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*Supplement of*

# Controls on the composition of hydroxylated isoprenoidal glycerol dialkyl glycerol tetraethers (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-isoGD GT-0 | OH-isoGD GT-1 | OH-isoGD GT-2 | OH-isoGD GT-3 | OH-isoGD GT-4 | isoGD GT-0 | isoGD GT-1 | isoGD GT-2 | isoGD GT-3 | isoGD GT-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-isoGD GT-0 | OH-isoGD GT-1 | OH-isoGD GT-2 | OH-isoGD GT-3 | OH-isoGD GT-4 | isoGD GT-0 | isoGD GT-1 | isoGD GT-2 | isoGD GT-3 | isoGD GT-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) |
| | *Ca.* Nitrososphaera gargensis Ga9.2 | Terrestrial thermophile | 35, 46 | 46 | - | - | - | Elling et al. (2017) |
| *Nitrososphaerales* | *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 |

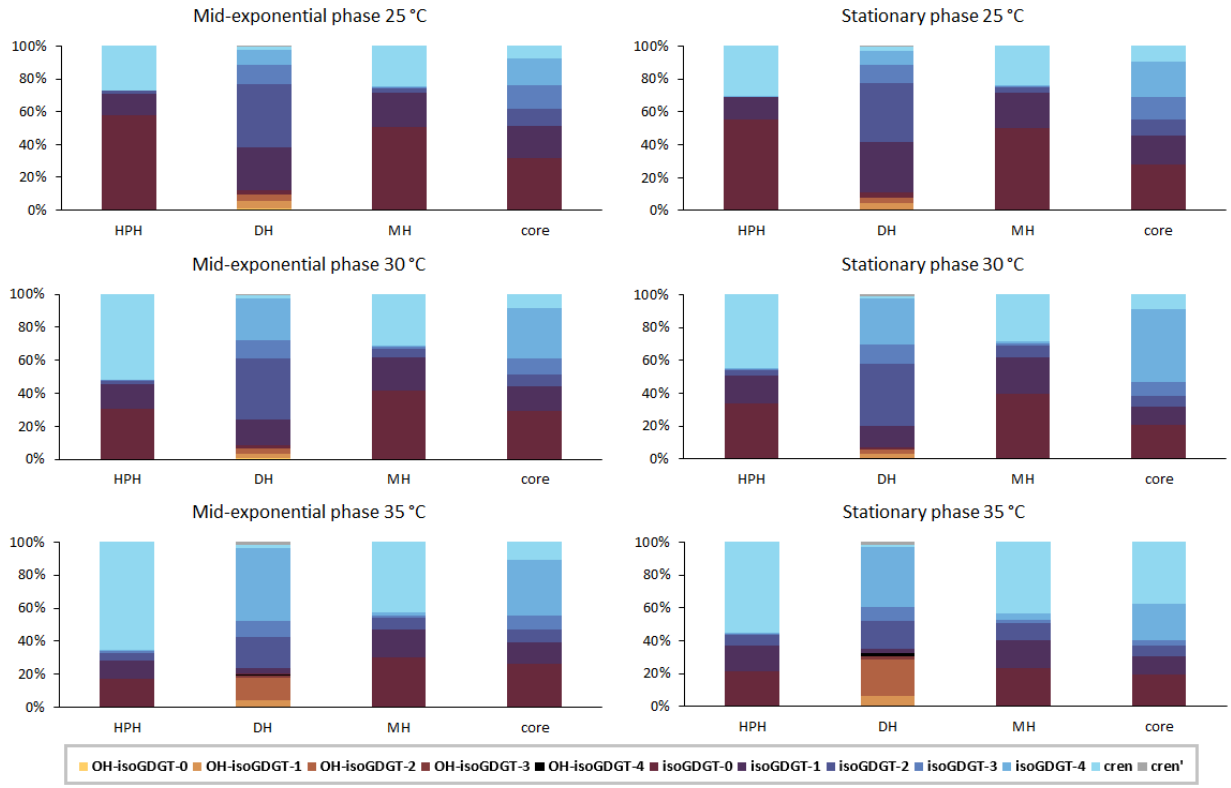


Figure S1. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from *Nitrosopumilus piranensis* D3C strain grown at 25, 30 and 35 °C and harvested at mid-exponential and stationary phases.

