Response to comments from reviewer #1

The authors present data from a detailed oceanographic survey in the Eastern Tropical North Atlantic, along a transect crossing a cyclonic eddy (CE). It is a highly valuable piece of data that contribute to better understand the impact of CE on microbial metabolism. Yet, the spatial resolution for some of the biological variables is limited (primary production, bacterial production and community respiration) and some of the metabolic rates were estimated (bacterial respiration) or measured at lower temperature than in situ temperature (bacterial production and community respiration), which may be distorting the relationship among variables. It is very difficult to understand why bacterial production or respiration could not be incubated at in situ, while Pp was incubated at in situ temperature. This is a major drawback, as the method used for BP estimation actually provides exactly the same BP at 22ºC than at 14ºC, which seems rather unlikely, at least in the absence of resource limitation. This definitely requires further explanation, or even using a different model for BP estimates. Therefore, the manuscript need a major revision to clarify and, eventually, reanalyze the results. The discussion should also be accordingly revised, and avoid repeating results or speculative statements. Also, the stations should be clearly identified in all the figures, and some figures should be revised. The English usage should be also carefully revised.

Thank you for the thorough review and support of our manuscript. We agree with the reviewer that it is favorable to incubate at in-situ temperatures. For technical constraints and due to the tight sampling schedule during the cruise, we have not been able to conduct the respiration measurements at in-situ temperatures; this would have required adaptation to a large number of different temperatures. In particular within the eddy vertical temperature profiles were very variable (Fig. 1). For example, the CTD was at times deployed every few hours, but incubation times were 36 h for CR measurements with the optodes. Additionally, only one incubator (fridge) with a temperature range between 4 and 16 °C was available to us. Bacterial processes are more susceptible to temperature than primary production. In order to obtain comparable results for BP, we incubated the samples at the same temperature as the samples for CR. The well-known relationship between temperature and these rates were then used to correct for the temperature difference.

Thank you for pointing us to the unrealistic BP rates after the temperature correction. We identified a mistake in the calculations and have corrected BP and BGE estimates. Temperature-corrected values for BP at in-situ temperature (22 °C) changed by a factor of 1.9 and are thus now 1.9 times higher than the values based on the incubations at 14 °C using the equation of López-Urrutia and Morán (2007). We will change the text, Figures, and Tables accordingly.

We will revise the entire manuscript to avoid simple mistakes and to use a more standard English.

Specific comments:

Title: I suggest changing the title, as the authors do not provide growth data and also the term "accelerates" is rather confusing. Moreover, primary production appears to be enhanced in a frontal zone, not in the CE, and this should be clear already in the title.

We agree with the referee that measured rates were particularly high at the eddy front. However, we would like to keep the title a bit more general, as future studies need to demonstrate whether frontogenesis is indeed the cause for enhancing PP. We will change the title to: "Eddy-enhanced primary production sustains microbial activities in the Eastern Tropical North Atlantic" This will include effects of the eddy on frontogenesis.

Abstract:

Line 19: revise "Mauretania" throughout the text and change to "Mauritania".

We will make the correction according to the referee's suggestion.

Line 21: revise the use of the term "cascading", which implies a temporal dimension, that has not been adequately addressed here.

We will change the term to "coupled".

Line 26: revise the use of the term parameter, which is not equivalent to the term variable. As an example, chl-a concentration is not a parameter.

We will use the term "variables" instead of "parameters"

Lines 25-27: please be more specific, and clearly indicate that the maximum concentration of phytoplankton occurred in the frontal zone.

The maximum Chl-a concentration was measured within the boundary of the eddy, while, for example, PP was highest in the frontal zone (see Fig. 2). The chosen interpolation between data points might have been misleading and will be slightly adjusted to make that clearer.

Line 36: indicate to what this percentage is referred to.

The percentage refers to % of PP_{DOC} and we will indicate this.

Lines 36-37: I do not think that PP/BCD reflects the metabolic state of the microbial community. I suggest either using PP/CR as an estimation of the metabolic state of the microbial plankton community, or use BCD/PP as indication of the fraction of PP production that is processed by bacteria. Please be specific. A PP/BCD>1 does not necessarily imply an autotrophic balance.

