

General comments from Referee #1

- 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.

- 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 resolution issues, which initially led to surface accumulations appearing solely derived from bottom water CH_4 advection, in response, our revised graphs now provide a clearer representation, 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 these occurrences. The challenge lies in deciphering whether the double peaks represent local net productions or if there are layers of net consumption intensifying these accumulations.

To address this, we've introduced a supplementary figure (Fig. S1) illustrating these dynamics more explicitly. Additionally, as suggested, a new panel of figures in profile format (Fig. S2) has been included, highlighting distinct increases in CH_4 concentrations (evidenced as "hot moments" or peaks) within the surface layer (0-20m).

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

Figure S1. Time series of vertical distribution of dissolved methane (nM) showing CH_4 hot moments in red ellipses at the surface layer at central Chile upwelling platform (ST18). It was from January 2018 to December 2021. The red dotted line indicates a depth of 20m. 0-20m generally oxygenated. White spaces indicate Not a Number (NaN).

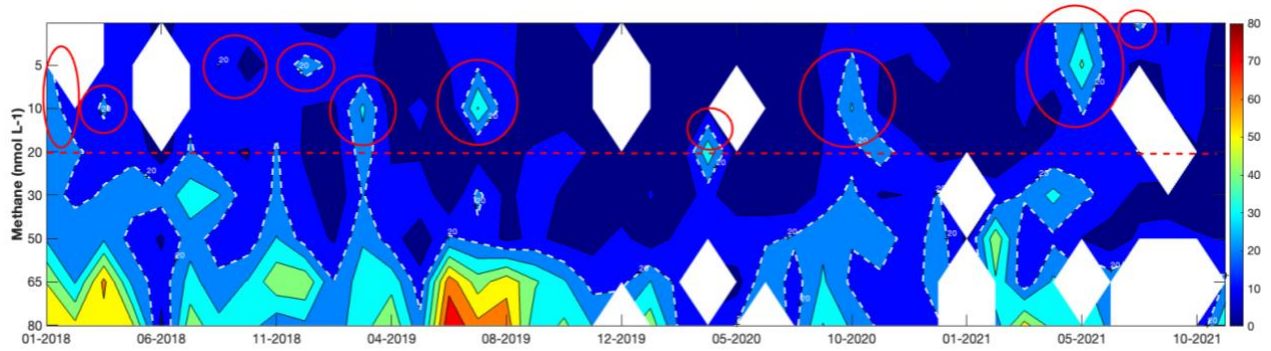
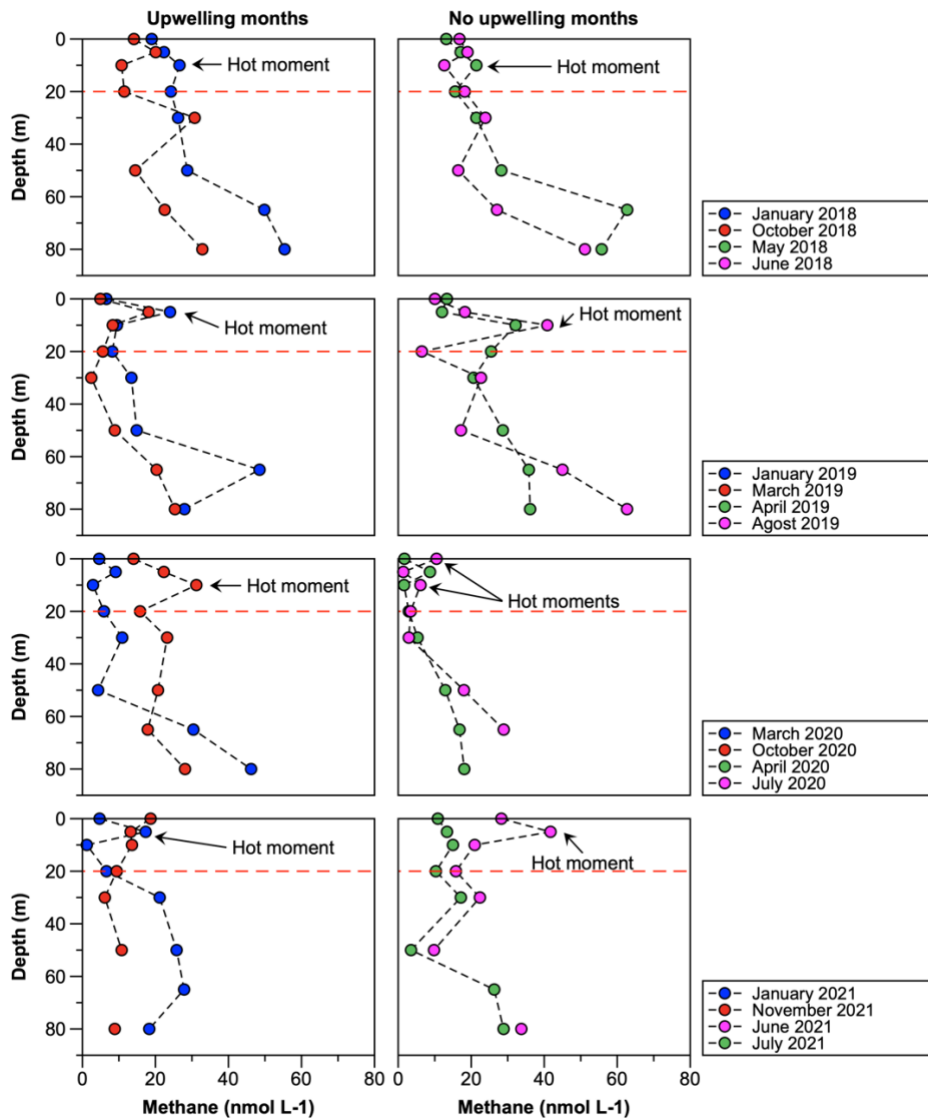


