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Dear Editors and reviewers,

We sincerely appreciate the time and effort that you and the reviewers have dedicated to evaluating the manuscript, titled "Picoplanktonic methane production in eutrophic surface waters", submitted for consideration in Biogeosciences.

We are delighted to receive your positive feedback and constructive suggestions, which have significantly contributed to the improvement of our work. Your insights and the reviewers' comments have been invaluable in refining the quality of the manuscript.

We have carefully revised and addressed each comment and making the necessary adjustments accordingly. The manuscript was modified at all sections to address the reviewers' comments, especially the introduction and first section of the results and discussion, which were completely changed. Certainly, we have been meticulous in ensuring the grammatical accuracy of the manuscript.

The following is a **list of changes** that were made in response to the reviewer's valuable comments:

Abstract

- The abstract has been modified regarding the exact date of methane paradox use.
- It is also note that the experiments were conducted in different phases of the primary productivity described for the study area.
- The key points have been reduced to three, since two of them conveyed the same message.

1. Introduction

- The introduction has been completely rewritten to reformulate the problem, providing a clearer understanding of the main message for the reader.
- A brief overview of the origin of the methane paradox has been described (paragraph 2).
- The coastal upwelling and why it is relevant to study CH_4 dynamics in this area have been described (paragraphs 4 and 5).

- The objective statement has been reworded (paragraph 6).

2. Material and methods

- A regional setting item has been added (section 2.1), describing relevant characteristics of the area.
- A location map of the study area has been included (Fig. 1).
- Third level subsections have been added in some sections.
- The methodology used for the calculation of the Brunt-Väisälä frequency (section 2.6.7) has been explained.
- A new analysis has been added: principal component analysis - PCA (section 2.7).

3. Results and discussion

- Hot moments have been described.
- A bar has been added to differentiate the productivity periods in Figure 3.
- To avoid confusion in the Table 2, the chlorophyll average has been eliminated, leaving only the inventories of biochemical variables.
- The scale of Figures 6 and 7 has been narrowed down.
- The results of correlations and PCA are discussed.

4. Conclusions

- Some words suggested by the reviewers were considered as being produced instead of recycled (paragraph 1).

5. References

- References have been updated to include additional relevant studies.
- The formatting of references has been standardized, omitting capital letters at the beginning of each word.

Supplementary material

- To enhance the visualize the hot moments, two figures have been added to the supplementary material (Figure S1 and S2).
- Additionally, a figure illustrating the results of the Principal Component Analysis has been included (Figure S4).

The following section provides a detailed account of the responses to each comment, observation, recommendation, and suggestion made by the reviewers.

Reviewer 1

Responses to reviewer's general comments 1

1. Reviewer's comment 1: "The writing can be improved for clarity. In some cases, the thread of the main message is lost and the contribution of a paragraph or a section of it to the main story is not clear. Additionally, some choice of words is inaccurate or incorrect"

   Thank you very much for your feedback. It is valuable to us and will contribute to more effective writing. We have addressed these concerns by revising certain paragraphs in the manuscript, meticulously checking sentence structures for grammar accuracy, condensing lengthy sentences, and engaging a native English speaker for a comprehensive review to improve consistency in the main message and clarify the contribution of each paragraph to the overall story. You will notice that the manuscript has been profoundly modified, for example the introduction was rewritten: therefore many of the original paragraphs no longer exist.

2. Reviewer's comment 2: "The methane profile in Fig. 2 does not clearly highlight temporal hotspots of oxic methane production. I am confident that oxic methane production occurs and that it plays a significant role, however, graphically, it appears as in most cases (not all) the surface methane results from the upwelling of methane-rich water. Hence, I encourage the authors to reconsider their presentation of that panel. highlighting the consumption of bottom-derived methane and the production of new methane in the surface layer. With this respect, please also review the units for methane concentration - is it nM as expected for oceanic environments and mentioned in the text, or is it µM as in Fig. 2 and Table 2?"

   We appreciate your feedback on the graph's scale and vertical resolutions, which initially led to surface accumulations appearing solely derived from bottom water CH_4 advection; our revised graphs now provide a clearer representation (Fig. S1 and S2), showcasing instances where significant surface accumulations exist independently, especially during non-upwelling periods with elevated CH_4 concentrations in the surface layer. Additionally, we have incorporated vertical profiles during some periods of substantial accumulation to illustrate the hot moment occurrences.

   Regarding the CH_4 concentration unit, it is nM; we recognize an oversight in consistently presenting it in nM. We have rectified this error in both Fig. 3 and Table 2 for accuracy.