We thank the reviewer for this valuable comment. We will follow the suggestion to use PP/CR as an estimation of the metabolic state of the microbial plankton community.

Introduction:

Line 90: indicate that you refer to bacterial biomass production.

We will make the correction according to the referee's suggestion.

Line 92: provide more recent references for the effect of DOM on BGE.

We will make the correction according to the referee's suggestion.

Line 100: provide references for BR on eddies.

We will add references for BR.

Lines 105-107: this part is somehow repetitive with information in lines 79-82. Please revise and avoid repetition.

Lines 79-82 will be merged with those in lines 105-107 of the original manscript to avoid repetition. Proposed new text:

"Yet, insight into the distribution of phytoplankton and their activities within mesoscale eddies is limited due to a lack of sufficient fine-scale vertical and horizontal resolution studies to adequately describe these distributions."

Lines 109-111: this part is also repetitive with that in lines 69-71. Please revise.

Lines 69-71 will be removed to avoid repetition.

Line 116: please specify the spatial resolution of the study.

We will specify the spatial resolution of the study:

"We studied the impact of a CE on microbial carbon cycling along a 900 km zonal corridor"

Materials and methods:

Lines 133-134: please clarify what you mean by "consecutive optimized identification of the eddy".

We will change the text to:

"[…], which made it difficult to identify the center of the eddy and required rerouting of the ship's track during the survey."

Lines 141-144: please provide also information about the temporal sequencing of the survey. A supplementary table indicating the sampling sate of each stations would be nice.

The sampling date and time will be added to Supplementary Table 1.

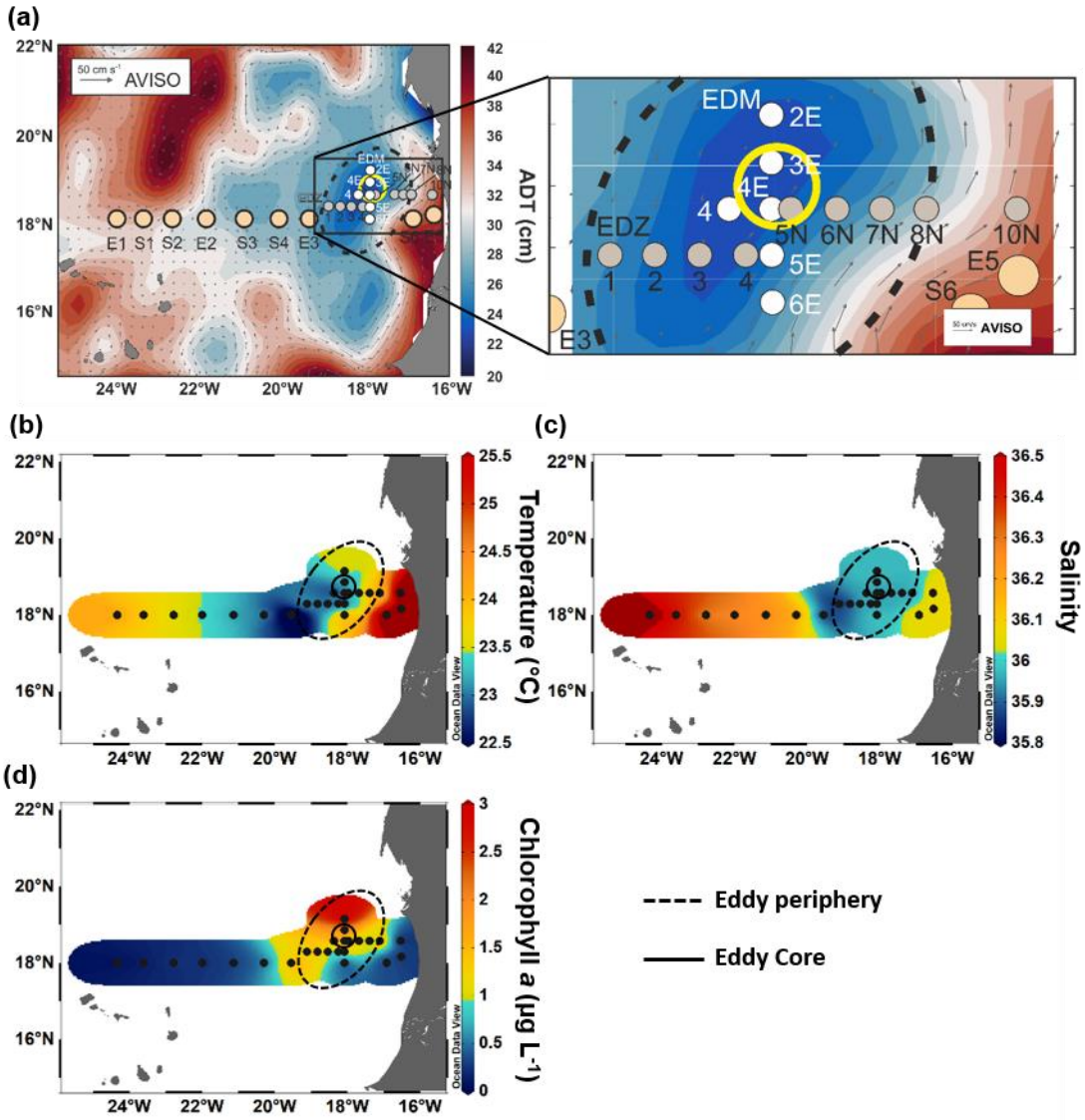
Lines 158-162: please re-write for clarity and English usage.

