Supporting Information for:

Environmental Controls on methanotrophic oxidation capacity in the southeastern North Sea

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This Supporting Information file contains detailed analytical procedures, one supporting table and one supporting figure.

Text S1. Analytical procedures

S1.1 Methane concentration measurements

Duplicate methane samples were immediately poisoned with 0.3 mL of 25% H2SO4 or 0.3 mL of 5N NaOH for freshwater to stop microbial activity. Samples were analyzed within one week in the laboratory. Dissolved CH_4 concentrations were determined using the headspace equilibration technique, in which a 20 mL N2 headspace was created in the sealed serum bottles. After equilibration, CH_4 concentrations were quantified by gas chromatographic analysis with flame ionization detection (GC-FID). Gas standards (Air Liquide) with concentrations of 10 and 100 ppm methane were used for calibration. The analytical precision of the GC was better than 5%.

S1.2 Fractional turnover rate and MOx measurements

The fractional turnover rate (k', d^{-1}) was determined following the radiotracer protocol described in Bussmann et al. (2015). Immediately after sample collection, 100 µl (10 µCi) of tritiated methane (^3H-CH_4; American Radiolabeled Chemicals, Inc.) was injected through the septum into each sample. Samples were processed in triplicates alongside killed controls. All samples were vigorously shaken for 60 s and incubated in the dark at near in situ temperature for approximately 24 h, after which live samples were also poisoned by the addition of in which 200 µl of 25% H2SO4 or 5 M NaOH.

In the laboratory, total radioactivity (^3H-CH_4 + ^3H-H_2O) was first determined by mixing a 2 ml aliquot with 5 ml scintillation cocktail (Ultima Gold™ LLT) and counting with a liquid scintillation counter (Tri-Carb® 2910 TR, Perkin Elmer). Samples were then sparged with air for 30 min to expel unreacted ^3H-CH_4, after which a second 2 ml aliquot was measured to quantify microbially produced ^3H-H_2O. The fractional turnover rate (k') was calculated by dividing the fraction of oxidized methane by the incubation time. The MOx rate (nmol $L^{-1}d^{-1}$) was then

obtained by multiplying k' by the in situ CH_4 concentration. Abiotic ^3H-H_2O formation estimated from killed controls (on average ~3.5% of total transformation) was subtracted from all measurements.

The limit of detection (minimum detection limit, MDL) had been determined with killed controls, the mean of the controls of each cruise + 3 times its standard deviation was set as the MDL. All values below the MDL were discarded. For the relevant cruises the MDL for k' was 0.010 ± 0.009. The coefficient of variation was high at low methane concentration (< 10 nM) and low at high methane concentrations (< 10 nM), i.e. 23% ± 11% and 7% ± 5%).

S1.3 Environmental parameters

During the Ludwig Prandtl cruises, temperature, salinity, and dissolved oxygen were measured immediately after sampling using a Universal Pocket Meter (Multi 340i). The measurement precision was ±0.1 °C for temperature, ±0.01 for salinity, and ±0.5 % for dissolved oxygen. Suspended particulate matter (SPM) was filtered on board immediately after sample collection, while subsequent processing was conducted in the laboratory. Filtration was performed using prewashed and pre-weighed Whatman GF/C filters. Filtered volumes ranged from 100 to 250 mL depending on turbidity. Filters were dried at 60 °C for 24 h and reweighed to determine total SPM concentrations.

Water samples for dissolved inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}) and silicate were filtered immediately after collection using GF/C filters (Whatman™). Filtrates were transferred into 50 mL Falcon tubes and frozen until laboratory analysis. Nutrient concentrations were determined using an autoanalyzer (AA3, Seal Analytical, Germany) following standardized procedures described in Wiltshire et al. (2010) and Grasshoff et al. (1983). Analytical uncertainty was estimated to be approximately 10 %.

Supporting Information for Table S1

Table S1. Coefficients of the selected second-order polynomial regression model (T + S + NO_3^-). Cross-validation (CV) means and standard deviations were calculated from repeated 5-fold cross-validations. Final coefficients were obtained from the complete marine-estuarine dataset. Predictor variables (T, S and NO_3^-) were standardized before polynomial expansion. VIF values are calculated among the base predictors.