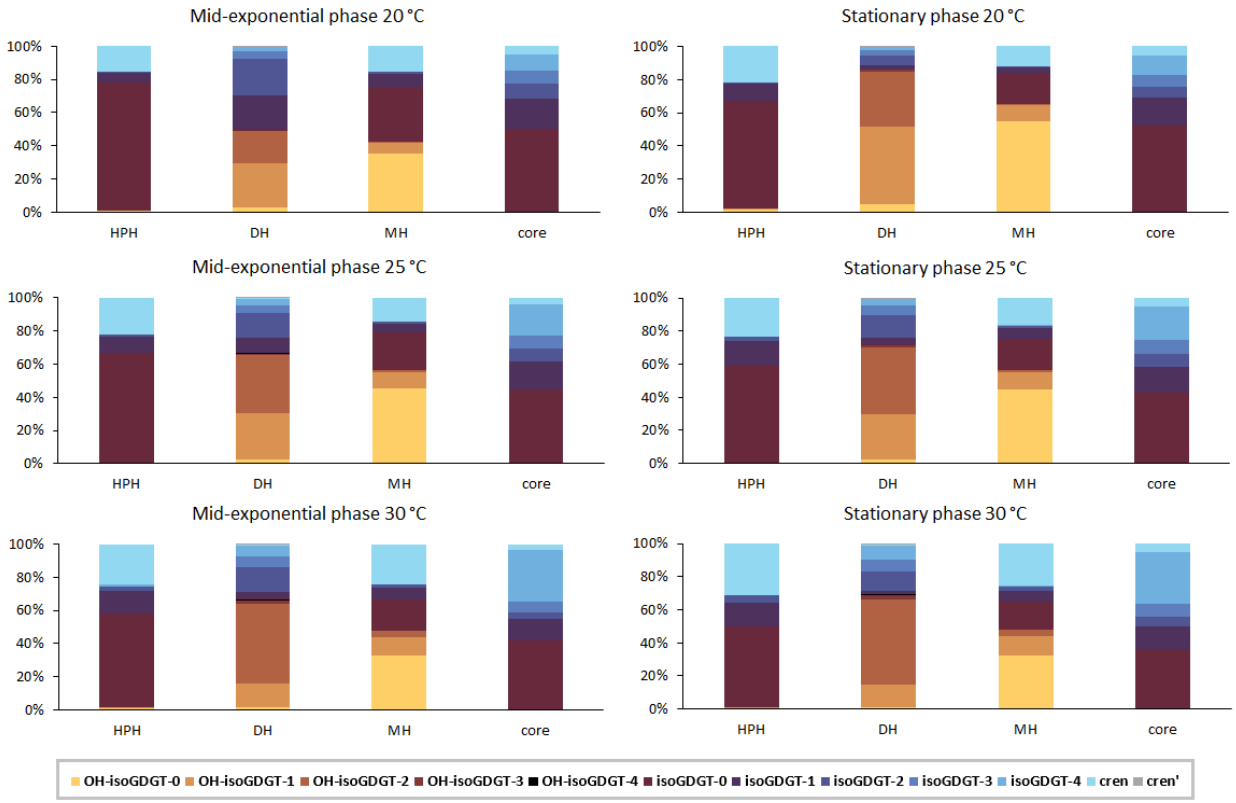


Figure S2. Relative abundance of isoGDGTs and OH-isoGDGTs per headgroup from *Nitrosopumilus adriaticus* NF5 strain grown at 20, 25 and 30 °C and harvested at mid-exponential and stationary phases.

