Supplementary materials to

Controls on the composition of hydroxylated isoGDGTs in cultivated ammonia oxidizing Thaumarchaeota

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Table S1. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from cultures of *Nitrosopumilus piranensis* D3C grown at three different temperatures and harvested at mid-exponential and stationary phases. N.D. = not detected.

Mid-exponential phase

	OH-isoGDGT-0	OH-isoGDGT-1	OH-isoGDGT-2	OH-isoGDGT-3	OH-isoGDGT-4	isoGDGT-0	isoGDGT-1	isoGDGT-2	isoGDGT-3	isoGDGT-4	Crenarchaeol	Crenarchaeol isomer
(a) 25 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	23.7	5.5	0.7	0.1	0.1	11.1	N.D.
DH	0.6	3.5	2.9	0.1	0.0	2.3	19.4	29.5	8.7	6.9	1.3	0.4
MH	0.1	0.0	N.D.	N.D.	N.D.	71.2	28.9	3.8	0.7	0.8	34.5	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	64.3	38.2	21.6	28.3	33.4	15.3	N.D.
(b) 30 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	17.8	8.6	1.3	0.2	0.2	30.1	N.D.
DH	0.3	1.7	1.8	0.0	0.0	1.0	8.4	19.8	6.2	13.6	1.0	0.4
MH	N.D.	N.D.	N.D.	N.D.	N.D.	27.6	13.4	3.4	0.6	0.7	20.6	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	24.1	11.9	6.2	7.9	24.6	7.0	N.D.
(c) 35 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	18.2	12.0	4.4	1.7	0.4	69.5	N.D.
DH	0.2	1.4	5.5	0.4	0.4	0.1	1.2	7.4	3.8	17.5	0.7	0.7
MH	N.D.	N.D.	N.D.	N.D.	N.D.	17.7	9.7	4.2	0.7	1.3	24.8	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	9.0	4.5	2.5	2.8	11.4	3.8	N.D.

Stationary phase

	OH-isoGDGT-0	OH-isoGDGT-1	OH-isoGDGT-2	OH-isoGDGT-3	OH-isoGDGT-4	isoGDGT-0	isoGDGT-1	isoGDGT-2	isoGDGT-3	isoGDGT-4	Crenarchaeol	Crenarchaeol isomer

(a) 25 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	55.4	13.6	0.2	0.2	0.1	30.6	N.D.
DH	0.6	3.7	3.1	0.1	N.D.	3.7	30.5	35.7	11.0	8.5	2.4	0.7
MH	0.0	0.0	0.0	N.D.	N.D.	50.1	21.8	3.3	0.5	0.5	23.9	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	27.9	17.3	10.1	13.5	21.3	9.8	N.D.
(b) 30 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	33.7	17.3	3.2	0.5	0.3	45.0	N.D.
DH	0.5	2.4	2.6	0.1	0.0	1.4	13.0	38.0	11.4	28.3	1.5	0.8
MH	N.D.	N.D.	N.D.	N.D.	N.D.	39.3	22.4	7.3	1.1	1.6	28.4	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	20.5	11.3	6.4	8.7	44.5	8.6	N.D.
(c) 35 °C												
HPH	N.D.	N.D.	N.D.	N.D.	N.D.	21.6	15.6	6.5	0.6	0.5	55.3	N.D.
DH	0.7	6.0	21.9	1.6	2.2	0.2	2.4	16.7	8.9	36.0	1.7	1.6
MH	0.0	0.1	0.2	N.D.	N.D.	23.1	16.7	10.6	1.7	4.1	43.5	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	19.3	11.1	6.5	3.5	22.0	37.6	N.D.

Table S2. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from cultures of *Nitrosopumilus adriaticus* NF5 strain grown at three different temperatures and harvested at mid-exponential and stationary phases. N.D. = not detected.

Mid-exponential phase

	OH-isoGDGT-0	OH-isoGDGT-1	OH-isoGDGT-2	OH-isoGDGT-3	OH-isoGDGT-4	isoGDGT-0	isoGDGT-1	isoGDGT-2	isoGDGT-3	isoGDGT-4	Crenarchaeol	Crenarchaeol isomer
(a) 20 °C												
HPH	0.6	0.2	N.D.	N.D.	N.D.	83.3	6.7	0.4	0.0	0.2	16.4	N.D.
DH	3.0	26.8	19.4	0.5	0.1	1.2	19.8	22.4	4.9	2.2	0.2	0.4
MH	35.4	6.3	0.6	N.D.	N.D.	32.8	7.6	1.3	0.1	0.1	15.1	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	75.6	28.1	13.1	12.1	14.7	7.4	N.D.
(b) 25 °C												
HPH	0.6	0.3	0.0	N.D.	N.D.	71.2	10.8	0.8	0.1	0.3	24.0	N.D.
DH	2.9	29.9	37.6	1.1	0.3	0.6	9.2	16.2	5.4	4.1	0.2	0.5
MH	56.8	12.1	1.4	N.D.	N.D.	28.0	7.1	1.0	0.1	0.3	17.9	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	50.1	18.5	8.4	9.2	21.0	4.3	N.D.
(c) 30 °C												
HPH	0.2	0.2	0.0	N.D.	N.D.	12.2	3.0	0.5	0.0	0.3	5.2	N.D.
DH	0.6	6.4	21.1	0.9	0.4	0.1	1.7	6.8	2.8	2.9	0.1	0.3
MH	17.8	6.2	1.9	N.D.	N.D.	10.5	3.6	1.0	0.0	0.1	13.3	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	19.7	6.1	1.8	3.1	14.7	1.5	N.D.

