The following supplement accompanies the article

Abundance, activity and diversity of methanotrophic bacteria in the Elbe Estuary and southern North Sea

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Text S1: Supplementary Materials and methods

2.2 Water sampling

The amount of suspended particulate matter (SPM) was determined by filtering the sample through pre-weighed GF/C filters, drying for 24 h at 60 °C, and weighed again. Samples for nutrient analyses were prefiltered (GF/C), stored frozen and analyzed with an auto-analyzer (Wiltshire et al. 2010).,

2.3 Determination of methane concentration and methane oxidation rates (MOX)

The MOX rate was determined as described in Bussmann et al. (2015). Radioactive, tritiated methane (0.1 ml of ^{3}H-CH_4, 2 kBq ml^{-1}, American Radiolabeled Chemicals) was added to triplicate samples. Samples were incubated for 24h near in situ temperature, in the dark. Incubation was stopped by adding 0.2 ml of 25% H_2SO_4. Abiotic controls were poisoned before adding the tracer. Radioactivity was determined with a liquid scintillation counter (Tri-Carb® 2910 TR, PerkinElmer) and Ultima Gold LLT (Perkin Elmer) as scintillation cocktail.

2.4 PCR amplification of methane monooxygenase genes

The environmental DNA samples were checked for the presence of methanotrophic DNA with the water column-specific primers wcpmoA189f (5´-GGNGACYGGGATTTCTGG) and wcpmoA661r (5´-CAGGMGCAACGTCYTTACC) (Tavormina et al. 2008) according to Bussmann et al. 2017.

2.5 Quantitative PCR (qPCR) of methane monooxygenase genes

The qPCR reaction mix (20 µL) contained 10 µL Master Mix (2 × LightCycler® 480 kit hot-start SYBR Green I Master, Roche, Germany), 10 mM of each PCR-primer (wcpmoA189f / wcpmoA661r) and 5 µL template DNA. The amplification was performed with an initial denaturation step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 10 s, annealing at 59 °C for 60 s and extension at 72 °C for 30 s. Fluorescence data were acquired during an additional temperature step (60 s at 65 °C). The relative abundance of MOB was calculated as the percentage of MOB-DNA in the total extracted DNA of each sample.

2.6 Methane monooxygenase intergenic spacer analysis (MISA)

PCR fragments from bulk environmental DNA were amplified using primers spacer_pmoC599f (5'-AAYGARTGGGGHCAYRCBTTC), spacer_pmoA192r (5'-TCDGMCCARAARTCCCARTC). A nested amplification was performed with the primer spacer_pmoC626_IRD (5'-RCBTTCTGGHTBATGGAAGA), and spacer_pmoA189r (5'-CCARAARTCCCARTCNCC) with purified PCR product from the first PCR as template. PCR conditions were as follows: in the first PCR, an initial denaturation at 94 °C for 180 s was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 60 s and elongation at 72 °C for 30 s. The final elongation step was at 72 °C for 300 s. In the second PCR, 2 µL of purified PCR product from the first PCR was used for amplification with modified and labelled primers (see above). The PCR program was modified as follows: initial denaturation at 94 °C for 180 s was followed by 5 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 60 s, elongation at 72 °C for 30 s and 25 cycles with an annealing temperature of 48 °C.

Amplified samples were separated on polyacrylamid gels using a DNA Analyzer 4300 (Licor, Germany). Running conditions on a 6.5% polyacrylamide gel (Lonza, Switzerland, 25 cm length, 0.25 mm thickness) were 1500 Volt, 40 mA, 40 W for 3.30 h at 45 °C. A 50–700 bp sizing standard (IRDye 700, Licor, Germany) was applied to the gel (Bussmann et al. 2017).

For the analysis of the MISA fingerprints (Bionumerics 7.0, Applied Maths, Belgium), size fragments of 350 to 700 bp were included (Schaal, 2016). Binning to band classes was performed with a position

tolerance setting of 1.88%. Each band class is referred to as a MISA operational taxonomic unit (MISA-OTU). Band patterns of the MISA-OTUs were translated to binary data reflecting the presence or absence of the respective OTU. The estimated diversity of MOBs was defined as the number of OTUs per station

Fig. S1: Methanotrophic abundance in bottom waters along the transect.