We will revise the sentence to read:

"Just beyond of the eddy periphery, at St. E3, a front was observed with surface temperature and salinity (not compensated by density) clearly different from the adjacent stations (Fig. 1b)."

Figure 1: The cruise track is not visible in the figure. Else, the positions of the stations in the CE are not fully visible, I suggest making a different graph for the stations within the CE. Finally, increase the suze of the symbols in plots b, c and d.

We will revise the caption by replacing "Cruise track" with "Sampling stations" and add a zoom-in showing the stations within the eddy. The size of symbols will be increased in panels (b), (c) and (d). Please see below for a draft of these changes.



Line178: nitrate and nitrite lack the symbol of the charge

Will be corrected.

Lines 179-181: please provide a reference for this statement.

We will add the following reference:

Carlson, C. A.: Production and Removal Processes. Chapter 4 in Biogeochemistry of Marine Dissolved Organic Matter, Editor(s): Hansell D. A., Carlson, C. A. AP, 805, 91–151. https://doi.org/10.1016/b978-012323841-2/50006-3. 2002.

Lines 187 and 196: please clarify if you measured dAA or dHAA.

We measured dHAA and will add this information accordingly.

Lines218-221: this is not equivalent to autotrophic plankton biomass, as it is including only pico and nanoplankton. Please use a term that clearly states this to avoid any possible confusion.

We will change the term to "autotrophic pico-and nanoplankton biomass".

Line 227: two duplicate samples and only one killed control is not really sufficient to get accurate estimates of BP.

Due to time, equipment capacity, and workload during the expedition we were only able to collect and analyse duplicates for BP. Yet, we entrust our data as the standard deviation for each sample were relatively low (see figure below for individual measurements of the duplicates).