3. Reviewer's comment 3: "In some cases, the view of the authors is somewhat too simplistic. For example, methyl phosphonates can be derived from many sources, while the authors suggest N maritimus as a sole source (see also typo in name in Fig 7). Similarly, TMA is one example of methylated amines, but there are papers discussing Mono and Di-methyl amines. Hence the discussion in the paper should present TMA as an example while stating that other similar compounds are likely present, and their degradation results in methane emission"

   Indeed, our focus on these specific compounds comes from their exclusive use in the experiment. However, we recognize the broader spectrum of methyl phosphonates and methylated amines, including various sources for methyl phosphonates beyond solely *N. maritimus* and the existence of other methylated amines such as Mono and Di-methyl amines. We appreciate your insight and have enriched the introduction and discussion to acknowledge these broader implications, emphasizing that while our study centered on these two compounds, it is crucial to recognize the likelihood of other similar compounds' presence and their potential contribution to CH_4 emissions.

4. Reviewer's comment 4: "The experimental results are in some cases confusing. Why does a fraction of the community produce more methane than the entire community (Fig. 3a)? Other questions regarding the experiments and their presentations are in the PDF"

   We appreciate your observation. While our findings strongly suggest the pivotal role of picoplankton in methane cycling, there are instances where the observed patterns remain somewhat puzzling. Our experiments primarily measured net recycling (production minus consumption), focusing on the role of the planktonic community as a whole.

   In this type of experiment, certain aspects require analysis. We acknowledge that the filtration process might act as a 'filter,' potentially influencing both the composition and quantity of microbial community. Additionally, the acclimatization process conducted before experimentation could alter the seawater composition. Particularly important is to investigate the specific role of methanotrophs, an area that warrants further exploration.

5. Reviewer's comment 5: "Font size is not uniform across the text, especially for references within the text. References are also not uniformly formatted"

   Yes, you are right, it was an oversight on our part. Thank you for noticing.

Responses to reviewer's specific comments 1

Line 11, comment: subscript for the 4 in CH_4

Answer (lines 11 to 25): it was rectified in the summary and in whole text.

Line 12, comment: I believe the first description of the phenomenon was in 1976-197.

Answer (lines 11 to 12): yes, you're correct. Our bibliographic search revealed Lamontagne et al. (1973), who first documented CH_4 concentrations in the surface layer during their cruise. However, we thought it appropriate to highlight the progress of the methane paradox in the last decade.

Line 22, comment: Metabolism or consumption - metabolization does not exists.

Answer: the word metabolization was changed to consumption in all text.

Line 53, comment: Font sizes

Answer: references to font sizes were rectified in all text.

Line 60, comment: Cyanobacteria and algae also produce CH_4 directly through photosynthesis. This should be mentioned here.

Answer (line 83 to 88): absolutely, in the new text, we highlight this autotrophic process and involved microorganisms.

Line 66, comment: And cyanobacteria. The Bizic 2020 paper you cite at the end of this sentence addresses these exclusively.

Answer (line 87): Bizic has a couple of papers in 2020 and 2021, the one you refer to is Bizic et al., 2021. Cyanobacteria were included.

Line 68, comment: Since the mechanisms of photosynthesis-associated methane production is not known I would say "directly linking photosynthesis and CH production" or "directly linking photosynthetic CO2 fixation with CH_4 production". Since 13C-CO2 was converted to 13C-CH_4.

Answer (lines 55 to 66): we rewrote that paragraph, and we eliminated the C isotope evidence, they were not conclusive for this manuscript.

Line 69 to 70, comment: Not clear - how is size of phytoplankton related - researchers have been following viral and bacterial processes so why would phytoplankton be a problem. These processes are not studied due to lack of awareness.

Answer (line 76 to 94): the entire paragraph has been changed. Please review the new paragraphs. We attempted to prevent the association of picoplankton biomass with production due to the low biomass maintained by this faction of phytoplankton.

Line 75, comment: What is the bottom depth at this location? This is very important to understand the methane flow from the sediment up.

Answer (line 106): the depth of this is 92 m.

Line 86, comment: meaning there were others?

Answer (line 121): no, there were only two substrates (MPn and TMA), the word "like" was used to refer to the substrates used. It was changes.

Line 87 to 88, comment: It appears from later in the paper that you killed the already sterile fraction (<0.22 µm) what would be the purpose of that?

Answer (lines 117 to 120): in fact, we carry out two negative controls, filtration with the nominal pore size of 0.2 µm. Sometimes passing other types of microorganisms and poisoning is usually more effective: we try to make sure to remove the uncertainty of abiotic CH_4 production and demonstrate that the CH_4 produced in our experiments is purely biological.

Line 109, comment: Normally below 0.2 µm there are viruses and very few bacteria.

Answer (line 136): exactly, that's precisely why it was utilized as a control treatment to showcase that the CH_4 production observed in our experiments originates from biological sources.

Line 260, comment: The text below shows values in nM which makes sense. Is the CH_4 concentration in the bottom water in the µM range? Is it not oxidized in the deep water due to the anoxia prevailing most of the time up to close to the surface? If the surface water CH_4 is in nM and the deep water CH_4 is in µM it may make sense to use a log scale to better highlight the hotspots.

