Dear reviewer, thank you for taking the time to review our manuscript.

P1 L 10 : “Estuaries are important natural sources of methane (CH4) to the atmosphere”. Word “important” is meaningless. Quantitatively CH4 emissions from estuaries (0.2 TgCH4/yr) are negligible (=0.4%) compared to freshwater wetlands (150 TgCH4/yr) and inland waters (rivers+lakes+reservoirs) (77 TgCH4/yr) according to Rosentreter et al. (2022).

We will remove the word “important” in the revised version.

Statement P1 L 13 “Salinity changes can inhibit methanotrophic activity” is an over-simplification of available literature. This statement should be re-phrased or removed from abstract.

We will re-phrase the sentence with “Increasing salinity in estuaries has been associated with lower methane oxidation rates”.

Osudar et al. (2015) and de Angelis & Scraton (1993) show indeed that specific MOX rates (or turnover time) decreased (or increased) along the salinity gradient of estuaries. However, as discussed by Osudar et al. (2015) this pattern is difficult to interpret because a lot of factors that can affect MOX co-vary with salinity such as nutrients, suspended matter, O2. Experiments of adding salt to a freshwater sample of sediment (Sherry et al., 2016) or of water (De Anglis & Scraton 1993) leads to a decrease of MOX. This is not surprising has there is an enormous osmotic stress to freshwater methanotrophs over a short time-period, as would be the case of any other type of freshwater micro-organism. This cannot be translated by an “inhibition of methanotrophic activity”. Osudar et al. (2017) shows that a gradual increase of salinity allows some of the more halotolerant methanotrophs to overcome the salinity stress, not leading to a collapse of MOX.

Some studies that looked into sediments with stable salinity conditions across sites with variable salinity. These studies do show that community composition of methanotrophs and rates of MOX are indeed different in response to salinity (Zhang et al. 2023).

The real question is if there is an interference at cellular level of salinity on methanotrophs (osmotic stress, enzymatic disruption) or are just marine conditions less favourable than freshwater to methanotrophs, due to dilution of microbial populations, of substrates (CH4), and of other potentially limiting factors (macro- and micro-nutrients)?

We agree with the comment of the reviewer about the effect of salinity on the activity of methanotrophs. Answering to the last question, our results suggest that increasing salinity does not necessarily lead to a general inhibition of methane oxidation, but rather promotes a shift in methanotrophic community composition towards more halotolerant taxa, while community members which cannot adapt, vanish. This observation is consistent with Osudar et al. (2017), who demonstrated that gradual salinity increases allow certain methanotrophs to adapt to elevated salinity conditions without a complete collapse of methane oxidation activity. Therefore,

salinity appears to act primarily as a selective pressure shaping methanotrophic communities rather than as a universal inhibitor of methane oxidation.

P2 L36: The work of Zhang et al. (2023) is mis-cited here. Their Figures 2 and 3 show very important changes with salinity in methanotroph community composition, as well as MOX rates.

We will remove the citation of Zhang et al.,(2023) from that sentence.

P11 L 8 : “our data, together with previous results, challenge the paradigm that CH_4 and salinity are anticorrelated in estuaries.” Such paradigm never existed, and if it did exist, then it was already shattered almost 25 yrs ago by Middelburg et al. (2002) who showed in several European estuaries all kinds of shapes in profiles of CH4 vs salinity. Same comment applies for sentence L11-13. No previous publication has concluded naively that “ CH4 dynamics (...) are only governed by a two-endmember mixing”

We thank the reviewer for this important clarification. We agree that previous studies, including Middelburg et al. (2002), have already demonstrated that methane distributions in estuaries often deviate from simple conservative mixing patterns. Our intention was not to suggest that previous work interpreted estuarine CH_4 dynamics solely in terms of two-endmember mixing, but rather to emphasize the complexity of the processes controlling methane distributions along estuarine gradients. We will therefore adjust the wording throughout the manuscript by replacing “challenge the paradigm” with more neutral phrasing. We will also modify the last sentence, which now reads “Our data further support previous observations that methane dynamics in estuaries are shaped by multiple biogeochemical and hydrodynamic processes beyond simple conservative mixing.”

P11L 23: The work of Poffenbarger et al. (2011) deals with fluxes of CH4 from sediments to the atmosphere in saltmarshes not MOX, there’s not a single measurement of MOX in this paper.