Stationary phase

	OH-isoGDGT-0	OH-isoGDGT-1	OH-isoGDGT-2	OH-isoGDGT-3	OH-isoGDGT-4	isoGDGT-0	isoGDGT-1	isoGDGT-2	isoGDGT-3	isoGDGT-4	Crenarchaeol	Crenarchaeol isomer
(a) 20 °C												
HPH	1.6	0.6	0.1	N.D.	N.D.	64.9	10.7	0.1	0.1	0.2	21.9	N.D.
DH	4.7	46.9	33.3	0.9	0.2	0.1	2.5	5.5	3.5	1.8	0.2	0.3

MH	54.9	9.4	1.0	N.D.	N.D.	18.1	4.0	0.6	0.0	0.1	12.0	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	52.1	16.8	6.6	7.5	11.2	5.7	N.D.
(b) 25 °C												
HPH	0.4	0.2	0.1	N.D.	N.D.	58.4	14.7	2.7	0.2	0.2	23.1	N.D.
DH	2.4	27.5	40.4	1.0	0.3	0.2	4.3	13.6	5.7	4.0	0.2	0.5
MH	45.0	10.2	1.5	N.D.	N.D.	18.5	6.5	1.6	0.2	0.2	16.4	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	42.6	15.8	8.1	8.1	20.1	5.4	N.D.
(c) 30 °C												
HPH	0.6	0.4	0.2	N.D.	N.D.	48.7	14.7	4.0	0.3	0.2	30.9	N.D.
DH	1.2	13.8	51.5	2.2	1.0	0.0	1.5	12.3	6.7	8.8	0.2	0.8
MH	32.6	11.5	3.7	N.D.	N.D.	17.1	6.6	2.7	0.3	0.5	25.2	N.D.
core	N.D.	N.D.	N.D.	N.D.	N.D.	35.9	13.9	6.1	7.5	31.8	4.8	N.D.

Table S3. Relative abundances (%) of core isoGDGT and OH-isoGDGT lipids inferred from mass spectral response of IPLs with mean and standard deviation from three replicate cultures of *Nitrosopumilus piranensis* D3C strain and *Nitrosopumilus adriaticus* NF5 strain grown at three different temperatures and harvested at two growth phases.

Nitrosopumilus piranensis D3C strain

	25 °C		30 °C		35 °C	
	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase
OH-isoGDGT-0	0.1± 0.1	0.2± 0	0.1± 0	0.1± 0	0.1± 0.1	0.3± 0.1
OH-isoGDGT-1	0.7± 0.3	1± 0.1	0.6± 0.3	0.5± 0.1	0.7± 0.6	2.4± 1.1
OH-isoGDGT-2	0.6± 0.3	0.8± 0.2	0.6± 0.2	0.5± 0.1	2.4± 2.6	8.6± 4.8
OH-isoGDGT-3	0± 0	0± 0	0± 0	0± 0	0.1± 0.2	0.6± 0.5
OH-isoGDGT-4	0± 0	N.D.	0± 0	0± 0	0.2± 0.3	0.8± 0.6
isoGDGT-0	44.6± 4.7	38.8± 3.3	34.3± 1	31.3± 1.4	22.2± 3.9	14.3± 0.3

isoGDGT-1	20.1± 2.1	23.1± 1.1	19.2± 1.1	20.1± 1.4	13± 1.8	10.8± 1
isoGDGT-2	7.8± 2.8	11.2± 2	10.4± 1	13.2± 1.3	10.1± 0.3	12.3± 3.1
isoGDGT-3	2.4± 0.8	3.2± 1.1	2.9± 0.3	3.2± 0.8	3.3± 0.6	4.3± 0.3
isoGDGT-4	2.3± 0.6	2.8± 0.9	5.8± 0.6	7.7± 2	12.1± 4.1	16.3± 2.9
Crenarchaeol	21.3± 2.1	18.8± 0.6	26.1± 3.6	23.2± 2.2	35.4± 2.8	28.7± 0.2
Crenarchaeol isomer	0.1± 0	0.2± 0.1	0.1± 0	0.1± 0.1	0.3± 0.3	0.6± 0.1

