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Supplement of

Adaptation of methane-oxidizing bacteria to environmental changes: implications for coastal methane dynamics

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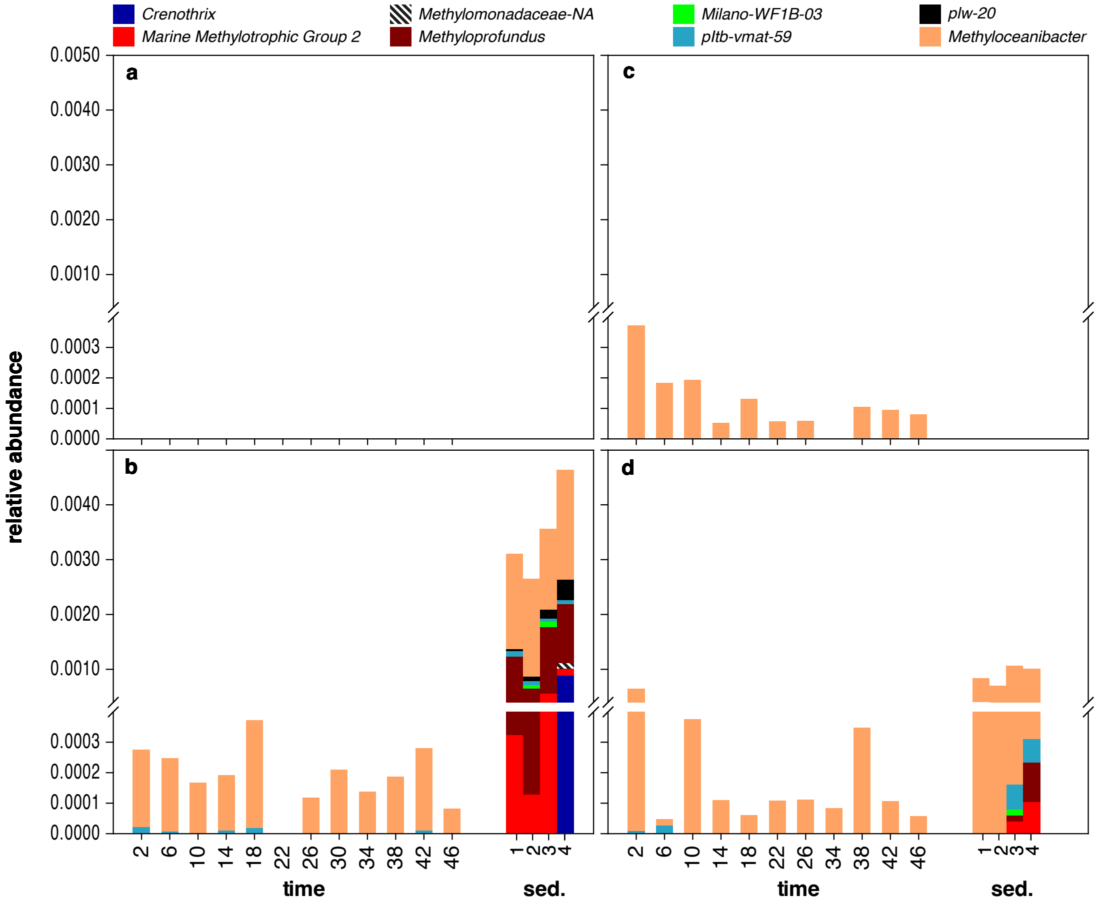

Figure S1. Temporal and seasonal variation of environmental MOB communities in the North Sea water column and sediment. The x-axis indicates the time (in hours) during a repeated 48 h time-series of sampling during summer at 10 m (a) and 35 m water depth (b) as well as during autumn at 10 m (c) and 35 m water depth (d). Surface sediments (sed.) sampled at the end of the time series during summer (b) and autumn (d) are indicated, too. MOB were below detection limit in summer at 10 m water depth; no samples were available for 35 m water depth at 22 and 34 h in summer as well as at 10 m water depth at 34 h in autumn. Note that not all MOBs found in the incubation experiments (Figure 1) could be detected in the initial water column or sediment samples.

