

General comments from Referee #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 the manuscript. We recognize that some sentences in the introduction may be too general or require clarification. Reviewer 1 had the same observation. We have revised the above sections to improve clarity and focus.

Specific comments from Referee #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: indeed, due to the potential masking of aerobic methanogenesis by empirical associations between phytoplankton biomass and CH₄, we find it imperative to highlight the need for considering alternative proxies or indicators. Specifically, if a portion of CH₄ generation is facilitated by picoplankton organisms, future investigations should explore additional indicators.

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.

Answer: thank you. Yes, we've completed the introduction, refining the section discussing CH₄ in upwelling areas and enhancing precision by improving the referencing.

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: we understand, the omission of the 2008 background back is 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 CH₄ paradox. Due to all the observations regarding the introduction, we have written a new introduction.

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: 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: we improved the map indicating several geographical points and useful information.

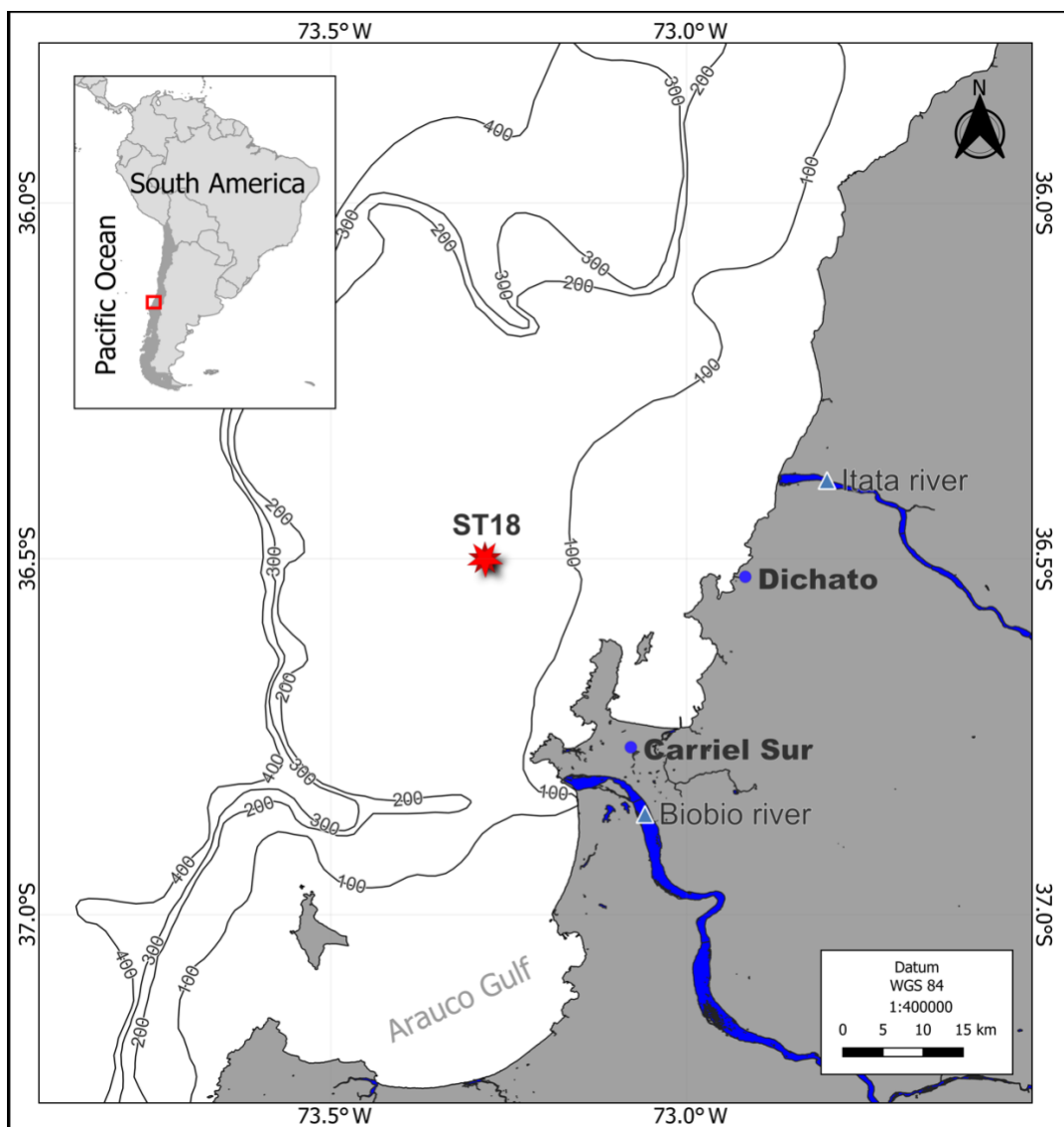


Figure 1. Time series location map (ST18) over the central Chile upwelling platform. The Itata and Biobio rivers, Carriel sur and Dichato are shown.