Answer (line 293): you are right. Here there was a mistake in the digitization of the units, the correct is nM.

Line 265 to 266, comment: In the figure the values are in uM and here in nM. The nM values make more sense.

Answer (line 30): indeed, there was an error in the digitization of the units, the correct is nM.

Line 266, comment: If this is a probability value resulting from a statistical test comparing upwelling and non-upwelling seasons, then something is wrong - p should be between 0 and 1

Answer (line 302): you are right, we made a mistake when writing the numbers. This was corrected.

Line 267, comment: You have used this term also earlier in the text. It is somewhat problematic. You are probably building on the term "hotspots" however, as two words and in a temporal context (moments) it may convey a different message. I would use directly the text in the parentheses. "...during short periods of high local accumulations. of CH_4"

Answer (line 306 to 310): we acknowledge the potential confusion caused by the term usage. We intended to introduce the concept of 'hot moment', drawing on microbial dynamics, where brief periods exhibit significantly higher reaction rates compared to longer intermediate periods, considering our study's fixed point. We aim to clarify and maintain consistency in our terminology throughout the text.

Line 270, comment: I am not certain that the term eutrophic is correct as it engulfs more parameters than bloom-level chlorophyll concentrations.

Answer (line 78): this zone corresponds to an upwelling area, characterized by high productivity and substantial phytoplanktonic biomass, leading to significant primary production. While 'eutrophic' might encompass broader parameters, in this context, it's classified as eutrophic specifically in terms of phytoplanktonic biomass and primary production. We'll incorporate references supporting this characterization in our study area (e.g. Testa et al., 2018), But this term has been omitted in the presentation of results, it is only mentioned in the introduction.

Line 270, comment: What is the bottom depth of the sampling area? Looking at the CH_4 and Chl profiles, there is no clear overlap or correlation. Also I do not see a clear DCM.

Answer (line 106 and lines 316 to 318): the sampling area's depth is 92 meters. Indeed, at the surface layer, there's no observable correlation between CH_4 and Chl-a profiles. This observation aligns with the time series (climatological study) described by Farías et al., 2021, conducted in the same area. Notably, both during upwelling and non-upwelling periods, hot moments of CH_4 occur independently. We've depicted this phenomenon more clearly in Figure S1 and S2.

Line 278, comment: is this the flux calculation mentioned in the methods section? If so replace the "assume" with we calculated... and rephrase the sentence to reflect that this is a result.

Answer (lines 261 to 271 and line 310): Yes, the methodology is included in the respective section. We appreciate the suggestion.

Line 278 to 282, comment: This sentence is too long and needs to be separated. One idea per sentence. 1) This is the flux we calculated 2) Based on different reasons - we link it to bacterial and microalgal metabolism. Note that so far the second part of this sentence has not been yet justified by the results presented so far. Also, the paper by Bizic et al 2020 is missing from the microalgae citations assuming that this includes cyanobacteria and the paper from Wang et al 2022 on methylamines in the bacterial part.

Answer (line 310 to 325): it was separate and rewritten.

Line 282, comment: I also believe that the paper by Bizic 2021 (JPR) highlights the possible significance of phytoplankton as global methane producers, and should also be mentioned here.

Answer (line 322): you are right. Bizic et al., 2021 was added.

Line 288, comment: See my next comment. Accordingly, I suggest to rephrase. So far, studies suggest that in this area DMSP demethylation may contribute to CH_4 but none of the other mechanisms was were quantified in this area.

Answer (line 55 to 66): yes, indeed those lines were skewed, there is not much history of aerobic CH_4 production in upwelling areas, and particularly in those areas, separating that coming from anaerobic from aerobic methanogenesis is particularly difficult. Finally, DMS could be one of the substrates used by picoplankton (e.g. heterotrophic. bacteria). As a result, that paragraph has been rewritten to reflect that DMS is a potential source of substrate for methylotrophic methanogenesis. Only mentioned in the introduction.

Line 288, comment: While I am confident that DMSP is a source of CH_4. There are several methodological problems (i.e. lack of appropriate controls) with this particular paper as a direct proof of the uptake and conversion of DMSP to CH_4.

Answer (line 65): we cited Florez et al., 2013's work as it stands as one of the few pieces of evidence indicating a potential link between CH_4 and DMS in upwelling systems (same study area as this manuscript). Their study suggested that a portion of the CH_4 produced in their experiments originated from the added DMS. They demonstrated the transformation of ^{13}DMS into $^{13}CH_4$, yet there remains uncertainty regarding the specific mechanisms, whether it occurs assimilatively or dissimilatively, and the microbial or functional group responsible for this conversion.