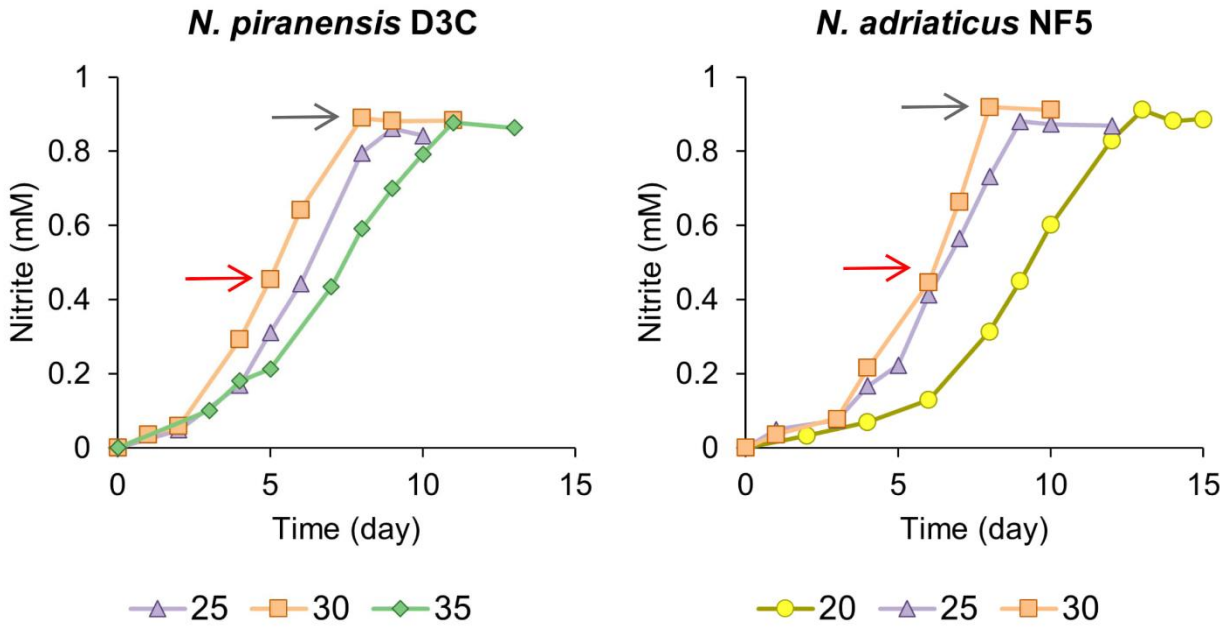

Figure S3. Nitrite production by cultures of *Nitrosopumilus piranensis* D3C strain and *Nitrosopumilus adriaticus* NF5 strain grown at different temperatures between 20 and 35 °C and harvested at mid-exponential phase as indicated by red arrows and stationary phase as indicated by grey arrows.

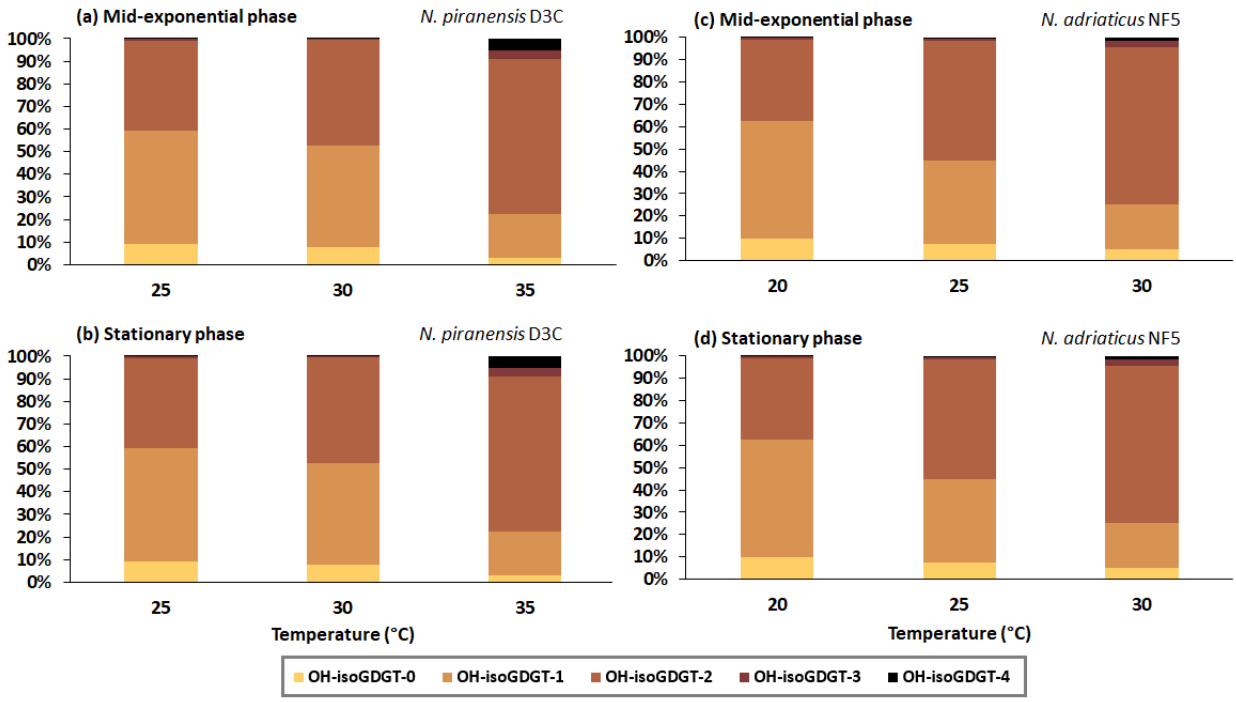


Figure S4. Core lipid composition from IPLs of OH-isoGDGTs from (a, b) *Nitrosopumilus piranensis* D3C strain and (c, d) *Nitrosopumilus adriaticus* NF5 strain grown at different temperatures and harvested at mid-exponential phase and stationary phase.