Nitrosopumilus adriaticus NF5 strain

	20 °C		25 °C		30 °C	
	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase
OH-isoGDGT-0	3.1± 0.7	5.7± 1.2	4.2± 1.4	3.2± 0.6	3.3± 0.8	2.4± 0.6
OH-isoGDGT-1	15.3± 3.7	29.4± 6.2	17.5± 4.8	15.9± 2.3	12.2± 1.9	9.5± 1.4
OH-isoGDGT-2	11± 1.3	20.6± 4.6	21.6± 6.1	22.7± 3.3	37.3± 4.9	33.3± 4.6
OH-isoGDGT-3	0.3± 0	0.6± 0.1	0.6± 0.1	0.5± 0.1	1.5± 0.1	1.5± 0.3
OH-isoGDGT-4	0± 0	0.1± 0	0.2± 0	0.1± 0	0.6± 0	0.7± 0.1
isoGDGT-0	31.8± 2.8	23.4± 7	24.9± 8.5	24.6± 2.9	11± 4.5	16.4± 3.2
isoGDGT-1	14.2± 2.1	5.5± 1.3	8.7± 0.8	8.5± 0.9	5.9± 0.8	6.1± 0.8
isoGDGT-2	13.2± 3.6	4± 0.8	9.1± 1.3	8.6± 1.6	12.2± 1.2	8.8± 1.1

isoGDGT-3	2.9± 0.4	2.2± 0.2	2.9± 0.3	3.2± 0.5	4.6± 0.3	4.2± 0.2
isoGDGT-4	1.4± 0.2	1.2± 0.1	2.3± 0.1	2.3± 0.4	5.4± 0.5	5.5± 0.4
Crenarchaeol	6.7± 1.6	7.3± 4.7	7.8± 4.3	10± 2.9	5.5± 2	11.2± 2.3
Crenarchaeol isomer	0.3± 0	0.2± 0	0.2± 0	0.3± 0.1	0.4± 0.1	0.5± 0.1

Table S4. Relative abundance based on total ion current intensity of different headgroups from the IPLs of isoGDGTs and OH-isoGDGTs with mean and standard deviation from three replicate cultures of *Nitrosopumilus piranensis* D3C strain and *Nitrosopumilus adriaticus* NF5 strain grown at three different temperatures and harvested at two growth phases. Core lipids without any headgroup are indicated by core isoGDGTs. N.D. = not detected.

Nitrosopumilus piranensis D3C strain

	25 °C		30 °C		35 °C	
	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase
HPH-OH-isoGDGTs	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
DH-OH-isoGDGTs	1.4± 0.6	2± 0.3	1.3± 0.5	1.1± 0.2	3.5± 3.7	12.5± 7
MH-OH-isoGDGTs	0± 0	0± 0	N.D.	N.D.	N.D.	0.1± 0.1
HPH-isoGDGTs	2.5± 2.6	9.2± 9.1	2.8± 2.3	3.6± 2.7	8.2± 12.6	7± 1.2
DH-isoGDGTs	12.3± 6.5	22.6± 7.6	15.2± 2.5	18.3± 7	18.2± 6.7	25.2± 6.5
MH-isoGDGTs	80.3± 9	64.3± 15.8	77.8± 4.5	74.8± 9.8	66.8± 21.7	51.3± 0.5
core isoGDGTs	3.5± 0.5	1.9± 0.6	2.9± 0.2	2.2± 0.2	3.2± 1.2	3.9± 0.8

Nitrosopumilus adriaticus NF5 strain

	20 °C		25 °C		30 °C	
	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase	Mid-exponential phase	Stationary phase
HPH-OH-isoGDGTs	0.3± 0.1	0.8± 0.2	0.3± 0.1	0.3± 0	0.2± 0.1	0.4± 0
DH-OH-isoGDGTs	27.9± 5.1	53± 11.3	40.8± 11.2	40.1± 5.8	51.7± 6.9	44.9± 6.4
MH-OH-isoGDGTs	1.5± 0.2	2.5± 0.6	2.9± 1.4	2.1± 0.6	3± 0.9	2± 0.5
HPH-isoGDGTs	38.3± 4.7	33± 13.6	33.8± 15.7	40.1± 7.7	16.3± 8.6	31.7± 7
DH-isoGDGTs	29.6± 6.2	9± 2.1	19.5± 2.6	15.5± 3	24.6± 2.2	18.2± 1.6
MH-isoGDGTs	2± 0.1	1.4± 0.4	2.2± 0.9	1.6± 0.4	3.4± 1	2.1± 0.5
core isoGDGTs	0.4± 0	0.3± 0.1	0.4± 0.2	0.4± 0.1	0.8± 0.2	0.6± 0.1

Table S5. Occurrence of OH-isoGDGTs (+ = presence, - = absence) in thaumarchaeotal cultures studied for OH-isoGDGTs. Empty cells denote where information was not available.