Figure S2. Vertical profiles of dissolved methane (nM) distribution at central Chile upwelling platform (ST18), in two periods of upwelling and non-upwelling during 2018 and 2021. This shows some months where hot moments are observed in the oxygenated surface layer (0 - 20m). Red dotted line represents 20m depth.



- 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 CH₄ emissions.

- 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 individuals. Additionally, the acclimatization process conducted before experimentation could alter the seawater composition. Moreover, we have yet to explore the specific role of methanotrophs, an area that warrants further exploration.

- 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.

Specific comments from Referee #1

Line 11, comment: subscript for the 4 in CH₄

Answer: it was rectified in the whole summary.

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

Answer: yes, you're correct. Our bibliographic search revealed Lamontagne et al. (1973), who first documented CH₄ concentrations in the surface layer during their cruise. We have made the necessary corrections based on this information.

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

Answer: the word metabolization was changed to consumption.

Line 53, comment: Font sizes

Answer: references to font sizes were rectified.

Line 60, comment: Cyanobacteria and algae also produce CH₄ directly through photosynthesis. This should be mentioned here.

Answer: absolutely. This paragraph specifically focuses on picoplankton, and in that context, we previously mentioned only cyanobacteria (bacteria), overlooking the inclusion of photosynthetic picoeukaryotes (eukarya). We've amended this omission in our discussion to encompass this important point.

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

Answer: 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 CH₄ production" or "directly linking photosynthetic CO₂ fixation with CH₄ production". Since ¹³C-CO₂ was converted to ¹³C-CH₄

Answer: we rewrite that paragraph in the context of initial evidence based on indirect relationships between phytoplankton biomass and methane supersaturation; and recent findings have unveiled direct evidence of CH₄ production and autotrophic carbon assimilation but focusing specifically on picoplankton, notably cyanobacteria and picoeukaryotes.

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: the entire paragraph has been changed. Please review the new paragraph; it was previously tangled and contained inconsistencies. We attempted to prevent the association of

picoplankton biomass with production due to the low biomass maintained by this fraction of phytoplankton.

This paragraph might be perplexing. Empirical relationships often link CH₄ accumulation to chlorophyll-a levels. However, these connections aren't direct indicators of phytoplankton producing CH₄; instead, they signify trophic conditions (eu- or meso-trophic) and the microbiome. Picoplankton, despite playing a crucial role, doesn't significantly reflect in chlorophyll-a accumulation due to its low biomass. These relationships can often obscure the actual dynamics at play.

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: the depth of this is 90 m.

Line 86, comment: meaning there were others?

Answer: 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: 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 CH₄ production and demonstrate that the CH₄ produced in our experiments is purely biological.

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

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

Line 260, comment: The text below shows values in nM which makes sense. Is the CH₄ 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 CH₄ is in nM and the deep water CH₄ is in μM it may make sense to use a log scale to better highlight the hotspots.

Answer: 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: 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: 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 CH₄"

Answer: 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: 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, where the term eutrophic implies a concentration >1 mg m⁻³ (Antoine et al., 1996).

Line 270, comment: What is the bottom depth of the sampling area?

Looking at the CH₄ and Chl profiles, there is no clear overlap or correlation. Also I do not see a clear DCM.

Answer: the sampling area's depth is 90 meters. Indeed, at the surface layer, there's no observable correlation between CH₄ and Chl-a profiles. This observation aligns with the time series (climatological study) described by Farias et al., 2021, conducted in the same area. Notably, both during upwelling and non-upwelling periods, hot moments of CH₄ 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: ok, we use "calculated". 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: 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: 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 CH₄ but none of the other mechanisms was were quantified in this area.

Answer: yes, indeed those lines are skewed, there is not much history of aerobic CH₄ production in upwelling areas, and particularly in those, separating that coming from anaerobic from aerobic methanogenesis is particularly difficult. Finally, DMS could be one of the substrates used by pico and bacterioplankton. As a result, that paragraph has been rewritten to reflect these considerations.