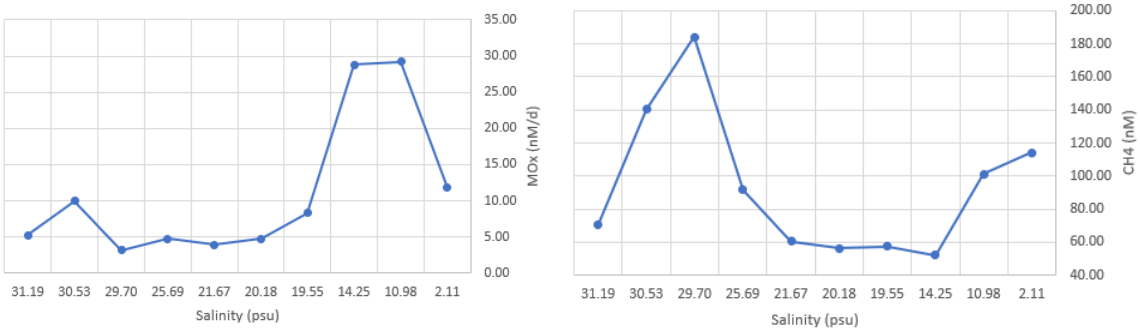
P11 L23: The work of HO et al. (2018) deals with methanotrophs in rice paddy soils. This seems irrelevant for discussing methanotrophy in the water column of an estuary.

We thank the reviewer for the corrections, we will replace the references with more suitable ones in the revised version.

P11 L 25: “In contrast, we found relatively high rates of MOx (up to 5 nM d^{-1}) throughout the entire estuary, apparently independent of salinity” There’s no plot of MOX vs salinity. Figure 3 only shows MOX vs station number. It is necessary to plot variables such as MOX and CH4 vs salinity and not just vs station number.

The sampling stations followed the estuarine salinity gradient, such that, plotting methane concentrations and methane oxidation rates against station number or salinity produces nearly identical spatial patterns. We originally chose station number to facilitate the identification of specific estuarine regions along the transect. However, we agree that this representation may

obscure the direct relationship with salinity. We will clarify this point in the text and add plots of CH_4 and MOx versus salinity in the Supplementary section.



P11 L 15-33: Here are discussed MOX rates, however, it is well established that MOX depends primarily on CH4 availability (in oxygenated conditions). Hence, MOX and CH4 should co-vary, and it is advisable to normalize for CH4 and look into the variations of specific MOX (MOX:CH4) or turnover (CH4:MOX). For example the work of De Anglis & Scraton (1993) shows a very distinctive decrease of specific MOX as a function of salinity. Based on the current analysis of MOX, it is difficult to understand what are the drivers of methanotrophy, it is then advisable to plot specific MOX as a function of other potential drivers.

We thank the reviewer for the suggestion. We explored the relationship between methane oxidation and potential environmental drivers, following the reviewer's recommendation. Based on these data, no significant correlation was observed between CH_4 and MOx (Pearson's r = -0.84, p = 0.07). When considering first order constant (k' or "specific MOx") and salinity alone, a strong negative correlation was observed (Pearson's $r = -0.867$, $p < 0.01$). However, when accounting for additional environmental variables, the strength of this relationship decreased substantially (partial correlation: r = −0.63, p = 0.25), suggesting that salinity alone may not be the primary control on methanotrophic activity. These results support our interpretation that increasing salinity does not necessarily lead to a general inhibition of methane oxidation, but rather to a shift in methanotrophic community composition. To improve clarity, we will add the statistical results to the Supplementary section.

P11 L 31-33: the paper of Zhang et al. (2023) shows very clearly in their Fig. 2 "changes in the composition of the MOB community as a function of salinity". This is what you do seem to state in P12L7 : "These shifts are likely driven by the differential sensitivity of MOB groups to a range of salinities, which was discussed to shape community composition of MOB (Osudar et al., 2017, Zhang et al., 2023)"

We are not sure what the reviewer's concern is here.

P12L21: "However, despite the differential water column MOB community composition, overall MOx was not substantially affected by salinity (see previous section), but instead followed MOB abundances." This statement is not supported by the data. Figure 3 shows that MOX rates change

by a factor of 10 between stations 3 and 9. It would be useful if you actually plot MOX and specific MOX (MOX:CH4) vs the abundance of methanotrophs.

This would allow to actually test the hypothesis given in the following sentence "This suggest that salinity may indeed exert a selection pressure on the composition of the MOB community but that system-level MOx is determined by the abundance of MOB rather than their identity".

I actually agree with this statement (CH4 will always be oxidized by whoever is present) but it would be nice you could actually test this with the data by plotting MOX or specific MOX vs the methanotroph abundance.

We thank the reviewer for the suggestion. We tested the correlation between MOx and methanotroph abundance. They are strongly positively correlated (Pearson's $r = 0.886$, $p < 0.001$). In addition, when considering specific methane oxidation rates (k'), the relationship with methanotroph abundance became even stronger in the partial correlation analysis (r = 0.89, p = 0.04). These statistical results will be added in the manuscript.