Order	Genus & Species	Organism type	Growth temperatures reported (°C)	Optimum growth temperature (°C)	OH-isoGDGT detection	OH-isoGDGT core lipids	OH-isoGDGT IPLs	References
Nitrosopumilales	Nitrosopumilus *sp.* AR	Marine mesophile	25	25	+	OH-0 to OH-1	DH	Pitcher et al. (2011); Sinninghe Damsté et al. (2012)
	Nitrosopumilus *sp.* SJ	Marine mesophile	25	25	+	OH-0 to OH-2	DH	Pitcher et al. (2011); Sinninghe Damsté et al. (2012)
	Nitrosopumilus adriaticus NF5	Marine mesophile	30	30	+	OH-0 to OH-5	DH, MH	Elling et al. (2017)
	Nitrosopumilus piranensis D3C	Marine mesophile	30	32	+	OH-0 to OH-5	DH, MH	Elling et al. (2017)
	Nitrosopumilus maritimus SCM1	Marine mesophile	15, 20, 22, 25, 28, 30, 33, 35	28	+	OH-0 to OH-5	DH, MH	Elling et al. (2015, 2017)
	Ca. Nitrosopumilus strain NAOA2	Marine mesophile	18, 22, 28, 35	28	+	OH-0 to OH-4	DH, MH	Elling et al. (2015, 2017)
	Ca. Nitrosopumilus strain NAOA6	Marine mesophile	18, 22, 28	28	+	OH-0 to OH-5	DH, MH	Elling et al. (2015, 2017)
	Ca. Nitrosarchaeum limnium SFB1	Marine mesophile	22	22	+		DH	Pitcher et al. (2011); Sinninghe Damsté et al. (2012)
	Nitrosarchaeum koreense MY1	Soil mesophile	25	25	+	OH-0 to OH-2	DH	Sinninghe Damsté et al. (2012)
	Ca. Nitrosotenuis uzonensis N4	Terrestrial thermophile	37, 46, 50	46	-	-	-	Bale et al. (2019)
	Ca. Nitrosotenuis chungbukensis MY2	Soil mesophile	25	30	+			Bale et al. (2019)
	Ca. Nitrosotalea devanaterra Nd1	Soil acidophilic mesophile	25	25	+	OH-0 to OH-3	DH, MH	Elling et al. (2017)
Nitrososphaerales	*Ca.* Nitrososphaera gargensis Ga9.2	Terrestrial thermophile	35, 46	46	-	-	-	Elling et al. (2017)
	Nitrososphaera viennensis EN76	Soil mesophile	37	37	-	-	-	Sinninghe Damsté et al. (2012)
	Nitrososphaera viennensis EN123	Soil mesophile	37	37	-	-	-	Elling et al. (2017)