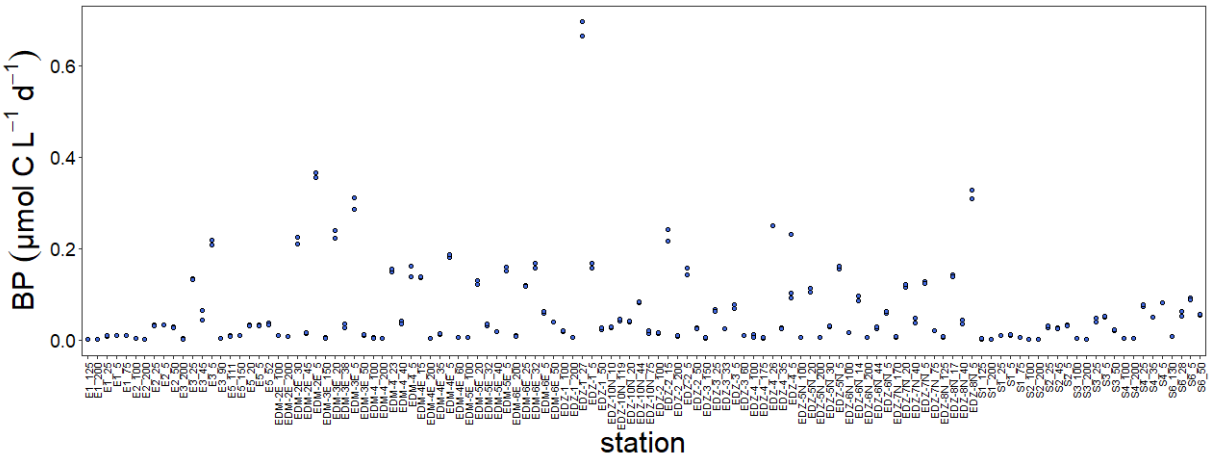


Figure: Individual data points of duplicate measurements of bacterial biomass production (BP) rates from 0-200 m depth from all samples.

Line 229: the authors should clearly explain why they did not measure BP at in situ temperature. This is a major limitation of their work, and is not sufficiently justified.

We agree with the reviewer that incubations at in situ temperature would have been favorable. However, due to technical issue, we could only measured CR in an incubator (fridge) with a temperature range of 4 to 16 °C.Both BP and CR rates are temperature dependent (e.g., López-Urrutia and Morán, 2007; Regaudie-de-Gioux et al., 2012, Yvon-Durocher et al., 2012) and in order to compare them, we decided to incubate them at the same temperature (14 °C).

Line 233: the author should consider to use a different model to estimate BP at 22ºC form estimates at 14ºC.

We thank the referee for pointing us to this. We found a mistake in our calculations which will be corrected and now provides a more realistic conversion factor of the BP rates. All results and figures will be corrected accordingly. Please see our response to your general comment for the changes in BP rates between the original and the revised version of the manuscript.

Line 236: again, the authors should justify the reason why they did not measure CR at in situ temperature. Also, they should explain why they conducted incubations > 24 h, and when. Finally, the number of replicates for CR should be also indicated.

Please see the explanation above concerning the incubation temperature.

For the incubation time, we measured the decrease of oxygen at several time points (0 h, 6 h, 12 h, 24 h and 36 h). The relatively long incubation time was chosen due to the low CR typically observed in oligotrophic water (e.g Reinthaler et al., 2006) and to be consisent throughout the cruise. The method is fully described in the supplementary information.

We will included the information that CR was measured in quadruplicate.

Lines 253-254: again, the number of replicates is too low.

While we understand that more replicates for PP rates would have been favorable, during sea-going expeditions, sample material and time is often limiting, restricting the number of replicates that can be analyzed. Our duplicate analysis showed highly similar results (see figure showing individual data points for each sample). Additionally, we have measured and published PP data from many oceanic regions with variable number of replicates and the obtained rates have always shown highly similar similar results. Thus, we believe that our PP data provide accurate estimates.