Line 288, comment: While I am confident that DMSP is a source of CH₄. 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 CH₄.

Answer: we cited Florez et al., 2013's work as it stands as one of the few pieces of evidence indicating a potential link between CH₄ and DMS in upwelling systems (same study area as this manuscript). Their study suggested that a portion of the CH₄ produced in their experiments originated from the added DMS. They demonstrated the transformation of ¹³DMS into ¹³CH₄, 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: this word was changed.

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: 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: 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: (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.

(2) 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 CH₄ productions. The fact that multiple species of phytoplankton produce CH₄ was already established in many previous studies and is not indicated from your data (so far).

Answer: indeed, we haven't established a link between chlorophyll-a and CH₄ 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 CH₄ 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 CH₄ in the upwelling waters cannot be established or linked to isotopic data.

Answer: 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 CH₄ 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 CH₄ accumulation during the incubation period are evident.

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 CH₄ production/cycling.

Answer: 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 synthesize this sentence.

Line 325, comment: please avoid such statements.

Answer: ok, thanks for the suggestion

Line 326, comment: is

Answer: it is done.

Line 346, comment: (1) this term must be replaced across the paper. Here for example you could use. several time points of high CH₄ accumulation. (2) primary / bacterial or CH₄ productivity?

Answer: (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: it is done.

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

Answer: this fraction was used as a control treatment.

Line 370, comment: This serves as control for viral CH₄ production or from abiotic processes - otherwise the <0.22 μ, water is considered (as you also state) 'sterile'

Answer: exactly, that was the end of it.

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: indeed, we have not exhibited CDOM with UV. This sentence was to discuss a bit that although abiotic CH₄ production has been demonstrated, in our experiments we did not find this to be the case, we just evidenced it with our control treatments of <0.2 μm and <0.2 μm + HgCl₂. We changed the sentence 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: That's right, it is pure seawater. The idea of doing this was to emphasize the importance of the picoplankton fraction in CH_4 production. We know that in the total fraction, we find methanotrophs and their efficiency regulates CH_4 accumulation.

Line 384, comment: insert spaces

Answer: 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: 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.

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

Answer: 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: 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: 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: 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: 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: 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: 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 CH₄ 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 CH₄?
- 2) If the potential to use MPn exists, why did CH₄ production stop at 20 nM. There was still plenty of MPn in the system that could be converted.

Answer: 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 CH₄ emission.

Answer: CH₄ production with TMA has been evidenced but at very low rates, as in our case. also, it may be the case that TMA metabolization is very fast and that the CH₄ 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 CH₄ story.

Answer: we believe it is necessary to mention since nutrient ratios are also important determinants for the production of oxic CH₄.

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: 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: it is okay.

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

Answer: that word was removed. It is remarkable because it shows the amount of chlorophyll-a expected for each fraction.

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

Answer: 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: is an explanatory sequence of the sentence.

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

Answer: 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: 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: We rewrote the paragraph.

Line 497, comment: Why "regeneration"? 2) Isn't this expected based on all knowledge on MPN metabolism that both heterotrophic bacteria and Cyanobacteria use it and emit CH₄ in the process? However, normally with MPN amendments, the CH₄ produced is at the same scale as the MPN added i.e. 1 μmol MPN --> ca. 1 μmol CH₄. 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 CH₄. Not all MPN is metabolized to CH₄ - e.g. by Prochlorococcus.

Answer: 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 CH₄.

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: here we are talking about the TMA treatment, and we say that CC+TMA and >h+TMA responded similarly compared to the NC+TMA control treatment.

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

Answer: you are right, this is not clearly visible in Figure 3C, although the differences between the rates are negligible, we rely on Table 4 to make such a statement.

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

Answer: 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: 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 CH₄ production? If so this was not shown in Klintzch 2023 but in Bizic 2020. 2) Klintzch 2023 studied the isotopic signature of photosynthetic associated CH₄ 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: (1) yes, that's right, we cite Klintzch because they corroborate the photosynthesis signal in CH₄ production. We have added Bizic et al 2020 (2) because in our case, the only existing cyanobacterium is *Synechococcus* 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: 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: you are right, the trends are almost identical, but there are differences in the concentrations of CH₄ produced, especially near the end of the experiment.

Line 555 to 556, comment: You are likely talking here about two mechanisms.

- 1) Photosynthesis associated CH₄ 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: may not provide MPN, but may provide other dissolved compounds for CH₄ 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 CH₄.

Answer: yes, it is appropriate, but at the limit of this size are also methanotrophs, besides our experiments we see the net production, we have not separated both processes, that is why we prefer to use "recycling".

Line 565, comment: production

Answer: answer similar to the previous one.

Line 566, comment: Not really a novel aspect.

Answer: We believe it is somewhat novel in the sense that they can maintain or sustain CH₄ hot spots in the oxygenated surface layer. CH₄ is not from sediments or anoxic waters but generated in situ.

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: 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: 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: 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: it is done.