Response to Christine Klass "Spatial patterns of biphasic ectoenzymatic kinetics related to biogeochemical properties in the Mediterranean Sea" by France Van Wambeke et al. ms BG-2020-253

Dear author,
The revised manuscript has tackled most of the main comments from previous versions in a convincing manner. There are still a few minor issues that need addressing. See comments below and annotated manuscript. The annotated manuscript also contains suggestion for improvement of the text which needs a thorough revision. I would urge you to take these comments into consideration (this has not always been the case in the last submitted version). I would accept publication of your manuscript pending these minor revisions.
Sincerely,
Christine Klaas

We would like to thank Dr Klass for her thoughtful comments and recommendations, which we address below in blue. We also took the recommendations from the annotated pdf into account in the revised version of our manuscript.

Comments (further comments are given in the attached annotated manuscript):

Line 381-382: referring to figure 4, which only shows one example for the argument is not sufficient.
We guess that there is a misunderstanding here, as the example is shown in Figure 3. On these lines, we refer to Figure 4, which shows the plots Vm_{50} vs Vm_{all} and Vm_1 vs Vm_{all} (and the same for Km) for the 3 enzymes. We also modified this paragraph (see below).

Similarly, in the following statements (whole paragraph, lines 382-388) you discuss the individual model parameters and their se, but in Table S2 present average values for each layer over several stations instead of the values discussed. Please show the actual data discussed here. I suggest that in supplement Table S2, instead of statistical data on average parameters for each layer, which is already partially shown in Table 2, you provide the individual Km1, Vm1, Km50, Vm50 and Kmall, Vmall with their uncertainties as estimated for each station and layer.
We understand this editor concern. However, showing all values will require a huge table including 10 stations, 4 layers, 3 enzymes, 3 models and 2 kinetic constants (and their errors). We chose to make the table simpler as the whole data set is available upon request in the INSU/CNRS LEFE CYBER database, as indicated in the data availability section of the ms. Nevertheless, for more clarity, this paragraph (lines: 528-534 of the marked-up revised version) was rephrased to better fit with Table S2, it now reads:
'For LAP and βGLU, Vm_{all} and Vm_{50} were close, and the distribution of these data fitted to the 1:1 axis (Fig. 4). For LAP and AP, Vm_{50} were subjected to higher errors than those of their corresponding Vm_{all} (Fig. 4), as the percentage of standard error (se%; Table S2) of Vm_{50} was higher than that of Vm_1 in most cases (40/40 for LAP, 24/25 for AP). At the opposite, for βGLU se% was higher only in 6 out of 20 cases. The relationships between Km_{50} and Km_{all} showed the same trend, although Km_{50} were generally slightly higher than their corresponding Km_{all}, in particular for βGLU. As noted for Vm, the se% was higher for Km_{50} than for Km_1 in most of the cases for LAP (39/40) and AP (25/25) and the opposite was seen for βGLU (5/20).'

I have the same comment for the next paragraph (lines 389-400), please show the individual values for the biphasic indicator in a figure or in the supplement Table 2.
We agree with this comment. We added 3 lines to the Table S2 (one per enzyme), to show the range of biphasic indicator for each layer.

In the text we developed the descriptions of biphasic indicators as follows (lines 598-604 of the marked-up revised version):
'The biphasic indicator was particularly marked for βGLU (means of 87 in SURF and 47 in DCM layers), but it was highly variable (Table S2). For LAP the mean indicator increased from ~9 in SURF and DCM layers to ~16 within LIW and MDW layers, however due to its high variability (Table S2) this increase was insignificant. For AP the biphasic indicator remained constant ($p > 0.05$) between the epipelagic layers (mean of 12 in SURF and of 6 in the DCM) and the deeper layers (mean of 5 in LIW and 9 in the MDW), with overall lower variability than for the 2 other enzymes, Table S2).'

p.16 includes a detailed discussion on Vm ratios between different enzymes, yet the data is presented nowhere. It would be helpful to include a figure similar to Fig. 9.
We agree with the Editor. A new figure showing AP/LAP and LAP/βGLU enzymatic ratios was added in the supplement material (please see Fig. S4).

Discussion lines 729-731 and Fig 11: It seems odd when you have BP data measured directly to include a 10% conversion efficiency factor. The BP is the actual carbon demand. The 10% factor would only make sense if you want to concert BP into growth rates or biomass accumulation. This needs to be corrected
We do not fully agree with this comment. Bacterial carbon demand (BCD) is the sum of bacterial respiration and bacterial production (BCD=BP+BR), and BCD=BP/BGE, BGE being the bacterial growth efficiency. As we don't know the fate of carbon in amino acids or carbohydrates after their uptake inside the cells (anabolism or catabolism), we need to compare the carbon sources issued from LAP and BGLU hydrolysis to BCD and not to BP. For the N demand, as we stated, if we assume no N excretion, heterotrophic bacterial N demand can be directly calculated from BP using the C/N ratio of 5.
As in the Piontek et al. (2014) study the contribution of hydrolysis was only was related to BP, therefore we used our value of BGE 10% to estimate a contribution of hydrolysis to BCD, in order to be able to compare the data of this study with our data set.