	Ca. Nitrososphaera strain JG1	Soil mesophile	37	37	-	-	-	Sinninghe Damsté et al. (2012)
	Ca. Nitrososcosmicus oleophius MY3	Terrestrial mesophile	30	30	+			Bale et al. (2019)
Ca. Nitrosocaldales	*Ca.* Nitrosocaldus yellowstonensis HL72	Terrestrial thermophile	72	72	-	-	-	Elling et al. (2017)
Nitrosopumilales	*Nitrosopumilus adriaticus* NF5	Marine mesophile	20, 25, 30	30	+	OH-0 to OH-4	DH, MH, HPH	This study
	Nitrosopumilus piranensis D3C	Marine mesophile	25, 30, 35	32	+	OH-0 to OH-4	DH, MH	This study
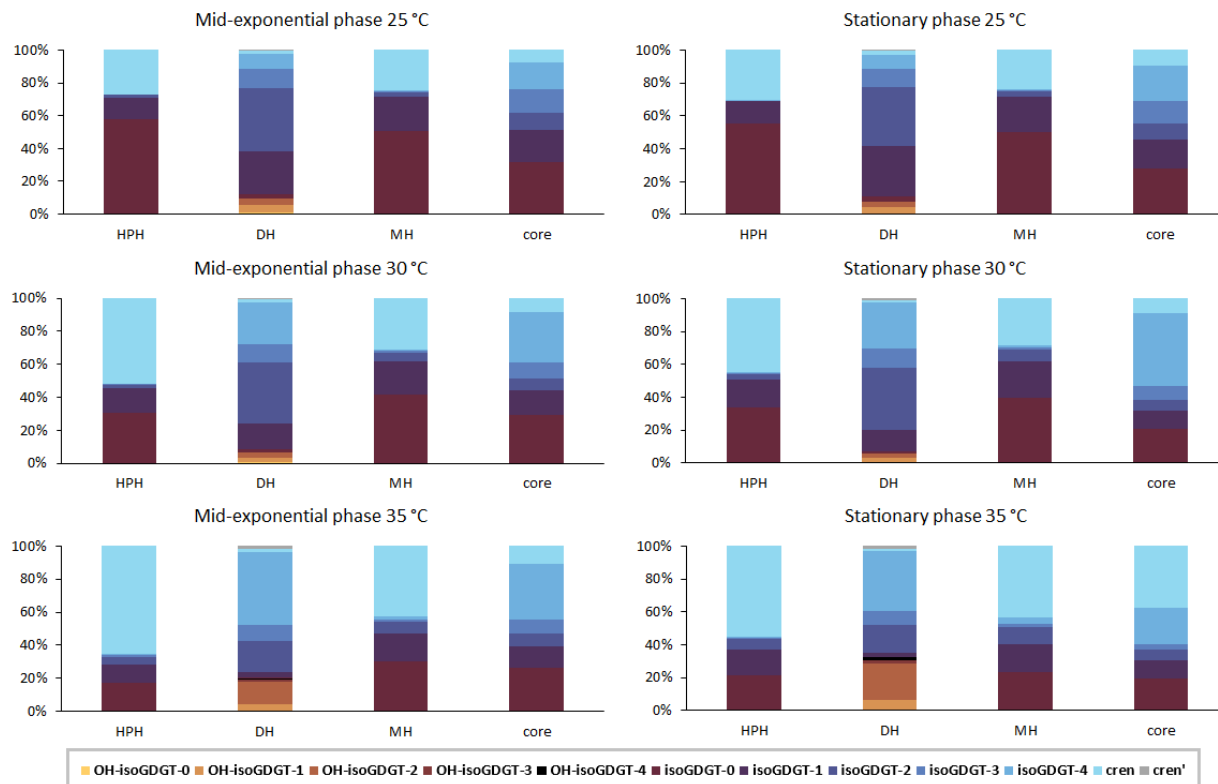
Figure S1. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from *Nitrosopumilus piranensis* D3C strain grown at 25, 30 and 35 °C and harvested at mid-exponential and stationary phases.
