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Biogeosciences
Discussions

Interactive comment on "Spatial patterns of biphasic ectoenzymatic kinetics related to biogeochemical properties in the Mediterranean Sea" *by* France Van Wambeke et al.

Anonymous Referee #1

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General comments: This article refers on the enzyme activity rates along the epipelagic and mesopelagic layers of a Mediterranean area, taking into consideration the variability of their kinetic properties in relation to the addition of different amounts of fluorogenic substrates. Particularly, two ranges are compared (0.025-1 μM with respect to 0.025-50 μM) to assess the degree of affinity between the enzyme and its substrate (in terms of Km Michaelis Menten constant). I think that the subject of this manuscript falls within the aims of Biogeosciences, This paper presents a large dataset of enzyme kinetics in different regions of the Mediterranean; their interpretations and conclusions are quite good, the supplied figures are explicative and the list of references is consistent, however the English language at some points is not very clear and could be

improved. Moreover, the structure of the Introduction could be improved as suggested in the Specific comments. However, the major doubt I have regarding its acceptance is that the Special Issue Atmospheric deposition in the low-nutrient-low-chlorophyll (LNLC) ocean- where it has been submitted - has a focus different from the Special Issue main theme and the subject of this manuscript (enzyme expression and its variability depending on the added substrate concentrations) refers on the effects of the atmospheric deposition or aerosol on marine processes in 4 lines of the discussion only (from 714 to 717). Therefore in my opinion this represent a too short and insufficient discussion to justify the inclusion of this manuscript in the Special Issue above reported.

Specific comments: line 20, the definition of the depth range of epi- and meso-pelagic layers should be indicated; line 23, and throughout the text, I suggest to refer to low and high affinity enzymes (instead of affinity systems); lines 28-29, please check this sentence, it remains without a conclusion (probably "although" should be deleted); lines 32 and successive: current and past interpretation of the three tested enzymes and their relative differences regarding the choice of added....; line 35. I suggest to change into: were different depending on activity estimates were derived from high or low concentrations of substrates were used; line 42, is a subject ; line 51, for the enzyme kinetics (instead of concentration kinetic), the minimum substrate concentration.

The Introduction should be re-organized, since on line 60 the authors talk about multiphasic kinetics, and they again return on the concept of biphasic kinetic on line 69 and also on lines 76-77, in a logical order it would be better to move lines 69 and 76 close to line 60 to discuss about multiphasic kinetics Again, sentence on lines 88-90 could be moved close to lines 80-83 (both talking about the enzyme assay); line 59, within a single species; line 81, prior incubation of the sample with the substrate; line 92, especially regarding the origin; line 97, interaction among different enzymes; line 100, since in the Abstract the acronyms of the enzymes are reported, please use them for aminopeptidase and phosphatase; line 104, the kinetics of three enzymes targeting;

line 118, in this manuscript (in full); line 130, epipelagic (0-250), what reference do the authors used to select these depth ranges? please include it; line 142, remove comma after rosette; lines 182, 186, 324 , NO3 (3 in small character) line 182,Within epipelagic, nutrient (DIP and NO3) depleted layers; line 182, LWCC in full (liquid waveguide capillary cells); line 202, Two replicates per each TCHO sample; line 214, acetonitrile as solvent B was used; line 250, The amounts of the products MCA and MUF, released by LAP.....; line 255, were produced instead of dispatched; line 260, The hydrolysis rate was calculated; line 279, Vm1 and Km1; line 290 correlations among variables; line 290, Results are reported as means $\pm$ standard errors; line 296, remove (the) , changing seasonal pattern; line 308, 38.48) were different from the EMDW water mass being less salty and colder; line 310, please include a reference for the identification of water masses (according to their physical-chemical properties); line 327, The depth of dcm (use acronym); line 333, the mean values of DOC/DON, line 334, the mean values of TAA; line 337, decreased from epipelagic to deeper waters; line 338, contribution of TAA-N to DON which ranged from 3 to 9% at surf and dcm and from 1.6 to 4.6% at liw and mdw respectively; line 342, according to; line 344, varied among the stations; line 345, the highest values were measured; line 352, were determined over highly variable trophic conditions; line 357, the finding that measurements at liw and mdw layers for GLU were below detection limits; line 365, please indicate how the CV coefficient of variation was calculated(mean/st. dev x100); line 370 AP Vm50 and Vm1 mean values; line 382, variable patterns (instead of inconsistent); line 390, their differences between two sets of concentrations (Vm50 and Vm1) were; line 394, with saturation rates occurring around 1 μM of added MUF-P (this repeats line 355); line 403, about the declining trend; line 411, Km 50/Km1 ratio (Fig.6b) decreased with depth, so enzyme affinity for the substrate increased (please check this sentence); line 417, the turnover times were the shortest and the longest; line 439, the mean values (instead of medians); line 440 and in Tab. 3, gC cell (instead of bact) ; Tab. 2 caption: Microbial abundances, activity rates and kinetics measured at the 4 layers first row, surface, dcm. liw. mdw (remove layers and waters); Tab.3 caption: in deep waters (liw and mdw , per BP/LAP

in nmolAA/nmolN (not C) and per BP/AP in nmolP/nmol P (not C); Tab. 4, first row: surface, dcm, liw, mdw (remove layers and waters); Fig. 8, per cell V50 or V1 GLU?; Fig. S1, include labels for a and b; lines 442-443,please check this sentence: while the specific activities per unit cell decreased with depth, the activity per unit BP increased (but from 46-136 they change into 1-14), since in Figure 8 a decrease with depth instead of an increase is shown ; line 444, according to stations; line 449, increased with depth (include reference to Fig.8) ; A better title for Paragraph 3.5 should be Hydrolysis versus carbon/nitrogen demand; line 452 higher than TAA concentrations (Table 2, Table S1); line 485, the differences between these two LAP systems could reach the differences?.(explain better this statement); lines 478 and 489, the difference between the two concentration sets of substrates (not two types of enzymes); It sounds very strange that the <0.2 micron fraction exceeded the total enzyme activity! how do the authors explain this strange result? line 508, expression of enzyme activities instead of development; lines 507-509, re-write or delete this sentence, it is unclear; line 529, This increases the importance of Km1 at low concentrations of substrate (is it right the meaning? just to avoid to repeat the risk of overestimation, already written in line 530); line 530, difference in the response; please check the references: Siokou-Frangou et al. 2010 (not included in the reference list), Zaccone et al. (2010) not cited in the text; line 555, remove for these authors; line 560, These authors suggested; line 604 (Limit of detection, not acronym); line 661 LLBN? please report in full; rewrite sentence on line 675; the direct influence on the determination of Km and Vm of the use of an appropriate set...; lines 679,680, 682, 683, 694 instead of affinity system I suggest affinity enzyme; line 691 Lemée 2002 or 2012?; line 704, supported by peptides and polysaccharides hydrolysed by enzyme activities; line 705, please report BGE in full (no acronym has been reported before)

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