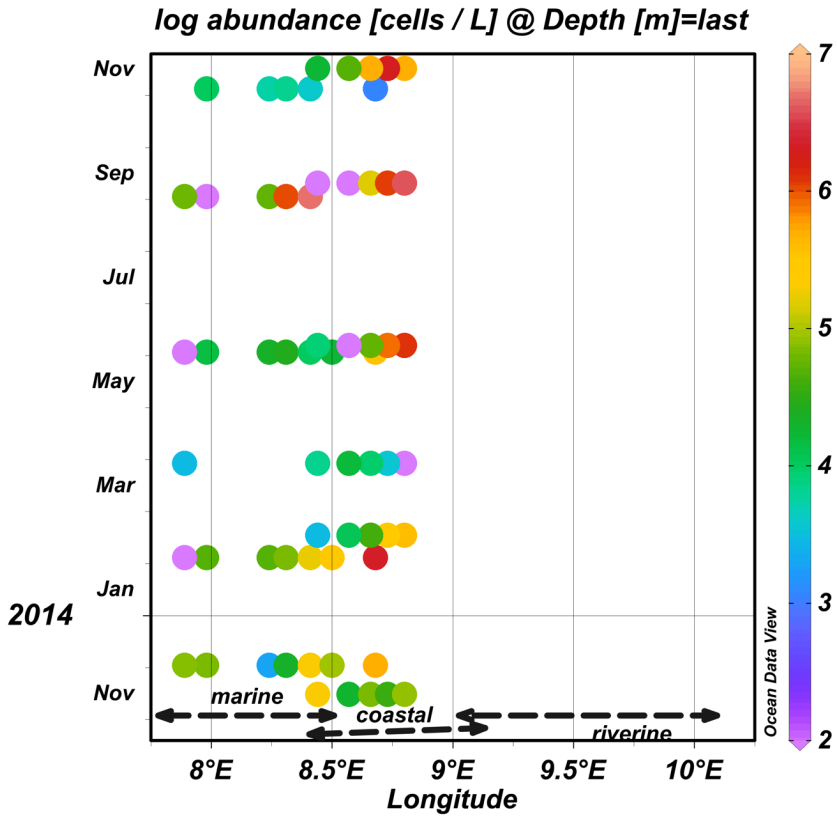

Table S1. Cruise information

Date	Research Vessel	Stations sampled
16.11.2013	FS Prandtl	EC 619 – Elbe VI
03.12.2013	FK Uthörn	Elbe I – Elbe VIII
04.02.2014	FK Uthörn	Elbe I – Elbe VIII
17.02.2014	FS Prandtl	EC 619 – Elbe VI
31.03.2014	FS Prandtl	EC 619 – Elbe VI + Elbe I
05.06.2014	FS Prandtl	EC 619 – Elbe VI
08.06.2014	FK Uthörn	Elbe I – Elbe VIII
03.09.2014	FK Uthörn	Elbe I – Elbe VIII
11.09.2014	FS Prandtl	EC 619 – Elbe VI
05.11.2014	FK Uthörn	Elbe I – Elbe VIII
17.11.2014	FS Prandtl	EC 619 – Elbe VI

Table S2. Multiple linear regression to explain MOX, k', abundance, relative abundance and diversity for the whole log-transformed dataset. Shown is the multiple r^2 (m-R2) and the respective levels of significance. Significant levels with p<0.05 are marked with (+) for positive and (-) for negative correlation and are in bold letters. Codes of significance: * < 0.05; ** < 0.01, *** < 0.001.

	MOX m-R^2 = 0.96	**k'** m-R^2 = 0.89	**Abundance** m-R^2 = 0.42	**Rel. Abund.** m-R^2 = 0.05	**Diversity** m-R^2 = 0.45
Abundance	0.430	0.614	-	-	0.297
Rel. Abundance	0.605	0.708	-	-	0.395
MOX	-	-	0.181	0.935	0.107
k'	-	-	0.814	0.540	0.690
Salinity	**< 0.001 *** (-)**	**< 0.001 *** (-)**	0.833	0.836	**0.008 ** (+)**
Temperature	0.105	0.235	**< 0.001 ***(+)**	0.364	0.096
CH_4	**< 0.001 ***(+)**	**< 0.001 ***(+)**	0.217	0.858	0.286
PO_4	**< 0.001 *** (-)**	**0.011 * (-)**	**0.038 *(+)**	0.637	**0.025 * (+)**
NO_3	0.929	**0.002 **(+)**	0.957	0.404	0.123
NO_2	0.791	**0.004 ** (-)**	**0.024 *(+)**	0.587	0.370
NH_4	0.317	0.728	0.448	0.933	0.482
SPM	0.940	**0.002 **(+)**	0.379	0.952	**0.009 ** (+)**
O_2	**< 0.001 *** (-)**	**0.030 * (-)**	**< 0.001 ***(+)**	0.248	**0.026 * (+)**

Table S3. Rank distribution (1 -13) of the sum of the presence / absence data (1 / 0) for all OTUs and within each of the four seasons (winter, spring, summer, autumn with their median temperatures).

	low MOX	medium MOX	high MOX	distribution pattern
MISA-OTU-677	12	10	13	rare & even
MISA-OTU-635	10	12	10	
MISA-OTU-471	11	12	10	
MISA-OTU-407	12	10	10	
MISA-OTU-363	9	9	9	
MISA-OTU-419	2	1	1	dominant & even
MISA-OTU-445	4	2	2	no clear pattern or prefers medium MOX
MISA-OTU-570	7	8	7	
MISA-OTU-536	3	5	3	
MISA-OTU-513	**6**	**2**	**3**	**prefer high MOX**
MISA-OTU-430	**8**	**7**	**6**	
MISA-OTU-486	**1**	**2**	**5**	**prefer low MOX**
MISA-OTU-560	**5**	**6**	**7**	

Table S4. Rank distribution (1 -13) of the sum of the presence / absence data (1 / 0) for all OTUs and within each of three levels of activity (low, medium and high with their median oxidation rates).

	winter (4.8°C)	spring (8.4°C)	autumn (14.1°C)	summer (16.2°C)	distribution pattern
MISA-OTU-677	12	10	13	11	rare & even
MISA-OTU-635	9	9	10	11	
MISA-OTU-570	8	8	7	8	
MISA-OTU-471	12	11	11	9	
MISA-OTU-407	10	11	12	13	
MISA-OTU-560	4	4	5	5	dominant & even
MISA-OTU-486	1	1	2	1	
MISA-OTU-419	3	2	1	2	
MISA-OTU-513	2	4	6	6	none
MISA-OTU-445	7	2	4	2	
MISA-OTU-536	**5**	**4**	**3**	**2**	**prefer warm**
MISA-OTU-430	**5**	**7**	**8**	**10**	**prefer cold**