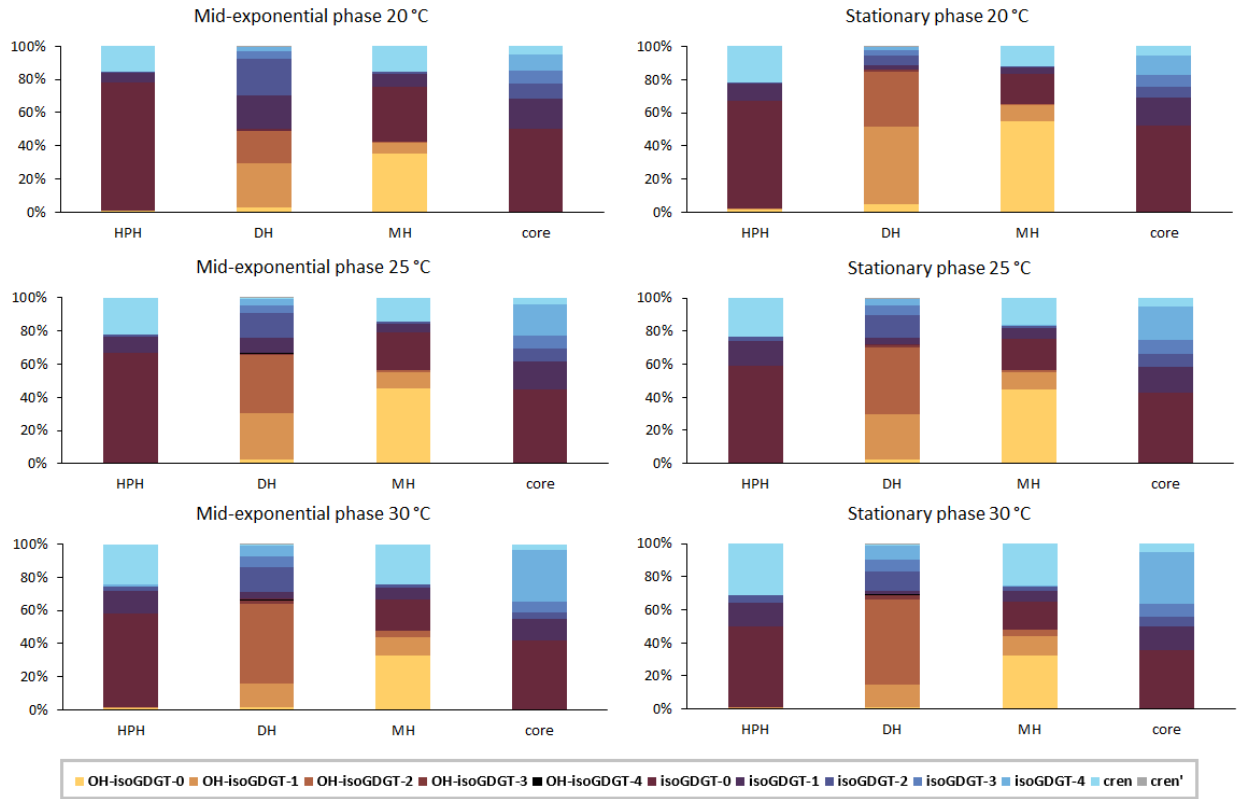
Figure S2. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from *Nitrosopumilus adriaticus* NF5 strain grown at 20, 25 and 30 °C and harvested at mid-exponential and stationary phases.
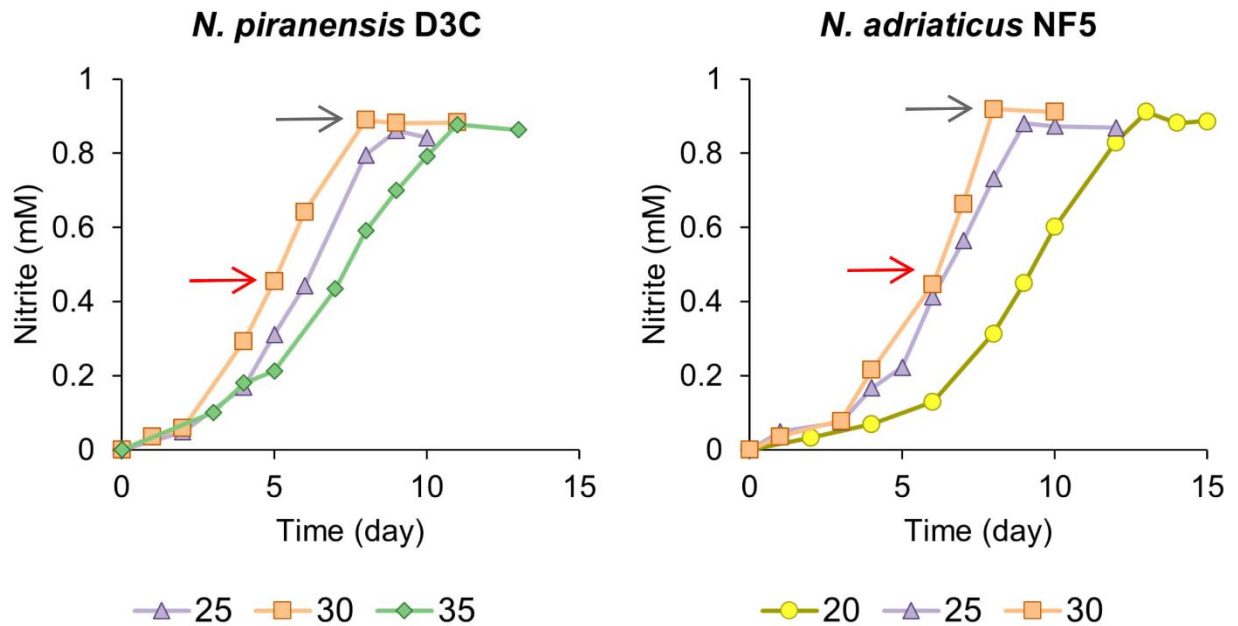
Figure S3. Nitrite production by cultures of *Nitrosopumilus piranensis* D3C strain and *Nitrosopumilus adriaticus* NF5 strain grown at different temperatures between 20 and 35 °C and harvested at mid-exponential phase as indicated by red arrows and stationary phase as indicated by grey arrows.
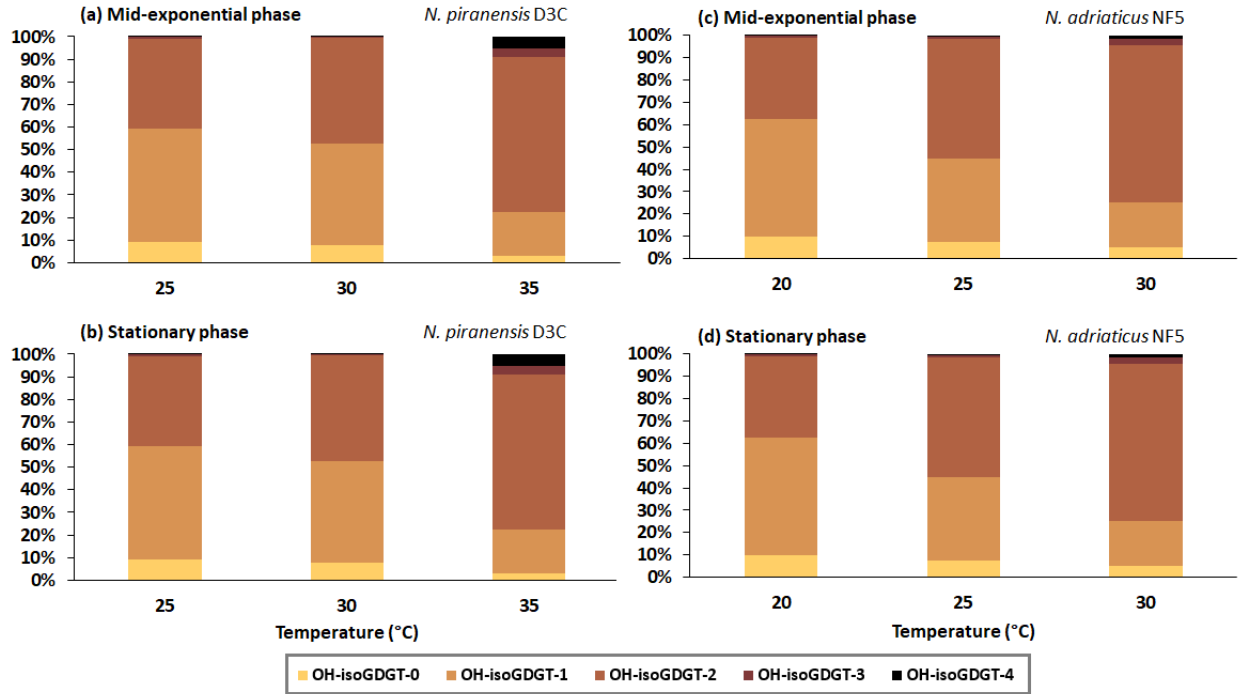