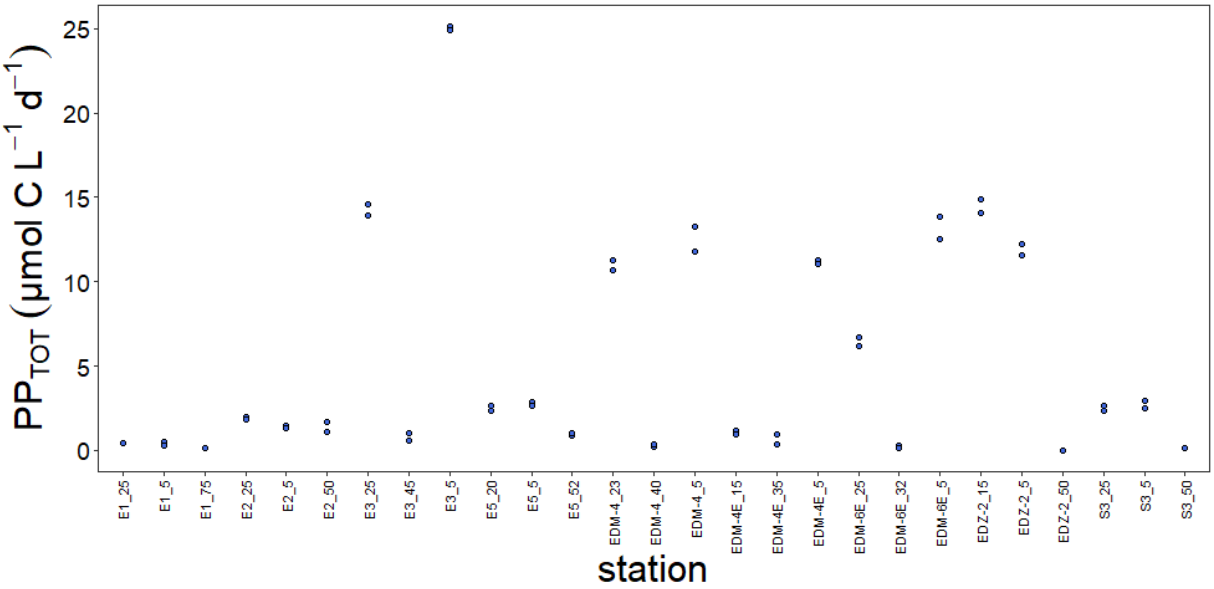


Figure: Individual data points of duplicate measurements of total primary production (PP$_{TOT}$) from all samples. When only one data point is visible, data point are overlying.

Line 254: indicate where PP incubations were done (controlled chamber?).

We will add the information that the incubations were done in an incubator.

Line 264: the author should justify the use of 0.4 instead of 0.2 microns PC filters to separate the dissolved fraction. Some bacteria can pass through 0.4 microns.

We agree with the reviewer that some bacteria might pass through 0.4 µm filters. However, filtration at 0.4 µm was initially (in the older literature) selected because it corresponds roughly to the upper limit size of viruses and the lower limit of bacteria. Comparisons of results from studies using different filter pore-sizes is highly uncertain. Thus, we decided to stick to the convetional method based on 0.4 µm filters.

Lines 269-270: please re-write for clarity.

We will revise the sentence. Proposed new text:

"To determine PP_{DOC}, 4 mL of filtrate were transferred to 20 mL scintillation vials and acidified with 100 µL 1N HCl. Scintillation vials were left open in the fume hood for 14 hours to remove inorganic carbon."

Line302-303: clarify the method of integration. Is the same as the trapezoid rule?

The midpoint rule approximates the definite integral using rectangular regions whereas the trapezoidal rule approximates the definite integral using trapezoidal approximations. We preferred to use the midpoint rule as it provides more accurate integrations especially when only three data points are used as for the 100 m integration that we did.

Results:

Figure 2: please clearly indicate in the plots the identification code of each station. Also increase the size of the dots.

We will increase the size of the dots and include all the station names.

Line 373: clarify that this is not autotrophic plankton biomass, it is only pico and nanoplankton using another term to refer to this.

We will change the term to "autotrophic pico- and nanoplankton biomass"

Lines 379-381: as stated above, it is better using a term that clearly define what this variable is, and thus, this sentence can be removed.

We will remove this sentence.

Table 1: please clarify how do you integrate down to 100 m in stations lacking samples below 50-75 m. Revise the use of the term "parameter". Why do the authors specify depths and sampling date for only some of the stations?

For all stations, samples for most parameters exist down to 200 m. The only exeptions are PP and CR. For the extrapolation, the shallowest value was extrapolated to 0 m and then the midpoint rule was applied down to 100 m. For PP only the top three depths were sampled. The fourth depth corresponded to the base of the photic zone based on Chl fluorescence profiles. This depth was extrapolated to a value of zero. The same was applied for CR. We will remove the information about the sampling depth and time point from the caption as they are disturbing here. Instead, we will provide this information in the SI Table 1 and will refer to the table in the caption of Table 1.

Table1: the differences between integrated chla- between EDZ1 and E3 are weird and not expected form what is presented in figure 3 (although in figure 3 the stations are not clearly indicated). Overall the results section is very difficult to follow due to the lack of station labels in figures.

We will add station labels to the Figures.

Figure 3: please add station labels and increase the size of dots. Rename the variable AutPI for clarity (it is only pico and nano plankton biomass).

We will make the change according to the referee's suggestion.