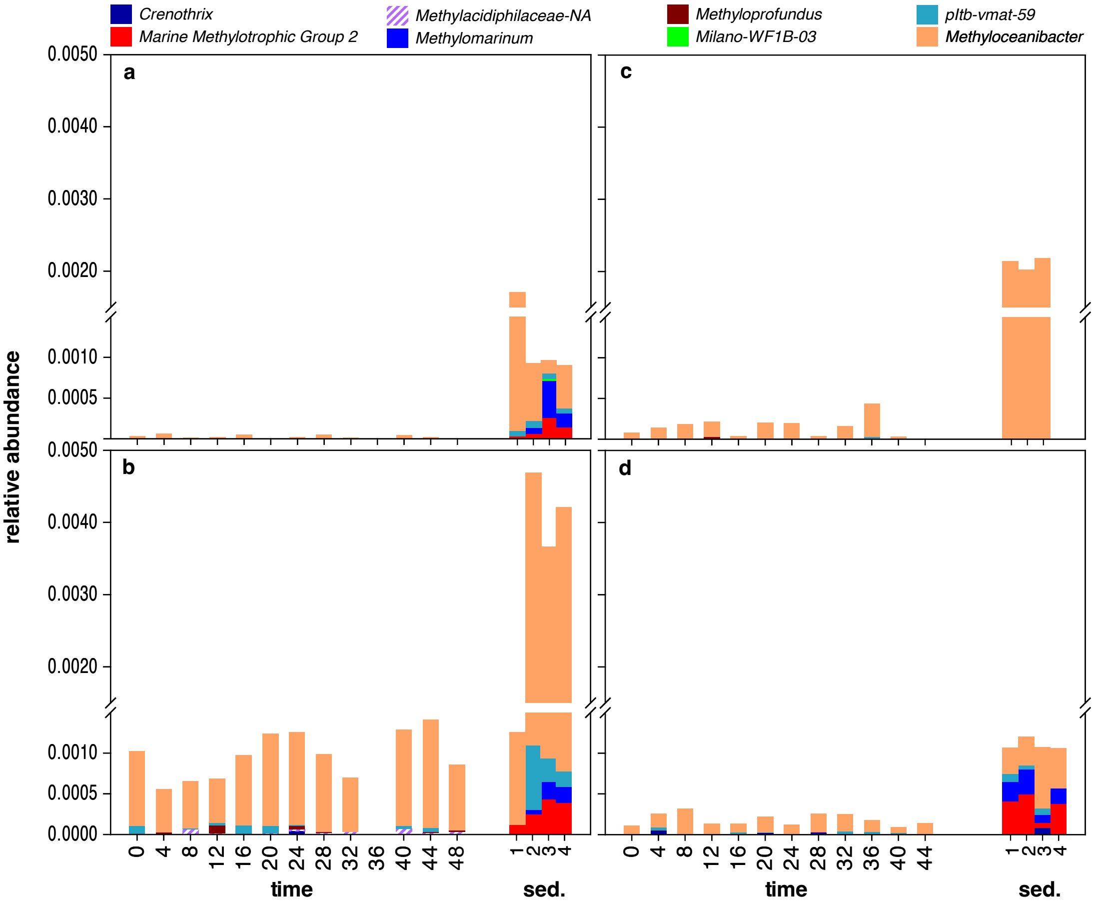

Figure S2. Temporal and seasonal variation of environmental MOB communities in the Wadden Sea water column and sediment. The x-axis indicates the time during a repeated 48 h time-series of sampling during spring (a), summer (b), autumn (c) and winter (d). Surface sediments (sed.) sampled at the end of the time series during summer (b) and autumn (d) are indicated, too. No samples were available for 36 h in autumn and only three sediment samples were available for summer. Note that not all MOBs found in the incubation experiments (Figure 2) could be detected in the initial water column or sediment samples. Conversely, unidentified genera of the Methylacidiphilaceae potentially comprising MOB were not detected in the incubation experiments.