Figure S4. Core lipid composition from IPLs of OH-isoGDGTs from (a, b) *Nitrosopumilus piranensis* D3C strain and (c, d) *Nitrosopumilus adriaticus* NF5 strain grown at different temperatures and harvested at mid-exponential phase and stationary phase.
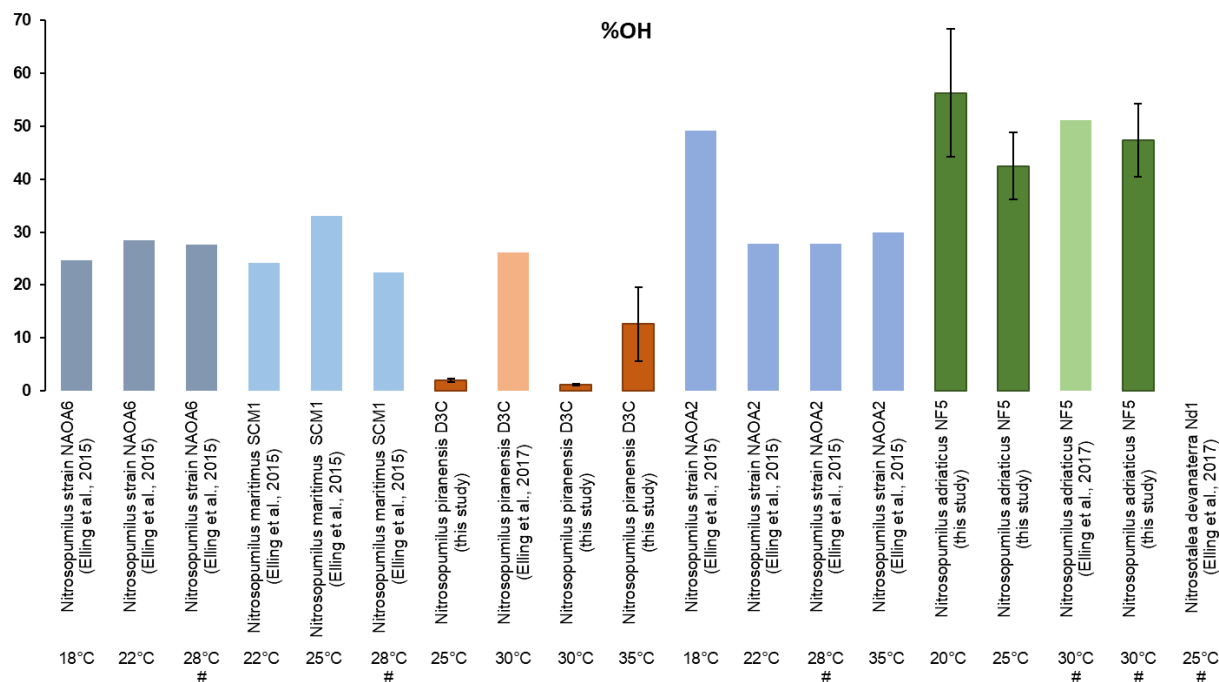
Figure S5. Relative abundance of OH-isoGDGTs relative to all isoGDGTs plus OH-isoGDGTs inferred from the IPLs of different cultures of Thaumarchaeota grown at different temperatures and harvested at stationary phase. Values from previous studies (Elling et al., 2015, 2017) were calculated according to IPL data reported. The optimal temperature of each strain is denoted by #. Error bars denote standard deviation for the triplicates used