Line 433: clarify is you refere to integrated or volumetric BP rates

We refered to volumetric BP rates and will include this information in the revised verion.

Line 430: PP/BCD < 1, does not indicate heterotrophic balance or conditions, it just indicates that concurrent PP is not fulfilling BCD. Revise and be more specific. I suggest either using PP/CR as an estimation of the metabolic state of the microbial plankton community, or use BCD/PP as indication of the fraction of PP production that is processed by bacteria.

We will make the correction according to the referee's suggestion and use PP_{TOT}/CR as an estimation of the metabolic state of the microbial plankton community.

Line 453: I suggest including this calculation (PP_{DOC}/BCD) in table 2.

We will include the suggested calculation in Table 2

Figure 4: please add station labels and increase the size of dots. Clearly state in the figure legend that BP and BR are stimates and indicate the method used for that estimation.

We will add the station labels, increase the size of the dots and change the legend to:

"CR and BP rates at in-situ temperature were estimated based on López-Urrutia and Morán (2007). BR rates were estimated from measured and temperature-corrected CR rates based on Regaudie-de-Gioux and Duarte (2012). Details are provided in the methods section and the SI."

Table 2: I suggest using PP/CR and BCD/PP as more insightful ratios than BCD/PP.

As mentioned above, we will follow the reviewer's suggestion.

Line 484: revise the usage of the term "indices" here, as it does not reflect the content of the section.

We will remove that section including the figures from the manuscript as it seems unnecessary.

Line 488: the correlation between cell-specific BR and BGE is spurious as both contain the variable BR. Please remove form the analysis.

See above.

Figure 5: I suggest representing BCD/PP, I find it more intuitive that the inverse.

We will use PP_{TOT}/CR as suggested above by the reviewer to estimate the metabolic state of the microbial plankton community.

Lines 491-493: I suggest removing also the correlation between chl-a and the biomass of pico and nanophytoplankton, as it is not necessary. It is enough indicating that the discrepancies are due to the fact that chl-a is total, and the biomass is only form small phytoplankton.

We will remove this section including the plot from the manuscript.

Figure 6: I suggest removing plot (a) (because it is spurious) and (b) (as iti is not necessary). Maybe the authors could add plots relating Chl-a vs. BP and/or BCD vs. PP_{DOC}.

See above.

Lines 513-524 and figure 7: please revise to eliminate the spurious correlations (e.g. BCD vs BR or BR; PPtot vs PPdoc). The authors could try to calculate correlation using data not affected and affected by the CE.

We will follow the referee's suggestion and make two correlation matrices using data not affected and affected by the CE (see below for proposed new figure). We will change the text accordingly and also remove the spurious correlations.