Line 295, comment: Please report on the data and let the readers decide if something is curious or interesting.

Answer: thank you for your suggestion.

Line 307 to 308, comment: double citation?

Answer: it was corrected.

Line 310, comment: Where is the cycle reflected in the table Do you refer to the different average values in different periods? I would term this as such without using the word cycle which is not evident from the table.

Answer (line 356): the average of the annual cycle (or climatological annual cycle) denotes the mean values observed across different months grouped within each season. For instance, spring-summer comprises Sep, Oct, Nov, Dec, and Jan; summer-autumn includes Feb, Mar, and Apr; and autumn- winter encompasses May, Jun, Jul, and Aug. Each season reflects distinct productivity phases in the area (Testa et al., 2018). These calculations were conducted over the study period from 2018 to 2021."

Line 311, comment: What is this unit? If it is flux/production than the time unit is missing, if it is concentration than it should be per volume not per area. Do you mean to sum up an entire water column? This is the same for other parameters.

Answer (line 357): these values represent integrated values per layer, as they are integrated in a depth range, the units in this dimensional analysis were mass per unit area, per time.

Line 314, comment: This is on ta table 2: (1) Why is Chl reported only for surface layer? There is data for SSL. What is the difference Chl-a here and two lines below? Not stated. (2) How were this defined?

Answer (line 360): (1) Chlorophyll-a is reported only for the surface layer as it's the sole area where photosynthetic biomass containing pigments exists. In the sub-surface layer, chlorophyll-a either doesn't exist or presents negligible to null values due to limited light penetration. The first chlorophyll-a value represents the average concentration solely in the surface layer, showcasing variability across different productivity periods. The second chlorophyll-a value refers to the integrated concentration across the entire water column but specifically in the surface layer. However, the average chlorophyll values were eliminated to avoid confusion.

Answer (line 244 to 252): (2) CH_4 Hot moments were defined based on anomalies observed in methane concentrations, indicating a significant imbalance exceeding three times the total monthly average throughout the study period, specifically occurring in the surface layer. Further details on this definition are provided in the methodology section.

Line 317 to 318, comment: So far based on the results presented so far no connection was established between Chl, phytoplankton species and CH_4 productions. The fact that multiple species of phytoplankton produce CH_4 was already established in many previous studies and is not indicated from your data (so far).

Answer (lines 342 to 345): indeed, we haven't established a link between chlorophyll-a and CH_4 production in our current findings. Future investigations could explore potential connections between methane and fractionated chlorophyll-a or other biomass indicators, like POC abundance. Recent advancements in methane research suggest spatial patterns that might offer insights into the communication among phytoplanktonic or bacterioplanktonic entities involved. Our study corroborates previous findings, indicating the involvement of autotrophic and heterotrophic microorganisms within picoplankton (<3µm) in CH_4 production, potentially sustaining hot moments within the oxygenated surface layer.

Line 321 to 322, comment: The fact that the isotopic signatures of phytoplankton are different than other source is clear. However, the results presented in this paper (so far) do not present isotopic data and therefore, the contribution of phytoplankton to the CH_4 in the upwelling waters cannot be established or linked to isotopic data.

Answer (lines 464 to 467 and lines 485 to 488): indeed, we do not have isotope data that can corroborate our hypothesis, however, the presence of cyanobacteria (*Synechococcus*) during the non-upwelling period could explain the presence of CH_4 hot moments on the surface and this correlates well in our microcosm experiments. We have cytometry samples where the presence of these microorganisms and the optimal response of the <3µm fraction in CH_4 accumulation during the incubation period are evident (Fig. S5).

Line 323 to 338, comment: This paragraph discusses factors that may explain the succession in phytoplankton. However, it is not linked to the topic of the paper which is the CH_4 production/cycling.

Answer (line 366 to 374): yes, the paragraph focuses on explaining the succession of phytoplankton and the varied taxonomic groups resulting from factors like nutrients, their N:P ratios, and seasonality. We included this information as it underscores the diverse factors contributing to phytoplankton succession, which could potentially relate to oxic methane production. Anyway, we synthetize this sentence.

Line 325, comment: please avoid such statements.

Answer: ok, thanks for the suggestion.

Line 326, comment: is.

Answer (line 367): it was omitted.

Line 346, comment: (1) this term must be replaced across the paper. Here for example you could use. several time points of high CH_4 accumulation. (2) primary / bacterial or CH_4 productivity?

Answer (line 306): (1) we believe that the term hot moments is the most appropriate for this study since according to the definition this is an accumulation that is above the surrounding medium at a given time and is triggered by microorganisms and high accumulation point can be interpreted as something given in a space (on a grid for example). (2) we refer to primary production, we have clarified this term throughout the document.

Line 348, comment: the occurrence of the "hot moments"?

Answer (line 307): this was corrected.