in this study. The bars were rearranged from Figure 4 according to more closely related strains (see phylogenetic analysis based on the 16S rRNA gene in Elling et al., 2017).
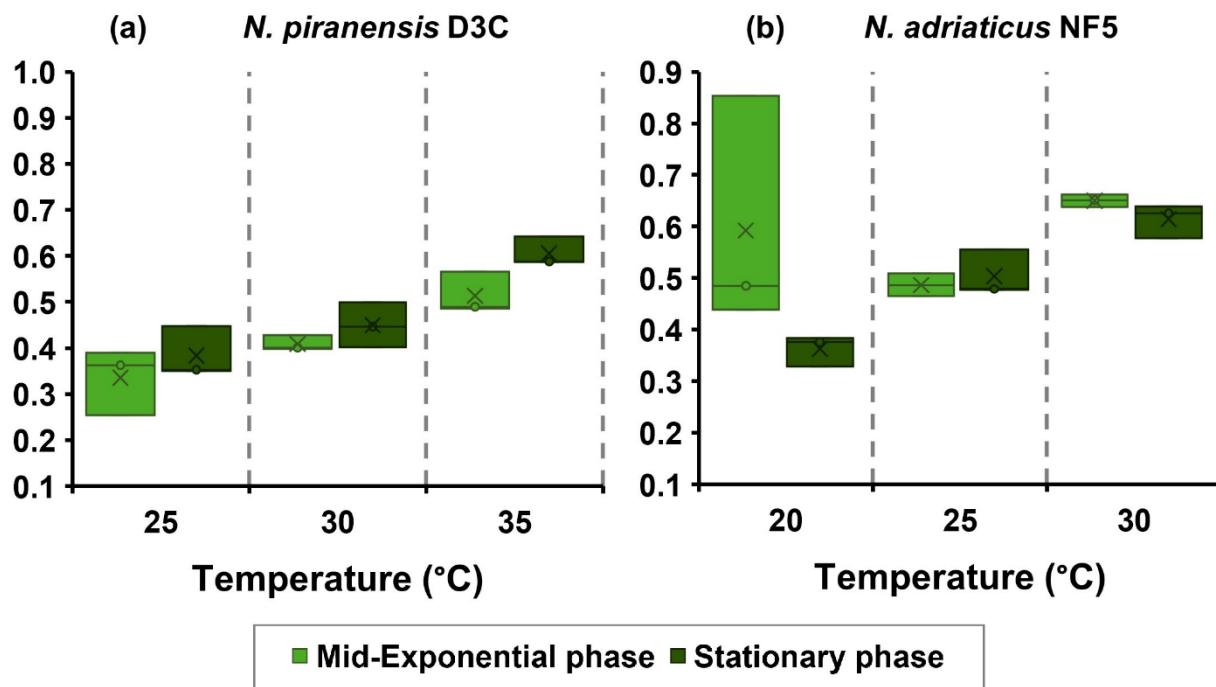
Figure S6. TEX_{86}^{OH} index with temperature calculated from IPLs of (a) *Nitrosopumilus piranensis* D3C strain and (b) *Nitrosopumilus adriaticus* NF5 strain, harvested at mid-exponential and stationary phases.
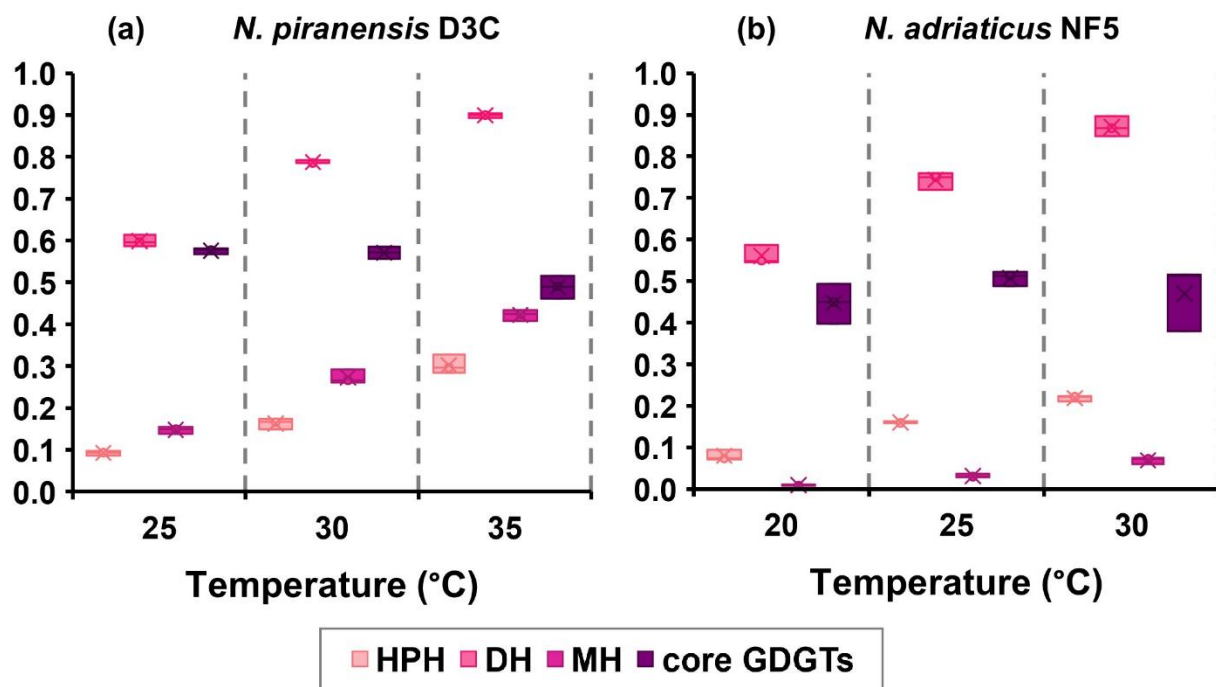
Figure S7. TEX$_{86}^{OH}$ index with temperature calculated for different headgroups of IPLs from (a) *Nitrosopumilus piranensis* D3C strain and (b) *Nitrosopumilus adriaticus* NF5 strain, harvested at stationary phase.