in Figures 2, 5-8 and 10-11 please use capital to indicate layers (SURF, DCM, LIW, MDW) as in the text.
Done

Figure 5b: x axis legends are missing
Corrected

Figure 9: in plot legends replace "per cell Vmall etc..." with "specific" or "cell specific Vmall " or Vm1, respectively. Or find a different abbreviation for cell specific Vm. (see annotated manuscript).
We introduced the abbreviation cs as suggested, in Fig. 9 and Fig 8.

Supplement Table S1: replace ld with "<dl" and explain the abbreviation in the legend: "<ld: below detection limit"
Done

Change legend Supplement Figure S2 to: "Nonlinear least squares regression fits of Michaelis-Menten kinetics for different and incremental ranges of substrate concentrations from 0.25 corresponding to a 0.025-0.25µM substrate concentration set to 50 corresponding to a 0.025-50µM substrate concentration set for a) LAP, b) βGLU and c) AP. Red dots correspond to the field measurements. The dataset is the same as in Figure 3 (DCM at station FAST). d, e f : corresponding distribution of the Vm and Km parameters calculated according to the different concentrations ranges tested."
Done

Supplement Figure S2: It would be helpful if the colors for the dataset used in panels a, b, c (0.25,05, 1 etc...) were used for the corresponding Vm and Km values in panels d,e,f.
The color codes of the plots presented in a, b, c correspond to the different concentrations indicated by the x axis in d, e, f. To be clearer, the last part of the legend was modified as: 'd, e f : corresponding distribution of the V and Km parameters plotted according to the maximum concentration added.'

Please find below the responses to some of your comments in the annotated version of the ms

Lines 438 : '…AP was the enzyme for which Vm_1 and Vm_{all} were the closest (average of Vm_{all} /Vm_1 ratio for the whole data set was 1.9 ± 1.2) (Fig. 7a), confirming that saturation rates occurred with 1 µM MUF-P addition … ' I do not understand how the values of V are an indicator for the level at which the rates saturate. If anything the argument should be based on the Km value or the slope (dV/dS) of the kinetic response
The sentence was reformulated (lines 634-637 of the marked-up revised version) as:
'AP was the enzyme for which Vm_1 and Vm_{all} were the closest (average of Vm_{all} /Vm_1 ratio for the whole data set was 1.9 ± 1.2) (Fig. 4c, 7a). Fits to model 50, using 2.5 to 50 µM concentration sets were often not significant (Table S2), because the rates stayed constant when adding these concentrations.'

Line 454 about turnovertime. Can you please explain were this this concept comes from (reference?). I understand the term turnover number (but to estimate it you also need to know the enzyme concentration).
The turnover time is defined by the ratio Km/Vm (conversely, Vm/Km defines turnover rate) and it has often been described and cited in different studies (e.g. Tholosan et al, 1999, Van Wambeke et al., 2002; Crottereau and Delmas, 1998, Unanue et al 1999; Misic et al., 2002). These ratios are used to estimate the ability of ectoenzymatic systems to be competitive at a low substrate concentration and were initially described in studies about monomer uptake or growth (Healy et al., 1980; Wright and Hobbie, 1966). We added a sentence in M&M section (line 362 in the marked-up revised version) as follows: 'The turnovertime was estimated as the ratio Km/Vm (Wright and Hobbie, 1966).'

Lines 712-716 about bacterial carbon demand and growth efficiency. 'Again clumsy and unnecessary'
These lines are not a repetition of results. In the results we describe in situ hydrolysis rates (section 3.5), but not nitrogen demand or bacterial carbon demand which are discussed here (paragraph starting line 1294 in the marked-up revised version).

Line 734 '…..as some cyanobacteria can also express LAP….' : what is the point here.

Part of the activity could be due to photosynthetic organisms, not only hprok, thus the TAA hydrolysis flux is probably not only devoted to heterotrophic bacterial N demand
The sentence was modified (lines 1387-1390 in the marked-up revised version) as:
'In our study, the contribution of TAA hydrolysis to bacterial N demand was higher in the DCM than in the SURF (10 to 40% based on the high affinity enzyme). Nevertheless, this calculation may be biased as not only heterotrophic microorganisms but also autotrophic cyanobacteria such as *Synechococcus* and *Prochlorococcus*, which are dominating phytoplankton groups in the Mediterranean Sea (Siokou-Frangou et al., 2010), can also express LAP (Martinez and Azam, 1993) to satisfy their N requirement.'

References not cited in the ms

Healey, F. P.: Slope of the Monod equation as an Indicator of Advantage in Nutrient Competition, Microb. Ecol., 5, 281-286, 1980.