Line 366, comment: Which communities are left below 0.2 µm filters. This fraction contains normally viruses.

Answer (line 416): this fraction was used as a control treatment.

Line 370, comment: This serves as control for viral CH_4 production or from abiotic processes - otherwise the <0.22 µ, water is considered (as you also state) 'sterlie'

Answer (line 418): exactly, eliminating all types of life, the only variation that could have appeared in this treatment would have been abiotic, a situation that did not occur.

Line 372, comment: This is occurring due to CDOM interaction with UV - did you expose your samples to UV? My impression is that you didn't

Answer (lines 416 to 421): indeed, we have not exposed CDOM to UV. This sentence aimed to discuss that, although abiotic CH_4 production has been demonstrated, in our experiments we did not find this to be the case. We only observed it in our control treatments of <0.2 µm and <0.2 µm + $HgCl_2$. We changed the paragraph to avoid confusion.

Line 378 to 379, comment: The NC community contains all that is in the <3 µm and more - so why there is the accumulation different (lower)?

Answer (lines 421 to 423): That's correct, it is pure seawater. The intention behind this was to emphasize the significance of the picoplankton fraction in CH_4 production. We are aware that within the total fraction, methanotrophs are present and their efficiency regulates CH_4 accumulation.

Line 384, comment: insert spaces.

Answer (line 428): it is done.

Line 396, comment: Why recycling? for recycling you would need to show a full cycle of production and oxidation - carbon transfer etc. Here you show concentrations over time, so far, with no data on processes (production or oxidation)

Answer (434): We understand your perspective. We used 'recycling' in the context of CH_4 generation from methylated compounds or other mechanisms, minus consumption through oxidation. In our experiment, we observed net accumulation or net consumption, but indeed, we didn't delve into specific processes like full cycle production and oxidation. However, we have changed the word to cycling.

Line 398, comment: perhaps cycling - or in the CH_4 cycle

Answer: (line 438) cycling seems correct to us. Thanks.

Line 401, comment: How - which DOC can be converted under oxic conditions to CH_4? If you have clear ideas you need to state these explicitly.

Answer (lines 439 to 442): of course. Within the pool of dissolved organic matter, certain C-1 compounds are produced by plankton during daylight hours. These compounds are then cleaved or utilized as substrates by heterotrophic bacteria, resulting in methane formation as a byproduct. Thank you for highlighting this clarification; we appreciate your observation.

Line 404, comment: suggested - not known. Have a look at this paper as well: https://onlinelibrary.wiley.com/doi/full/10.1002/edn3.441

Answer (lines 443 to 446): it is done.

Line 409, comment: Once more the question arises - why did the entire community which includes the 3-0.22 μm fraction produce less methane.

Answer (line 451): it could be that the size limit of the methanotrophs is in this range and that in the total fraction there are more methanotrophs, which is why there is little variability.

Line 411, comment: You should refer generally to methylamines (even though you added TIMA) For methylamines you should cite Wang et al 2022 (PNAS) and Bizic-Ionescu et al 2018. Karl and Repeta are addressing only MPN

Answer (line 455): You are right, we had only focused on the compounds in isolation. We have corrected this.

Line 414, comment: MPn is a part in many phosphonates.

Answer (lines 457 to 459): as this work only uses MPn, we thought it convenient to focus only on the evidence that Nitrosopumilus maritimus encodes proteins for the synthesis of MPn. Your suggestion to mention that MPn is part of other phosphates is valid.

Line 416, comment: Here once more you should refer to the entire family of methylamines MMA, DMA, TMA

Answer (lines 459 to 462): ok, we have talked about methylamines in general and we have specified TMA as the substrate we have used in our experiments.

Line 421, comment: Just state: The ammendment experiments were in...

Answer: it is done

Line 432, comment: This jump in CH_4 over the course of less than an hour looks as an artifact. 1) Why did it happen just as the end of the light period following a decrease in CH_4? 2) If the potential to use MPn exists, why did CH_4 production stop at 20 nM. There was still plenty of MPn in the system that could be converted.

Answer (line 474): this isn't an artifact; these results stem from our experiments. While we acknowledge the possibility of contamination, it's essential to note that the three replicates yielded similar outcomes. (1) The specific cause of this jump at the start of the dark period isn't precisely known. We hypothesize that in dark conditions, microorganisms might become more efficient at metabolizing dissolved organic carbon, and there is not competition relationship with phytoplankton. as evidenced when compared to the control (<3µm).

(2) The tendency to decrease toward the end of the experiment in March was followed by an increase in the subsequent period in May. Although uncertain, we attribute this shift to the changing planktonic composition between these contrasting months.

Line 439, comment: It may be that bacteria in that environment cannot use TMA or that TMA uptake did not result in CH_4 emission.