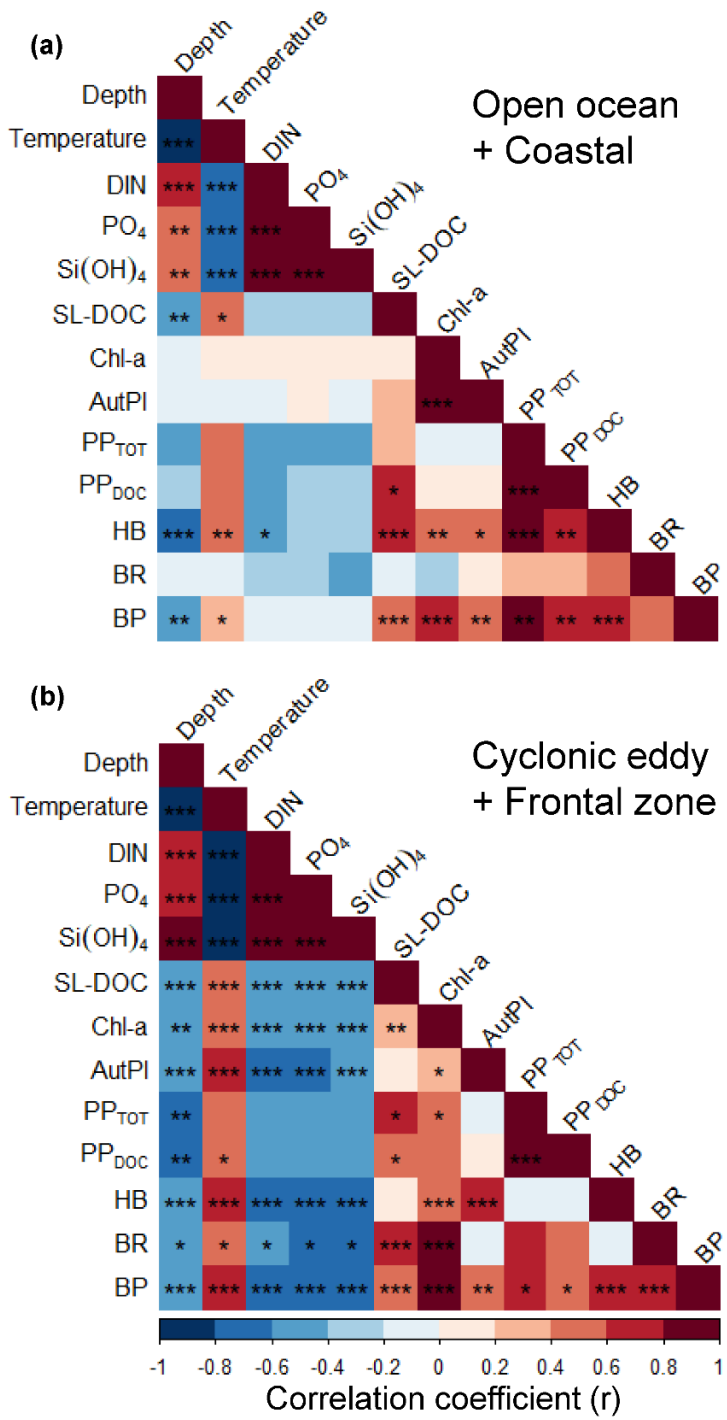


Figure: Correlations of biochemical parameters, metabolic activities, and bacterial abundance in the upper 100 m in (a), the transect excluding eddy-influenced samples, (i.e., coastal and open ocean stations) and (b) the eddy influenced samples. Statistical significance: '***'< 0.001, '**'< 0.01, '*'< 0.05.

Discussion:

Lines 545-546: this is speculative as the authors do not have data about the fraction of large phytoplankton. The relation between chl-a and biomass are also affected by factors such as photoacclimation. The authors can only guess that in more productive stations large phytoplankton is likely more relevant, but they do not have data to support that statement.

We will follow the referee's suggestion and remove the statement.

Line 553-555: this is again speculative, the authors do not have date on the contribution of small planktons, they only have total chl-a and the biomass of the small fraction, but the relation between chl-a and biomass is not straightforward. I suggest eliminating this statement

We will follow the referee's suggestion and remove the statement.

Lines 565: I suggest using an alternative to "compression", such as e.g. "uplifting".

We will make the correction according to the referee's suggestion.

Lines 567-568: revise for English usage.

We will revised this sentence to read:

"Similar uplifting of Chl-a isolines towards the surface have been reported for other eddies (Lochte and Pfannkuche 1987; Feng et al., 2007; Noyon et al., 2019) and might result from phytoplankton relocation through intense vertical mixing by strong surface winds (Feng et al., 2007; Noyon et al., 2019)."

Lines 573-575: revise the sentence, it is hard to follow the reasoning.

We will revise this sentence and integrate the statement into the previous ones. Proposed new text:

"Similar uplifting of Chl-a isolines towards the surface have been reported in other eddies (Lochte and Pfannkuche 1987; Feng et al., 2007; Noyon et al., 2019) and have been suggested to rusult from phytoplankton relocation through intense vertical mixing from strong surface winds (Feng et al., 2007; Noyon et al., 2019). Before our eddy survey, strong surface winds occurred offshore (SI Fig. S7), which might explain the high Chl-a concentration (>0.5 µg L-1) that we found at the surface (5 m) in all stations within the CE."

Lines 576-580: delete as this is mostly results.

We will delete this statement.

Lines 594-598: again very speculative. The authors do not have data about the presence of diatoms or dinoflagellates in this study. Delete or rewrite.

We will delete this statement.