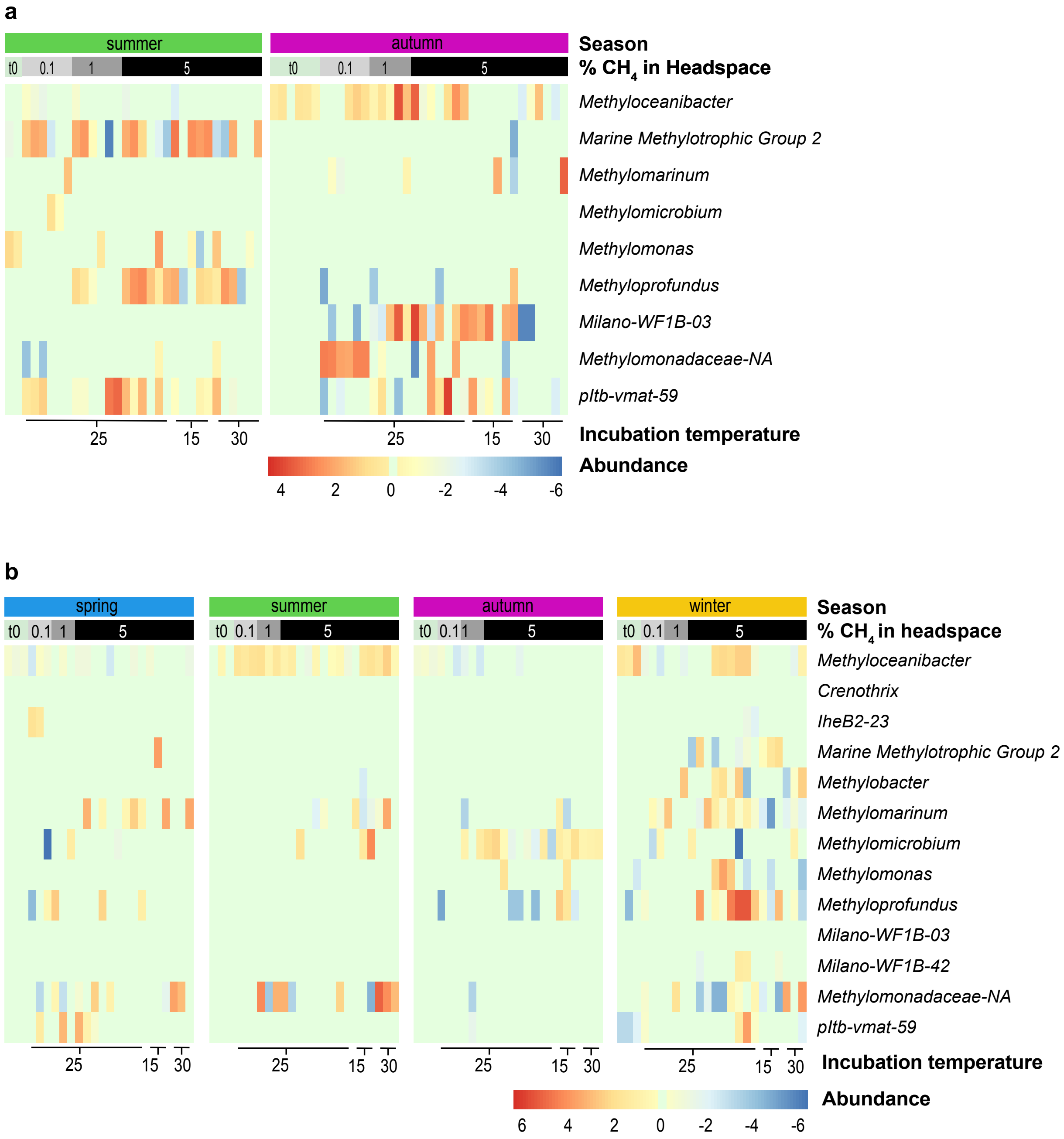

Figure S3. Heatmap of MOB abundances (centered log-ratio transformed counts) observed in North Sea (a) and Wadden Sea incubations (b), categorised by season and headspace methane concentration. Red color indicates higher abundance, blue indicates lower abundance of a particular MOB genus compared to the geometric mean of the respective sample.

Table S1. Experimental conditions for incubations with North Sea inocula, collected from 10 m and 35 m water depth during summer (su) and autumn (aut). The table shows incubation duration in days, CH_4 concentrations (in µM) at the start and end of each experiment and oxygen levels (in % air saturation) at the end of the experiment. Oxygen concentrations at the beginning of the experiment were 100% air saturation Each cell is formatted as: incubation time (days), CH_4-start/CH_4-end, O_2-end.