Answer (lines 479 to 481): CH_4 production with TMA has been evidenced but at very low rates, as in our case, it may be the case that TMA metabolization is very fast and that the CH_4 produced by TMA is more "flashy" to methanotrophs, but we have no way of demonstrating the latter.

Line 441 to 445, comment: This may be correct - however not linked to the CH_4 story.

Answer: we omitted this paragraph.

Line 448 to 449, comment: There are plenty of descriptions of MPN demethylation in cultures and in situ also in the presence of P.

Answer (lines 531 to 535): yes, you're right.

Line 478, comment: To highlight the changes you want to show - I suggest to scale the Y axis between 10-25 nM.

Answer (lines 507 and 555): it is okay.

Line 483, comment: Remove the word. Besides, why is this remarkable that the Chl concentrations match the phytoplankton community.

Answer (512): ok. It is remarkable because it shows the amount of chlorophyll-a expected for each fraction. But this word was omitted.

Line 485, comment: Why only eukaryotes? What about Prochlorococcus and other pico cyanobacteria?

Answer (line 513): this was added.

Line 486, comment: What is "basal" if we assume mortality than this happens over time and not at T0 - therefore what defines the basal level.

Answer: that word was removed.

Line 487, comment: Here point and new sentence.

Answer (lines 515 to 517): the paragraph has been changed for a better understanding, thank you very much for your suggestion.

Line 487 to 488, comment: To concentrate DOM normal tangential filtration at 30 KD is used.

Answer (line 516): that's right.

Line 491, comment: Here you intend to say: 1) The pattern was similar but 2) There were statistically significant differences. If so please rephrase.

Answer (lines 517 to 520): it is done.

Line 494 to 496, comment: (1) I do not agree - the starting point of the NC at 40h is lower - but the increase to the next time point is nearly identical. (2) Do not agree - see previous comment.

Answer (lines 522 to 523): We rewrote the paragraph.

Line 497, comment: Why "regeneration"?

Isn't this expected based on all knowledge on MPN metabolism that both heterorophic bacteria and Cyanobacteria use it and emit CH_4 in the process? However, normally with MPN amendments, the CH_4 produced is at the same scale as the MPN added i.e. 1 µmol MPN --> ca. 1 µmol CH_4. Here it seems to be not the case. This to me shows that the community is generally not P limited under natural conditions and/or the community as a whole is not equipped to metabolize MPN to CH_4. Not all MPN is metabolized to CH_4 - e.g. by Prochlorococcus.

Answer (529 to 543): Indeed, the studied environment is not limited by P. According to the literature, this does not mean that there are microorganisms that use MPn, but that the utilization rates are slower. Also, the area is not inhabited by *Prochlorococcus*, only *Synechococcus*.

According to the literature reviewed, the concentration of MPn in the natural environment is below that added in our experiments, in that sense, we would expect to enhance the response of accumulation and/or net production of CH_4.

Line 499, comment: Do you refer to the initial jump in concentration? A similar jump is seen in the CC+MPN treatment and CC-control. This may be an artifact.

Answer (lines 525 to 528): we understand, here we are talking about the TMA treatment, and we say that CC+TMA and <3 µm +TMA responded similarly compared to the NC+TMA control treatment.

Line 499 to 500, comment: Where is this visible in Fig. 5C?

Answer (507): you are right, this is not clearly visible in Figure 6C, although the differences between the rates are negligible, we rely on Table S4 to make such a statement.

Line 505, comment: this is unlikely....

Answer (line 531): this was corrected.

Line 506, comment: not entirely correct - see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5103098/ as well as papers on Nodularia.

Answer (lines 529 to 535): of course, the C-P lyase complex, manifests itself in P restricted conditions, to obtain the missing nutrient (P).

Line 509, comment: 1) Do you mean that this is photosynthesis associated CH_4 production? If so this was not shown in Klintzch 2023 but in Bizic 2020. 2) Klintzch 2023 studied the isotopic signature of photosynthetic associated CH_4 production - which you have not investigated. 3) If MPN usage is low in the presence of P - why would it be different for MPN-using cyanobacteria?

Answer (lines 539 to 543): (1) yes, that's right, we cite Klinktch because they corroborate the photosynthesis signal in CH_4 production. We have added Bizic et al 2020 (2) because in our case, the only existing cyanobacterium is *Synecococcus* and it is photosynthetic.

Line 530, comment: The figure is smaller than Fig. 5. Also, as mentioned for Fig. 5 change the scale of the Y axis

Answer (line 555): it is ok.

Line 532, comment: Here the trends between all treatments are nearly identical, suggesting that MPN and TMA had minimal or no influence in this period.

Answer (line 558): you are right, the trends are almost identical, but there are differences in the concentrations of CH_4 produced, especially near the end of the experiment.