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

Answer: thank you for the suggestion, but we believe that a general description of this chapter is not necessary, because the methodology is detailed in subchapters, however, the suggestion of adding subchapters in some items was taken.

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: we have addressed the calculation method for the Brunt-Vaisala frequency. We appreciate your observation regarding the omission of this detail, and in response, we've provided clarification on how this parameter was calculated.

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

Answer: 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: we have marked the different productivity periods in bars.

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

Answer: 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: indeed, within this study, correlation analyses were conducted between variables during both upwelling and non-upwelling periods, revealing no clear correlation between these variables. We include a table 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 CH₄ production and transport in greater depth.

Figure S4. Spearman correlations matrix between biochemical variables at the surface layer (20m). Values on the red and blue color scale represent positive and negative values of the Spearman correlation (ρ). Variables are: T: temperature, S: salinity, DO: dissolved oxygen; NO₃⁻: nitrate; PO₄³⁻: phosphate; Si(OH)₄: silicic acid; Chl-a: chlorophyll-a; CH₄: methane; N:P ratio, Si:N ratio and DOC: dissolved oxygen carbon. * represents a significant correlation of 0.05.

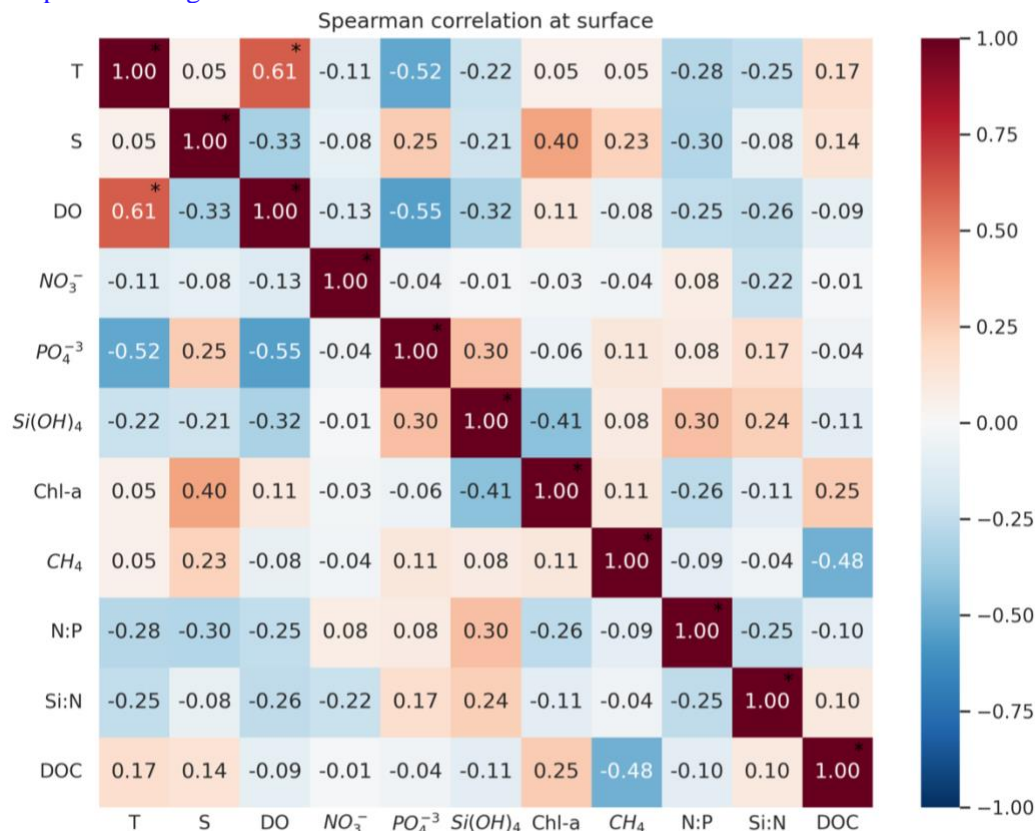


Figure 2 C shows the Brunt-Vaisala frequency but has not been discussed in the text. How does this frequency relate to the methane profile, what can be deduced from Figure 2 C?

Answer: the use of the Brunt-Vaisala frequency was to guide us and visualize the periods of mixing and stratification, which can help to interpret the physical conditions under which the planktonic communities develop and generate change and therefore the conditions under which the hot moments form.

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: 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 CH₄ could ascend with the upwelling to the surface. However, during periods without upwelling (autumn-winter), one might expect lower CH₄ concentrations at the surface. Contrarily, heightened CH₄ 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 CH₄ 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: thank you for your observation, we have added background information on CH₄ production in sediments in rivers.

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

Answer: a correlation analysis has been performed that affirms the sentence. This is in the supplementary material (Fig. S4).

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: you are correct, in our monthly time series we have not found a relationship between chlorophyll-a and CH₄ 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 have shown CH₄ 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 CH₄ formation in oxygenated environments”

Answer: we believe it is convenient to make clear to the reader why amino compounds are precursors for the formation of CH₄, thank you very much for the suggestion.