentration range, up to 10 Km at least. A specific literature exists on this particular bias which could be cited by authors in the Discussion part. However, the authors discussed their results with published literature, considering these methodological aspects, which is scarcely made while necessary for meaningful comparison.

In the revised version of the manuscript, we now address the substrate concentration bias as suggested by the referee line 473-475:

'.. as Km and Vm depend on the concentration of fluorogenic substrate added, with recommendations to add up to 10 times the Km value to calculate Vm appropriately (Urvoy et al., 2020). In most cases only one single substrate concentration….'

Details

Line 27: Define the significance of DIP the first time it appears rather than Line 42

Indeed, DIP appeared in the abstract without having been identified. We wrote 'dissolved inorganic phosphorus' in the abstract, line 27.

Line 49, 692: Labry et al. 2016 rather than 2021

Yes sorry for the mistake, this was corrected in the text line 52 and in the reference list

Line 58: precise under optimal conditions of concentrations of what ? enzyme ?

Yes it is. The sentence was modified line 62 as : *'… under optimal condition of enzyme concentration, pH and temperature…'*

Line 120: nitrite rather than « nitrites » and use « DOP » rather than its significance

The sentence was modified line 133 as : *' Other nutrient analyses (nitrate, nitrite, DOP, DIP with the classical method) were sampled….'….)*

Line 141, 637: Djaoudi et al. 2018 rather than 2017

Yes sorry for the mistake, we corrected the reference to that of 2018a in the text, as there is another Djaoudi et al. 2018 cited in the ms which became 2018b. The reference list was corrected too (lines 89, 160, 438, 767, 773)

Line 225 : concerning winter depth of Pcline, refer to Fig. 3b,c

The sentence was modified line 268 as: *' In winter, the depth of the Pcline… showing a great variability among stations (Fig 3b, c)'*

Line 395 : a little more exhausted literature on P diesters composition would be informative

A list is cited lines 504. We moved it earlier lines 67-71 as suggested as follows*: ' In aquatic environments, typical P-diesters identified are nucleotides, nucleic acids, and phospholipids coming from microorganism's intracellular material (Karl and Bjorkman, 2015), but the methodology used to estimate the P-diester pool (using also a commercially purified phosphodiesterase enzyme (Monbet et al., 2007; Yamaguchi et al., 2019)) does not allow to determine the in-situ P-diesters chemical composition in detail'*

Line 408-412 : The difficult comparison with previous studies also comes from the different substrates used, MUF- derivates (Thomson et al. 2020, Sato et al. 2013) vs paranitrophenyl-derivatives (Huang et al. 2022), corresponding to different enzyme affinity. Conditions of incubation, particularly temperature may also differ between studies, optimal versus in situ temperature.

We fully agree with this comment. Part of the discussion is already dedicated to the problem of the different types of substrates used (lines 482-485 about Km, lines 505-513 about TT).

As suggested by the referee, we considered other difficulties encountered for literature comparison adding a new sentence at the end of this paragraph (lines 477-482) as: *'In addition, while some authors used MUF-derivates (Sato et al., 2013; Thomson et al., 2020), others used paranitrophenyl- derivatives (Huang et al., 2022), corresponding probably to different enzyme affinity. In addition, conditions of incubation may differ, some authors using in situ or close-to in situ temperature (Sato et al., 2013; Suzumura et al., 2012; Yamaguchi et al., 2019; Thomson et al., 2020) and others optimal temperatures (Huang et al., 2022).'*

Line 425 : Thomson et al., 2020 rather than 2019

Yes sorry for the mistake, we have corrected this reference line 498

Line 478 – 483 : discussion on Km PME >> LDOP : do the authors mean that enzymes experience locally higher substrate concentrations due to intermittent and patchy distribution of organic Phosphorus ? Could the authors explain it more precisely

The sentence was modified lines 589-602 as: *'Possibly intermittent sources and patchiness of L_{DOP} composition and concentration could explain high Km relative to L_{DOP} so that microorganisms maximize their PME activities at high L_{DOP} concentrations. Patchiness is the consequence of the organic matter continuum of size with different molecular composition from low molecular weight to high molecular weight (Young and Ingall, 2010). Patchiness is provoked for instance, during the passage of sedimenting particles with their associated plumes (Kiørbe et al., 2001, phases of intense lysis of cells, egestion of food vacuoles by grazers (Nagata and Kirchman, 1992), or hydrolysis of particulate detritus. In addition, since most PME comes from intracellular or periplasm of cells (Luo et al., 2009), they are probably adapted to higher concentrations of DOP than that estimated by the bulk DOP measurement.'*

Figure 7 : The frame around the legend on the Km versus DIP graph could be removed

This has been done, as well as for Fig S2.

New references added in the ms:

Karl, D.M., and Björkman, K.M.: Dynamics of Dissolved Organic Phosphorus. In: Hansell D.A., and Carlson, C.A. (eds.) Biogeochemistry of Marine Dissolved Organic Matter, pp. 233-334. Burlington: Academic Press, 2015

Kiørboe, T., Ploug, H., and Thygesen, U.H.: Fluid motion and solute distribution around sinking aggregates. I. Small-scale fluxes and heterogeneity of nutrients in the pelagic environment. Mar. Ecol. Prog. Ser 211, 1-13, 2001.

Nagata, T., and Kirchman, D.L.: Release of dissolved organic matter by heterotrophic protozoa: implications for the microbial food webs. Arch. Hydrobiol. Beih. Ergebn. Limnol. 35, 99-109, 1992.

Urvoy, M., Labry, C., Delmas, D., Layla, C., and L'Helguen, S.: Microbial enzymatic assays in environmental water samples: impact of Inner Filter Effect and substrate

concentrations. Limnology and Oceanography: Methods 18, 725-738. https://doi.org/10.1002/lom3.10398, 2020.

Young, C.L., and Ingall, E.D.: Marine Dissolved Organic Phosphorus Composition: Insights from Samples Recovered Using Combined Electrodialysis/Reverse Osmosis. Aquat Geochem 16, 563-574. https://doi.org/10.1007/s10498-009-9087-y, 2010.