Line 555 to 556, comment: You are likely talking here about two mechanisms. 1) Photosynthesis associate CH production by Synechococcus 2) MPN demethylation. Form the sentence as it is now, it can be falsely understood that also Synechococcus provides MPN to heterotrophic Bacteria

Answer (lines 576 to 577): may not provide MPn, but may provide other dissolved compounds for CH_4 formation, such as TMA, as this is a waste product of all organisms.

Line 563: Wrong wording in my opinion - produced may be more appropriate. Recycling - would mean picoplankton also oxidized CH_4.

Answer (line 583): yes, you are right, we have decided to use the word produced.

Line 565, comment: production

Answer (line 585): answer similar to the previous one.

Line 566, comment: Not really a novel aspect.

Answer (line 584 to 586): We believe it is somewhat novel in the sense that they can maintain or sustain CH_4 hot spots in the oxygenated surface layer, since CH_4 is not from sediments or anoxic waters but generated in situ. However, we have rewritten this sentence.

Line 568 to 572, comment: TMA is one example - The literature supports methane generation from other methylated amines MMA and DMA. So you could leverage your conclusions to generalize this for other decomposition products by writing "... such as various methylated amines".

Answer (lines 587 to 591): yes, we only focus on the compounds used, and extrapolate these results to other amines, it boosts the conclusion, thank you very much.

Line 577, comment: is

Answer (line 596): it was added.

Line 577 to 580, comment: This is not an ideal last sentence. It deviates from the main message and reads as an "excuse" why some of the results are as they are.

Answer (lines 596 to 599): yes, it may sound like that, but the idea is to reinforce the hypotheses arising from our experimental gaps and to be taken into account in further studies.

Line 590, comment: This is in title case (Capital letter on each word) while other titles are not. Please use a uniform style.

Answer (line 609): it is done.

Reviewer 2

Responses to reviewer's general comments 2

In the manuscript by Tenorio and Farías, the methane saturation in the depth profile was monitored together with other parameters such as oxygen, chlorophyll and nutrient concentrations in the upwelling area off Chile over seasonal cycles. In addition, oxic methane formation experiments were conducted with the precursor compounds TMA and MPn, which had already been identified as precursors for oxic methane formation in previous studies. Overall, I think that the data are interesting for the readership of Biogeoscience. However, I also have some criticism that should be addressed.

The introduction contains many sentences that either need to be deleted or clarified as they are too general. This concerns Line 38-39, 56-59, 61-65, 70- 72, 69-70.

Thank you for taking the time to review our manuscript, and we appreciate your positive feedback on the overall interest in the data for the readership of Biogeosciences. Your input is valuable to improve this manuscript. We recognize that some sentences in the introduction may be too general or require clarification. Reviewer 1 had the same observation. we have reviewed this section and improved it according to your comments.

Responses to reviewer's specific comments 2

Line 69, comment: "however, since picoplankton are small in size and biomass, it is difficult to observe this relationship" not necessary to mention.

Answer (line 88): these lines have been omitted because they do not contribute significantly to the manuscript, however, the size of the picoplankton is detailed for general knowledge.

Line 39-43, comment: Oceanic methane biogeochemistry is oversimplified. Add a few sentences, e.g. what typical methane depth profiles look like and why. I also recommend moving the information about the studied upwelling region from the Results and Discussion chapter to the Introduction. The selected literature references are also not always well chosen, e.g. the publication by Weber et al. 2019, which deals with an improved estimation of global oceanic methane fluxes into the atmosphere, is incorrectly referenced here. In addition, the previous state of research on the pathways of oxic methane formation needs to be revised in the introduction. Overall, the section is too short, and the studies cited are not presented in a differentiated manner. This aspect is important as potential methane precursor compounds were also investigated in this study. Methane formation in the gut of zooplankton or in anoxic micro size of particles is not mentioned in the introduction but should be discussed in the manuscript when investigating TMA as a potential methane precursor.

Answer (lines 38 to 94): thank you for your recommendation. The omission of the 2008 background back was because we only focus on methylotrophic methanogenesis involving methylated substrates. We appreciate your suggestion, looking at the big picture will help the reader to better understand the CH_4 paradox. Due to all the observations regarding the introduction, we have written a new introduction, ensuring a coherent order and careful inclusion of bibliographic citations.

Line 70- 72, comment: It would be better to formulate hypotheses or objectives of the present studies at this point. It would also help the reader to provide a brief overview of what was done to answer the hypothesis or achieve the objectives.

Answer (lines 88 to 94): ok, we introduce an objective and a summary of the contents.

Material and methods. It would be good to have a geographical map of the study area in which the sampling site is marked.

Answer (line 112): we enhanced the map by indicating various geographical points and useful information.

Line 73, comment: A brief overview of this chapter and moderation of the text would also improve the reader's understanding here.

Answer (lines 96 to 103): thank you for the suggestion. However, we believe that a general description of this chapter is not necessary as the methodology is detailed in subchapters. Nonetheless, we have incorporated a subsection: regional setting, within the methodology, as well as sub-chapters in some items.

