Biogeosciences Discussions

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Comment on bg-2022-188

Anonymous Referee #2

Referee comment on "Extracellular enzyme activity in the coastal upwelling system off Peru: a mesocosm experiment" by Kristian Spilling et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-188-RC2, 2022

Spilling et al. report a series of measurements from highly complex mesocosm experiments, in which water was initially filled into multiple mesocosms, supplemented part way through with other water, and followed for a considerable time period. Doing fieldwork – especially with mesocosms – is a difficult and often frustrating task, since there is inherent variability in natural waters, and often one (or more) mesocosms will go their own way, despite scientists' efforts to have multiple replicates of specific treatments. From this perspective, it is understandable that Spilling and colleagues have a somewhat messy data set in which multiple parameters were measured from many mesocosms throughout a time series; figuring out what story the data are telling one is not an easy task.

However, the authors really need to spend a bit more time with their data in order to understand how the pieces do – and don't – fit together, and above all, in order to make the readers' journey through the manuscript as straightforward as possible. In the current version, essential information (e.g., depths from which the water for various analyses was collected) is missing or hard to find, and after reading the Methods and the Results, the reader is left confused, instead of having a general roadmap as to which parameters are being compared and how the treatments may or may not make a difference. For example, from just looking at the figures, it appears that the red and blue mesocosms (low and very low OMZ water addition) do not differ from one another in a systematic manner – sometimes the M4 mesocosm is an outlier, but not always; sometimes the M1 mesocosm might be an outlier. Does the main story lie then in the time course of evolution of these different mesocosms? Reading Bach et al leaves the impression that there might be a story in this direction; certainly the differences between the low and very low OMZ water addition does not seem to show robust differences.

Specific comments:

The abstract does not follow a clear line – it reads like a listing of observations/parameters. The authors need to portray a more coherent overview. As an example, the first sentence of the abstract discusses climate change – if this point is not carried through the manuscript, alter the introduction of the abstract so that it points the reader in the direction that does.

The explanation of the setup of the mesocosms and introduction of deep water was extremely confusing. Why was water exchanged in the mesocosms?

The authors should also discuss the effects of the introduction of brine on the microbial community. From reading Bach et al 2020, it seemed to be a very strong brine solution, so the activity/composition of organisms in this lower part of the water column was probably considerably affected. Much of the brine addition part of the manuscript (lines 117-124) was only really understandable after reading Bach et al 202; this part of the manuscript should be re-worked so that the main points are clear without reading the other manuscript for details.

Note also that any data that are re-used from Bach et al 2020 (at least some of the nutrient data?) should be noted in the methods.

It is very difficult to figure out which part of the mesocosm was measured – where did the water come from (which depths) for each analysis? This information is unclear for measurements of nutrients, FDOM, flow cytometry, chl a, and L-MCA measurements. Only the sequencing description also explicitly includes this information (line 199).

Detailed comments

Line 23: "….extracellular enzyme production of leucine aminopeptidase…" (measured activities, not production of the enzyme; reword)

Line 30: note that LAP does not degrade amino acids; it hydrolyzes terminal amino acids from larger units (the N-terminus of peptides or proteins). The amino acids themselves are degraded by other enzymes.

Line 64: organisms are productive, surface layers are not. Reword "the productive surface layer is driven by recycled production."

Line 71: wording such as "two of the most studied ones" requires references as examples, for example ' (e.g. author 1, year; authors et al. , year)'

Line 73: note that the Leu-MCA substrate integrates the activities of a wide range of peptidases (Steen et al. 2015, Substrate specificity of aquatic extracellular peptidases assessed by competitive inhibition assays using synthetic substrates Aquatic Microb Ecol 75:271. In any case, there are also a wide range of leucine aminopeptidase enzymes, so it is not 'a' protein degrading enzyme.

Lines 108-116: explain explicitly why the deep water was put in on days 11. The entire water exchange section is very confusing; rewrite or put in a figure as a supplement to guide the reader as to which water had what characteristics

Line 204: wording: what is meant by 'properly' homogenizing a sample?

Line 252: How much seawater was added to each replicate, compared to the L-AMC solution? How many replicates were measured? What was the maximum time of incubation (only minimum is given)? Was fluorescence of killed controls subtracted from the live incubations?

From what depth was the water used to measure L-AMC activity? How much time elapsed between sample collection and measurements of enzyme activities?

Line 261: Where was the 20 ml subsamples obtained? What depth was this water collected at?

Line 273: what are the two sample treatments (at this point in the manuscript, the differences are not clear)

Line 296: It is not clear from which depths the measurements were made, so the effects of the addition of the deep water are a bit confusing.

Line 326: at what depth was chl a measured?

Line 343: Are any sequences available for the initial mesocosm, or for water outside the mesocosms? Why were these particular time points selected for sequencing?

Line 366: What is the difference between deep water and OMZ water? The varying terminology is confusing.

Note that a rate of 359 nmol L-1 h-1 is not low – it is far higher than most rates reported in the literature for water column measurements of LAP.

Line 369: what is the rationale for plotting 'cumulative LAP' activities? Presumably if rates had been measured at even more time points, then the cumulative LAP rates would have been even higher, but it is difficult to understand the biological or biochemical rationale for summing the rates in this fashion.

Line 382 – line 390: use of statistics in this manner leaves the reader with the impression that the authors have run out of ideas. For example, the LAP activities are extraordinarily high. The statistical link with the bacterial community and biogeochemical variables is ok, but what underlying biological explanation would the authors like to put forward? This point would be far more interesting than just the statistics.

Line 405: the authors state they wish to "relate the biogeochemical and microbial community to the extracellular enzyme activity and a more detailed description of the temporal development and biomass comparison of microbial groups will be presented elsewhere in this special issue (e.g. Bach et al., 2020)". Bach et al discuss the phytoplankton, but the bacterial data could use some discussion, since presumably they are also the source of the LAP enzymes.

Line 449: first mention of integrated 0-10m sampling? Should have been easy to find in the Methods.

Line 472: what was the incubation temperature of the LAP samples? How long was the interval between water sampling and measurement?

The final part of the discussion contains considerable repetition.

Fig 1 caption: what is the difference between deep water and OMZ water? In addition, should note that the Pacific water was measured from water outside the mesocosms (as explained in Bach et al, but this term should be explained here, the reader shouldn't have to continually refer to Bach et al.)

Fig 2 caption: see above with respect to Pacific water measurements

Fig 5 is extremely hard to read. Maybe try a bubble plot, or group the colors in a non-random manner to make it easier to determine which sequences are which colors (use perhaps patterns on the colors to distinguish them)

Figs 6 and 7: cumulative enzyme activities are meaningless (they depend on the frequency of measurement), so these panels should be deleted.