P12 L 26 : "week" ?

Our apology, this is a typo, the correct word is "weak"

P12 L11 : "MOB abundance primarily governs overall MOx" this is wishful thinking, it is necessary to test this rigorously with the data rather than giving a vague account of patterns in Figure 3

The results of the Pearson's test are discussed in the comments regarding P12L21. As we are reporting observational data, we obviously cannot make causal inferences, but we can generate hypotheses.

P12 L 31 "Considering the current velocity of the Scheldt (~95 km d-1, Meire et al., 2005), a typical doubling time of MOB (~9 days, Mayr et al., 2020) and the length of the estuary investigated here (87 km) it seems unlikely that shifts in MOB composition are primarily driven by growth within the water column." This statement is incorrect. 95km/d should correspond to the average of tidal currents per day (or something equivalent). The residence time of water in this estuary is 1-3 months (de Brye et al. 2012) and not 0.9 days (=87/95).

According to Mayr et al., 2020, ~9 days is the average doubling time of MOB. Still, when considering a doubling time of 9 days, it seems unlikely that a community shift is only explained by the growth of the MOB community from whatever is inoculated into the estuary from upstream.

P12 L35: The sediment and water are tightly linked in such an environment. In the Scheldt, sediment deposition and resuspension are strongly tidally driven, with large amounts of fine sediment alternately eroded, suspended, and deposited during each semidiurnal tide (Baeyens et

al. 1997). So there is very active exchange benthic-pelagic of particles (and attached micro-organisms).

A simple way to test this is to plot specific MOX versus TSM.

We assume that TSM stands for total suspended matter. If so, unfortunately we do not have these data.

P12 L 37: “bubbles peculating” ?

Our apology, this is a typo, the correct word is “percolating”

Section 4.4. Another consideration is that the sediments might transfer different quantities of CH4 with similar 13C/12C ratios at different sites in the estuary. So the source isotopic signature might be the same but the resulting concentration variable. On top of that there’s fractionation by MOX. This makes the interpretation of water column d13C-CH4 extremely tricky.

We are not completely sure if we understood this comment. We agree that interpreting water-column $\delta^{13}C\text{-}CH_4$ signatures in estuaries is challenging because the observed isotopic composition reflects the combined effects of source signatures, mixing processes, and isotope fractionation during methane oxidation. However, we argue that differences in CH_4 release rates alone are unlikely to explain the pronounced isotopic differences observed in the estuary.
In our sediment incubation experiments, both sediment types were incubated under identical conditions (same temperature and sterile seawater), yet produced markedly different $\delta^{13}C\text{-}CH_4$ signatures (−57.8‰ at station 8 and −46.5‰ at station 10). These values closely matched the corresponding environmental observations (−55.2‰ and −46.4‰, respectively), supporting the interpretation that different sedimentary methane sources and/or methanogenic pathways contributed to the observed isotopic variability.
We already considered isotope enrichment caused by methane oxidation. Based on the measured isotopic shift, approximately 50% CH_4 consumption would be required to explain the observed enrichment solely by oxidation. However, the measured methane oxidation rates were too low to support such extensive fractionation. We therefore conclude that methane oxidation likely influenced the isotope signatures but cannot fully explain the observed spatial differences in $\delta^{13}C\text{-}CH_4$.

P15 L12 : “Similarly, considering the river’s discharge (110 m3s-1, Rijkswaterstaat) and our measured CH4 concentration of 71 nM (station 1), CH4 export into the North Sea (Fout) is ~674 mol CH4 d^{-1}”.
This is not correct and makes little sense. The concentration at the mouth of the estuary results from mixing of mostly seawater and a little bit of upstream water and you cannot simply multiply this concentration by the freshwater discharge to compute the outgoing flux.
Let’s take an element that is only present in seawater and absent in freshwater such as SO42-. The river input is 0. If you apply your computation, then the outflow from the estuary to the ocean would be massive, because the SO42- concentration would be very high at the mouth. But such large outflow does not make sense because the river input is zero, and there’s no generation of SO42- in the estuary. What is really happening is that you have an inflow of SO42- from the

ocean to the estuary at the mouth. This can be computed using a simple box model, such as the LOICZ approach (initially developed for phosphate but can be applied to any element).

We considered the concentration of 71nM as a sampling point that reflects the combined influence of upstream riverine input, internal estuarine production and consumption, sediment exchange, and marine mixing. Importantly, even considering lower CH_4 concentrations near the estuarine mouth (e.g. ~50 nM reported by Jacques et al., 2021), the overall conclusion remains unchanged: most CH_4 produced within the estuary is lost to the atmosphere, while only a comparatively small fraction is transported toward the North Sea. We will make this part clearer in the text.