Chapter 2.4 it would be better to add subchapters.

Answer: it is done.

Explain the Brunt-Vaisala frequency and how you measured/calculated it

Answer (lines 272 to 275): we have addressed the calculation method for the Brunt-Vaisala frequency. We appreciate your observation regarding the omission of this detail.

Line 239-252, comment: It would be good if some of the information were included in the introduction.

Answer (lines 96 to 103): thank you for your suggestion. This was relocated to the material and methods section, in the regional setting.

Line 253-257, comment: I suggest marking upwelling and non-upwelling periods in the Figure 2.

Answer (line 292): we thought it convenient to mark the different periods of productivity (Phase I, II and III) with bars in the upper part of figure 3.

Line 270, comment: Instead of mentioning euphotic, it would be more precise to refer to the chlorophyll concentration.

Answer (line 78): perhaps you meant eutrophic. The term was used to describe the nutrient load of a water body. However, there is a connection between chlorophyll-a concentration and eutrophication since chlorophyll-a is an indicator of primary productivity. In this sense, we rely on Antoine et al., 1996, who classify the ocean into provinces according to the annual mean levels of chlorophyll-a concentration in oligo, meso, and eutrophic, where eutrophic is an area with chlorophyll-a greater than 1 mg m^{-3}, coincident with our study area.

Line 270-277, comment: This discussion is quite speculative and subjective. Can the lack of correlation be statistically proven with the data? e.g. by correlating upwelling parameters and methane concentration? While in-situ production of methane is thinkable, a lateral influx of methanogenic methane from the coast is conceivable. Ultimately, a mass balance, such as that undertaken by Hartmann et al, 2020, is required to determine whether methane was produced aerobically in situ. This should be included in the discussion.

Answer (lines 375 to 399): indeed, within this study, correlation analyses were conducted and a PCA was added to discern patterns of variation in our study area. We include a figures S3 and S4 in the supplementary material.

We greatly appreciate your suggestion regarding a mass balance, as done by Hartmann et al. (2020). However, verifying in-situ CH_4 production requires a comprehensive consideration of various factors such as vertical and lateral advective transport, like Ekman transport and pumping, and the influence of continental inputs as well as to resolve CH_4 consumption (methanotrophs). At present, our immediate focus revolves around enhancing river monitoring techniques and installing buoy sensors to investigate variability and transportation scales. We believe these endeavors will lay a robust foundation for our future research, potentially exploring the complexities of CH_4 production and transport in greater depth.

Line 274, comment: Explain how the lack of a seasonal correlation indicates inflow from a river. Here it would be good to have a geographical map, as already mentioned.

Answer (lines 311 to 315): in this region, the influence of two primary rivers, namely the Itata and the Biobio, is significant. The new figure (Fig. 1) indicates the position of the rivers. During the upwelling period (spring-summer), a portion of CH_4 could ascend with the upwelling to the surface. However, during periods without upwelling (autumn-winter), one might expect lower CH_4 concentrations at the surface. Contrarily, heightened CH_4 levels are observed, potentially attributed to in-situ production (as evidenced in this study across both periods); but additionally, heavy rainfall during these times could transport organic matter to the coastal zone, serving as a substrate for CH_4 generation, either through biotic or abiotic processes.

Line 276, comment: Methane from sediments could also be introduced by the river input, this should also be taken into account / included in the discussion.

Answer (lines 313 to 315): thank you for your observation, the input of CH_4 from sediments to the ocean surface, brought by rivers, we consider as a single source (rivers).

Line 296, comment: This could be proven by a correlation analysis.

Answer (line 375 to 387): a correlation analysis has been performed that affirms the sentence. This is in the supplementary material (Fig. S3).

Line 316-322, comment: This reasoning is a bit confusing to me, since you found no correlation between chlorophyll and methane formation, right? Even if methane formation by phytoplankton is possible in principle and is rightly discussed here, the point that there was no correlation between methane and chlorophyll should be taken up again here.

Answer (lines 383 to 387): you are correct, is our monthly time series we have not found a relationship between chlorophyll-a and CH_4 and we believe that processes on a short time scale (on the order of days or hours) could be masking such a relationship, as recent studies has shown CH_4 formation from photosynthesis.

Line 410- 412, comment: The reference for TMA is missing. Delete "because they have a methyl radical (-CH3), a potential precursor for CH_4 formation in oxygenated environments"

Answer (line 451 to 455): we believe it is convenient to make clear to the reader why amino compounds are precursors for the formation of CH_4, thank you very much for the suggestion.

We appreciate once again the opportunity to improve our manuscript and are willing to make additional adjustments if necessary. We hope that these modifications will meet the expectations of the journal and the reviewers.

Yours sincerely,

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