Line 603: the use of the term "diversity" is not appropriate here, as the authors only provide data of a couple of functional phytoplankton groups.

We will avoid the term "diversity".

Lines 603-606: revise English usage as it is very difficult to understand the sentence.

We will revise the sentence. Proposed new text:

"Our flow cytometry data (SI Fig. S7) showed that Cyanobacteria (Synechococcus) and eukaryotic pico- and nanoplankton within the CE were unevenly distributed. This, suggest that the phytoplankton community of the CE was likely distinct from the surrounding waters, but also variable on the submesoscale within the CE. This is consistent with previous studies on phytoplankton distributions in eddies (e.g., Lochte and Pfannkuche, 1987; Lasternas et al., 2013; Hernández-Hernández et al., 2020)."

Lines 607-608: delete this sentence, the authors do not have data on phytoplankton taxonomy, only flow cytometry counts of different groups based on scatter and fluorescence.

This sentence will be deleted.

Lines 619-621: this sentence is just repeating results. Please, delete.

This sentence will be deleted.

Line 621-625: please revise English usage.

The sentence will be revised. Proposed new text:

"We emit two hypotheses regarding this distribution: 1) the lower PER was due to a higher proportion of larger phytoplankton (e.g., diatoms) which have lower turnover rates and therefore lower PER and/or 2) the upwelling of nutrients generated by the CE might have enhanced the physiological health of the phytoplankton community (Agustí and Duarte, 2013; Laternas and Agustí, 2014)."

Lines 630-631: please indicate where this correlation is found in the results, as the correlation matrix in figure 7 was calculated including all data.

We will divide original Figure 7 into two correlation matrices as suggested by the reviewer in an earlier comment and add the reference to the figure in the main text.

Lines 631-633: revise English usage. Else, it is hard to see such continuous trends in HB or PP in the figures.

We will change the sentence to:

"Along the zonal transect, in the stations not affected by the eddy (open ocean+coastal stations), a significant positive correlation between HB abundance and PP_{TOT} rates was observed (Fig. 6A)."

Line 635: delete this first sentence.

Will be deleted.

Line 636: explain the acronym CanUS.

We will explain the acronym.

Lines639-641: re-write for clarity. Again, avoid statements about phytoplankton compositions, as the authors are not reporting such data (they only have cytometric groups).

We will remove this statement.

Line 656: please town down, change “state” to “suggest”.

We will make the change according to the referee's suggestion

Lines 661-662: certainly BGEs are very low, which may be partially related to a severe underestimation of BP (see general comments and comments to the materials and methods section).

As outlined above, BP rates and BGEs will be corrected due to a mistake in the calculations.

Lines 686-688: please delete references tp the presence of diatoms and/or dinofñagellates as these data are not provided. Also town donw the statement.

We will change the sentence to:

“As stated previously, the upwelling induced by the CE and the Frontal Zone led to higher phytoplankton biomass, which was likely responsible for this increase in BP.”

Lines 689-706: all this discussion must be revised once BP estimates are clarified. Also engñish usage should be revised.

The paragraph will be revised after the correction as outlined above.

Lines 707-714: all this paragraph is about an spurious relationship. In addition, the authors do not have data on bacterial community compostion. I suggest deleting it.

We will delete this part of the discussion according to the referee’s suggestion.

Line 715: revise the usage of the term “growth” as this variable was not included in this study.

We will revise the use of the term “growth”. Proposed new text:

"Our results show that BGE is not proportional to the amount of DOM received through exudation but rather depends on the different requirements between respiration and biomass production."

Lines 720-722: revise as it is very difficult to follow the reasoning, as phytoplankton taxonomic composition is not provided in this study.

We will change the sentence to:

"Here we hypothesise that in CEs, which cross oligotrophic waters in the ETNA, BGE variability depends on both BP through phytoplankton biomass and BR through the amount and quality of SL-DOC."

Lines 732-736: revise English usage. In addition, revise statements about temporal dynamics, which does not seem to be adequately resolved in this survey.

We will change the sentences to:

"Here we showed that both autotrophy and heterotrophy can occur at the same time within a single eddy. This urges the need for more high-resolution eddy studies in order to better estimate their impact on plankton metabolic activities and carbon cycling."