$k' = \beta_0 + \beta_1 T + \beta_2 S + \beta_3 N + \beta_4 T^2 + \beta_5 S^2 + \beta_6 TS + \beta_7 TNO_3^- + \beta_8 SNO_3^-$				
Term	coefficient	CV coefficient mean ± SD	Final coefficient	VIF
Intercept	β_0	0.044 ± 0.006	0.043	-
T	β_1	0.043 ± 0.003	0.043	1.05
S	β_2	-0.046 ± 0.004	-0.047	1.61
NO_3^-	β_3	-0.047 ± 0.006	-0.047	1.67
T^2	β_4	-0.013 ± 0.003	-0.013	-
S^2	β_5	0.026 ± 0.007	0.027	-
T × S	β_6	-0.014 ± 0.004	-0.014	-
T × NO_3^-	β_7	0.074 ± 0.008	0.075	-
S × NO_3^-	β_8	-0.008 ± 0.004	-0.008	-

Supporting Information for Figure S1

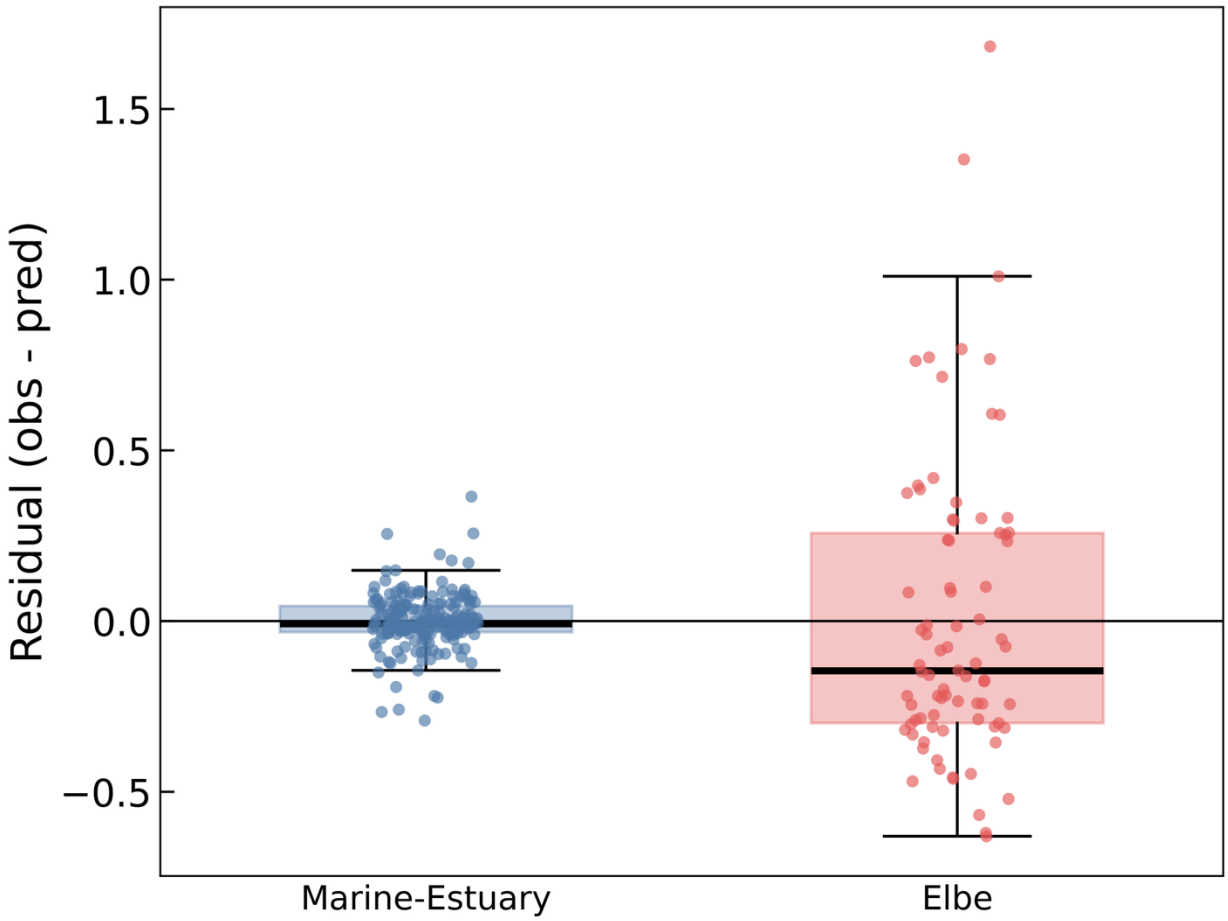

Figure S1. Regional residual distributions for the k' model (T + S + NO_3^-, polynomial degree = 2) trained using all samples (river-estuary-marine). Residuals were calculated as observed minus cross-validated predicted k' and then grouped by regions (Marine-Estuary and Elbe). The bold line within each box indicates the median residual.

References

Bussmann, I., Matousu, A., Osudar, R., and Mau, S.: Assessment of the radio^{3} H-CH_4 tracer technique to measure aerobic methane oxidation in the water column, Limnology & Ocean Methods, 13, 312–327, https://doi.org/10.1002/lom3.10027, 2015.

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