

**Title: Environmental controls on methanotrophic oxidation capacity in the southeastern North Sea**

This manuscript presents a valuable investigation into parameterizing the fractional turnover rate ( $k'$ ) of aerobic methane oxidation using routinely measured environmental variables across a land-sea gradient. This work addresses an important knowledge gap within coastal carbon budgets, by seeking to replace static fixed-rate constants with a dynamic formulation dependent on ambient environmental conditions. The statistical analysis is methodologically rigorous, deploying polynomial regression, repeated five-fold cross-validation, out-of-fold prediction metrics, and variance inflation factor diagnostics to mitigate overfitting and assess multicollinearity. Nevertheless, multiple conceptual and region-specific points require further elaboration and clarification prior to publication.

**Major comments:**

1. While the second-order polynomial model ( $T+S+NO_3^-$ ) successfully reproduces approximately 80% of the spatiotemporal variability in the estuarine and marine regions, it completely fails in the riverine Elbe where  $k'$  is highest and most variable. The authors attribute this failure to a distinct regional response regime or uncaptured abiotic variables. However, this limits the applicability of

the proposed framework across the entire coastal continuum. The manuscript would be strengthened by a more thorough discussion or secondary analysis exploring why standard abiotic predictors break down entirely in the riverine zone, and whether factors such as high turbidity, short water residence times, or specific organic matter inputs dominate the Elbe region.

2. The selected predictors (T, S, and  $\text{NO}_3^-$ ) perform exceptionally well in terms of predictive metrics ( $R^2 = 0.82$  for validation), but they largely act as macro-environmental proxies for seasonality and water-mass mixing rather than direct physiological controls on methanotrophs. Given that  $k'$  is inherently an integrated outcome of active methanotroph abundance, community composition, and cell-specific activity, the authors correctly point out in the conclusion that future work must incorporate microbial data. The discussion should more explicitly address how these statistical proxies link to underlying microbial community shifts, especially since community composition can change rapidly across salinity and nutrient gradients. Additionally, please address whether high collinearity among nutrient variables within the riverine subset could impair model-selection outcomes, even though multicollinearity was evaluated using VIF metrics computed over the full dataset.

3. The finding that ambient methane concentration contributes no

predictive information for  $k'$  runs counter to classical enzyme kinetics (such as Michaelis-Menten frameworks). While the authors attribute this to oxidation capacity being driven by broader environmental conditions rather than substrate supply, the authors should clarify whether the narrow concentration ranges sampled in certain sub-regions or analytical limitations could have masked a kinetic response.

4. Nitrate improves model performance primarily via the temperature-nitrate interaction term ( $T \times \text{NO}_3^-$ ). The text hypothesizes that warm, high-nitrate conditions support higher methanotroph biomass. However, the present dataset cannot disentangle whether nitrate functions as a direct nutritional resource supporting methanotroph growth, or merely acts as a passive tracer for river-derived water masses and associated co-varying unmeasured variables. This critical ambiguity should be explicitly acknowledged within the Discussion, and overinterpretation of nitrate's direct causal biological effects should be avoided

**Minor comments:**

Table 2: Please standardise the formatting of ion chemical symbols throughout the table.

Figure 2: This figure uses non-linear y-axes for visualization. While this is reasonable, please add explicit annotation within each panel

to alert readers about non-linear scaling, to avoid visual misinterpretation of box-plot magnitudes.

Line 57: Seasonal differences in the fractional contribution of MOx to total methane loss are attributed primarily to variability in  $k'$ . However, the environmental drivers modulating  $k'$  also simultaneously alter other relevant variables (e.g. dissolved methane concentrations, nutrient availability). This confounding co-variation renders the original statement overly simplistic and requires revision.

Line 128: High statistical goodness-of-fit does not equate to established causal mechanisms. Some predictors incorporated in the optimal model may represent confounding covariates that correlate spatiotemporally with true underlying drivers of  $k'$ , rather than exerting direct biological control.

Line 132: Correlation cannot establish causation. Please clarify how the authors rule out the possibility that predictors retained within the final optimal model are confounders rather than mechanistic drivers shaping  $k'$ ?

Line 224: Is nitrate a direct causal driver of  $k'$ , or a confounding variable whose spatiotemporal pattern merely coincides with that of the genuine underlying driver?

Line 278: Note that temperature and salinity act as proxies for water-

mass mixing processes.

Line 297: Please add brief consideration that ammonium may exert inhibitory effects upon MOx activity.

Line 267: How do these environmental factors modulate  $k'$ ? Do these factors exert consistent directional effects (uniformly positive or uniformly negative) upon  $k'$ ?

Line 305: Please expand discussion regarding the potential role of ammonium?

Line 351: Ambient methane concentration contributed no predictive information, suggesting that oxidation capacity is determined by the environmental conditions experienced by the methanotrophic community rather than by substrate supply?

Lines 393, Line 390: Formatting inconsistencies appear across the reference list (including inconsistent journal-abbreviation conventions). The reference section should be carefully proof-read to enforce uniform formatting.