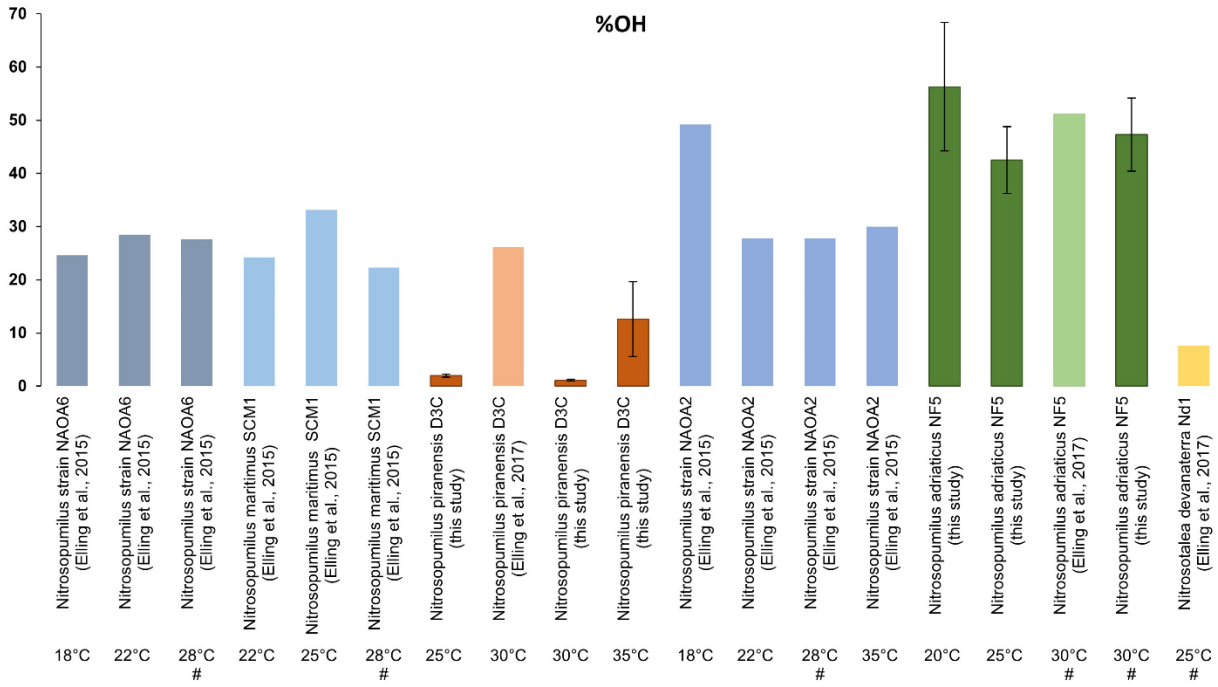


Figure S5. Relative abundance of OH-isoGDGTs relative to all isoGDGTs plus OH-isoGDGTs inferred from the IPLs of different cultures of Thaumarchaeota grown at different temperatures and harvested at stationary phase. Values from previous studies (Elling et al., 2015, 2017) were calculated according to 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).