CH_4	**0.10%**	**1%**	**5%**	**5%**	**5%**
T	**25°C**	**25°C**	**25°C**	**15°C**	**30°C**
10 m, su	25, 3/1	25, 28/5	34, 162/61, 64	54, 165/22	34, 161/90
35 m, su	25, 3/1	25, 33/2	34, 183/23, 65	34, 162/61	34, 161/77
10 m, aut	34, 3/1	25, 32/13	34, 171/113, 97	84, 194/52	34, 214/43
35 m, aut	25, 4/1	84, 34/29	25, 200/87, 98	84, 199/148	34, 202/166

Table S2. Experimental conditions for incubations with Wadden Sea inocula, collected during spring (spr), summer (su), autumn (aut) and winter (win). The table shows incubation duration in days and the corresponding CH_4 concentrations (in µM) at the start and end of each experiment and oxygen levels (in % air saturation) at the end of the experiment. Oxygen concentrations at the beginning of the experiment were 100% air saturation Each cell is formatted as: incubation time (days), CH_4-start/CH_4-end, O_2-end.

CH_4	**0.10%**	**1%**	**5%**	**5%**	**5%**	**5%**	**5%**
T	**25°C**	**25°C**	**25°C**	**15°C**	**30°C**	**25°C**	**25°C**
sal	**30psu**	**30psu**	**30psu**	**30psu**	**30psu**	**15psu**	**35psu**
spr	28, 3/2	13, 42/22	28, 130/75	28, 112/57	28, 98/40	28, 155/117	28, 242/146
su	43, 3/1	43, 40/22	43, 126/63	43, 132/67	43, 141/58	43, 158/92	43, 158/112
aut	27, 3/1	27, 22/9	27, 184/74	27, 199/88	27, 192/63	27, 197/123	27, 108/27
win	34, 2/1, 89	34, 21/4	34, 118/40, 63	34, 97/27	34, 120/16, 53	43, 97/77	43, 91/86

Table S3. Phylogeny of known aerobic methane oxidising bacteria (note that Candidatus Mehtylomirabilis utilises nitrate/nitrite for methane oxidation; Ettwig et al., 2009, 2010). See also Dedysh and Knief (2018), and Haque et al. (2020).

Type	Phylum	Order	Family	Genus
I	Gammaprteobacteria	Methylococcales	Methylomonadaceae	Crenothrix
				ET-SHO
				IheB2-23
				Incertae Sedis
				Marine Methylotrophic Group 2
				Methylicorpusculum
				Methylobacter
				Methyloglobulus
				Methylomarinum
				Methylomicrobium
				Methylomonas
				Methyloprofundus
				Methylosarcina
				Methylosoma
				Methylosphaera
				Methylotuvimicrobium
				Methylovulum
				Milano-WF1B-03
				Milano-WF1B-42
				pItb-vmat-59
				pLW-20
			Methylococcacea	Candidatus Methylospira
				Incertae Sedis
				Methylocaldum
				Methylococcus
				Methylogaea
				Methylomagnum
				Methyloparacoccus
				Methyloterricola
			Methylohalobiaceae	Methylohalobius
				Methylomarinovum
				Methylothermus
II	Alphaproteobacteria	Hyphomicrobiales	Beijerinckiaceae	Methylosinus
				Methylocystis
				Methylocella
				Methylocapsa
				Methyloferula
			Methyloligellaceae	Methyloceanibacter
III	Verrucomicrobiota	Methylacidiphilales	Methylacidiphilaceae	Methylacidiphilum
				Methylacidimicrobium
	Methylomirabilota	Methylomirabilales	Methylomirabilaceae	Candidatus Mehtylomirabilis

Supplementary files available at DAS under DOI Nr: 10.25850/nioz/7b.b.6h (https://dataportal.nioz.nl/doi/10.25850/nioz/7b.b.6h)

- configuration file of the Cascabel pipeline (Suppl_Information_1_config_Cascabel.yaml)
- ASV table (asv_table.csv)
- Taxonomy assignment of ASVs (taxotable.csv)
- sample metadata (filtered_metadata.csv)
- R-script for filtering out MOB and relative abundance calculation (asv_mob_RA.R)
- R-script for DESeq of North Sea samples (DESeq_NS.R)
- R-script for DESeq of Wadden Sea samples (DESeq_WS.R)
- R-script for NMDS of whole community (nmds_whole community.R)
- R-script for NMDS of MOB (nmds_MOB.R)