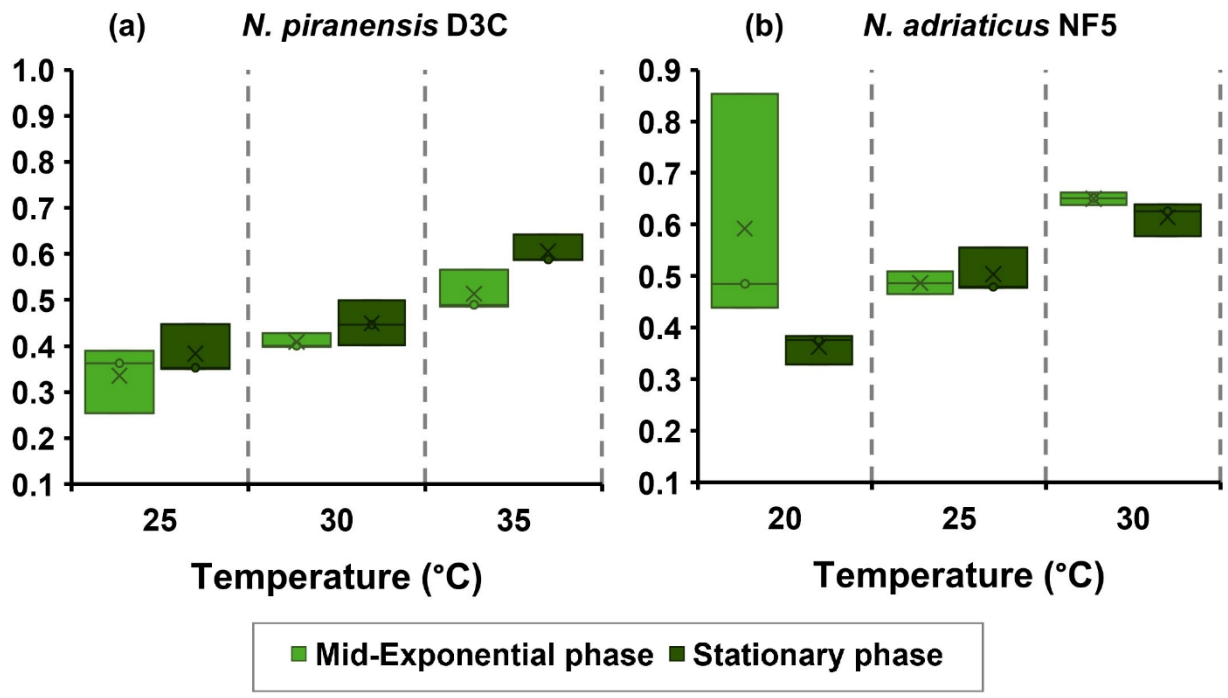


Figure S6. $TEX_{86}^{OH}$ index with temperature calculated from IPLs of (a) *Nitrosopumilus piranensis* D3C strain and (b) *Nitrosopumilus adriaticus* NF5 strain, harvested at mid-exponential and stationary phases.

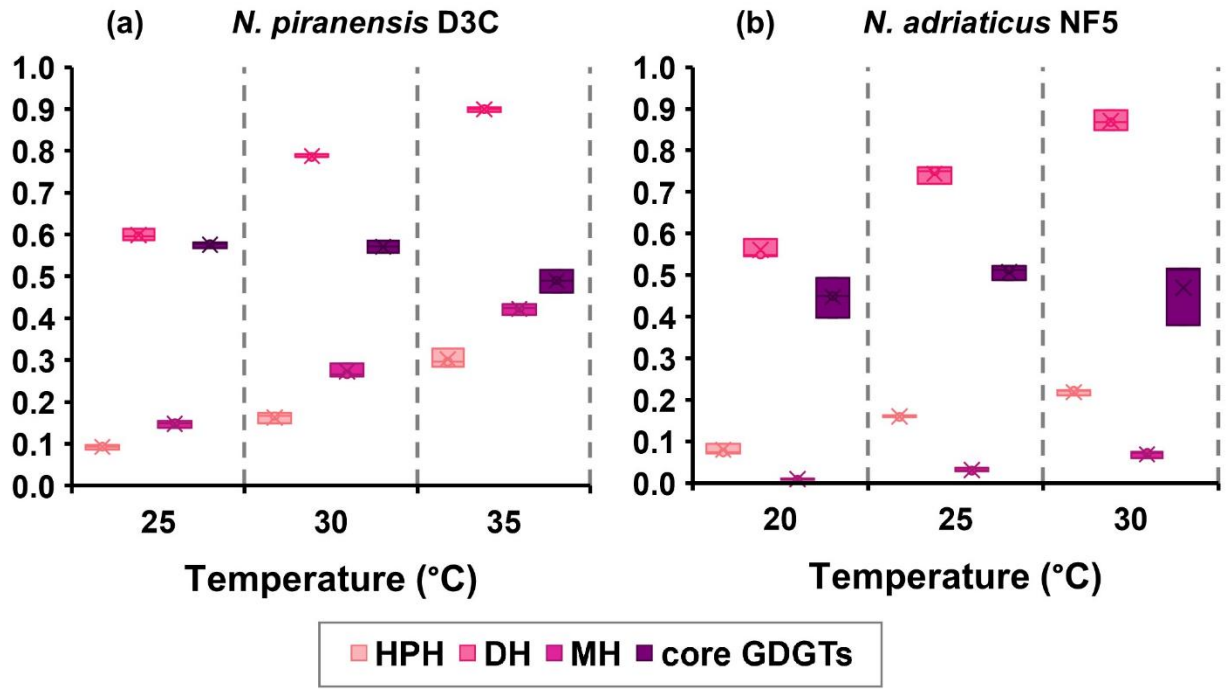

Figure S7. $TEX_{86}^{OH}$ index with temperature calculated for different headgroups of IPLs from (a) *Nitrosopumilus piranensis* D3C strain and (b) *Nitrosopumilus adriaticus* NF5 strain, harvested at stationary phase.