**The response of estuarine ammonia-oxidizing communities to constant and fluctuating salinity regimes**

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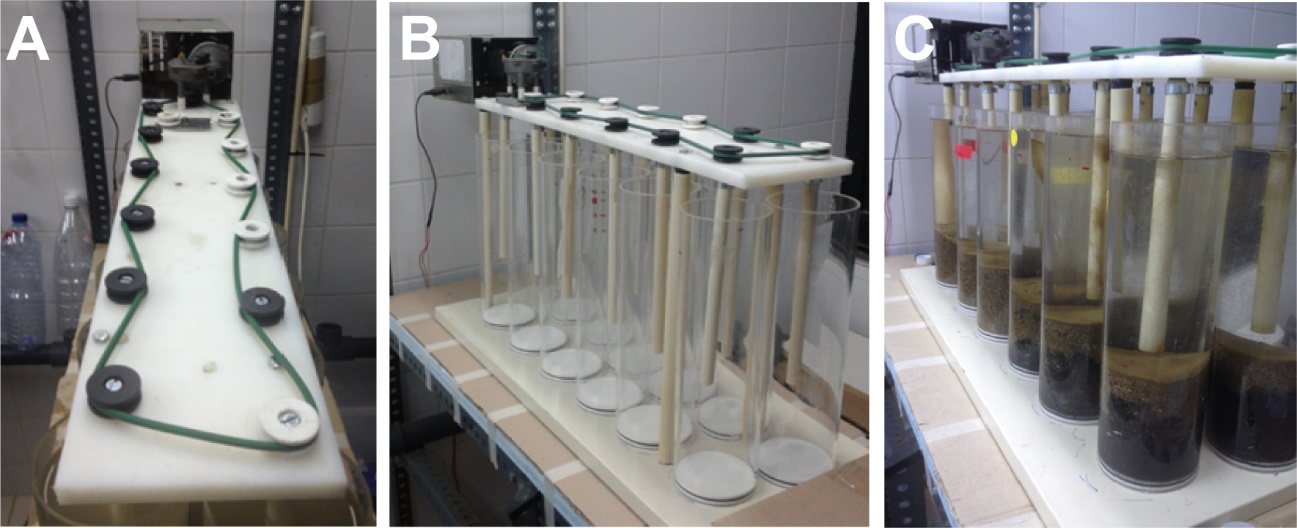
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Supplementary Figures

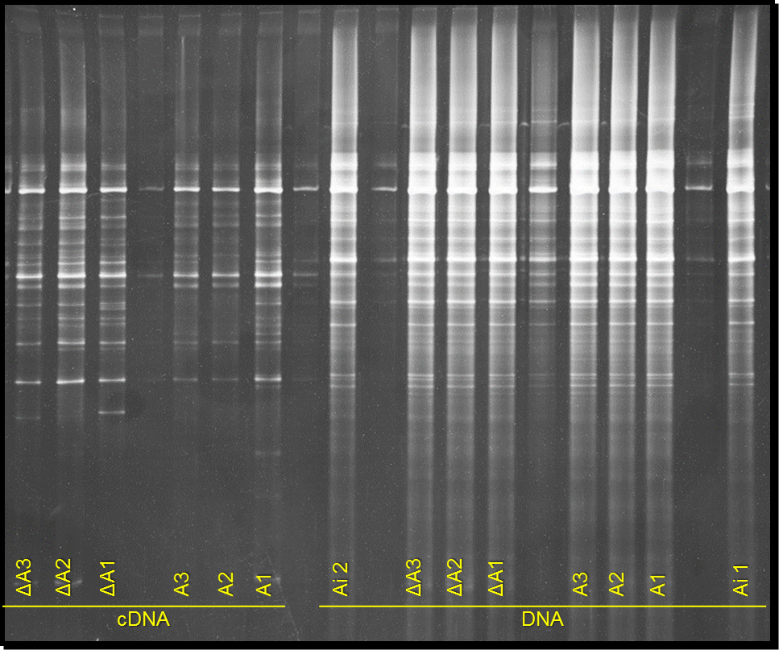


**Figure S1.** Pictures of the upper part (**Figure S1-A**) of the autonomous water agitation system for 12 acrylic reactors before (**Figure S1-B**) and during the incubation (**Figure S1-C**) experiment.

**Figure S2.** Hierarchical cluster analysis using the average linkage of Bray-Curtis similarity for the presence or absence of archaeal *amoA* transcripts (cDNA) and genes (DNA) under different salinity treatments (constant – CR\_C and AF\_C, and fluctuation – CR\_Δ and AF\_Δ) from Afurada (**Figure S2-A**) and Crestuma (**Figure S2-B**) sediments. Archaeal *amoA* genes include initial *in situ* sediment (AF\_i and CR\_i samples). The relative “richness” of *amoA* transcripts and genes is given based on the number of bands of each PCR-DGGE and RT-PCR-DGGE profile generated (gray bars).



**Figure S3.** Hierarchical cluster analysis using the average linkage of Bray Curtis similarities for the presence or absence of *β-*proteobacterial *amoA* transcripts (cDNA) and genes (DNA) under different salinity treatments (constant – CR\_C and AF\_C, and fluctuation – CR\_Δ and AF\_Δ) from Afurada (**Figure S3-A**) sediments and only proteobacterial *amoA* genes for Crestuma (**Figure S3-B**) sediments. Proteobacterial *amoA* genes include initial *in situ* sediment (AF\_i and CR\_i samples). The relative “richness” of *amoA* transcripts and genes is given based on the number of bands of each PCR-DGGE and RT-PCR DGGE profile generated (gray bars).



**AF\_Δ1**

**AF\_Δ2**

**AF\_Δ3**

**AF\_C3**

**AF\_C2**

**AF\_C1**

**AF\_i2**

**AF\_C3**

**AF\_C2**

**AF\_C1**

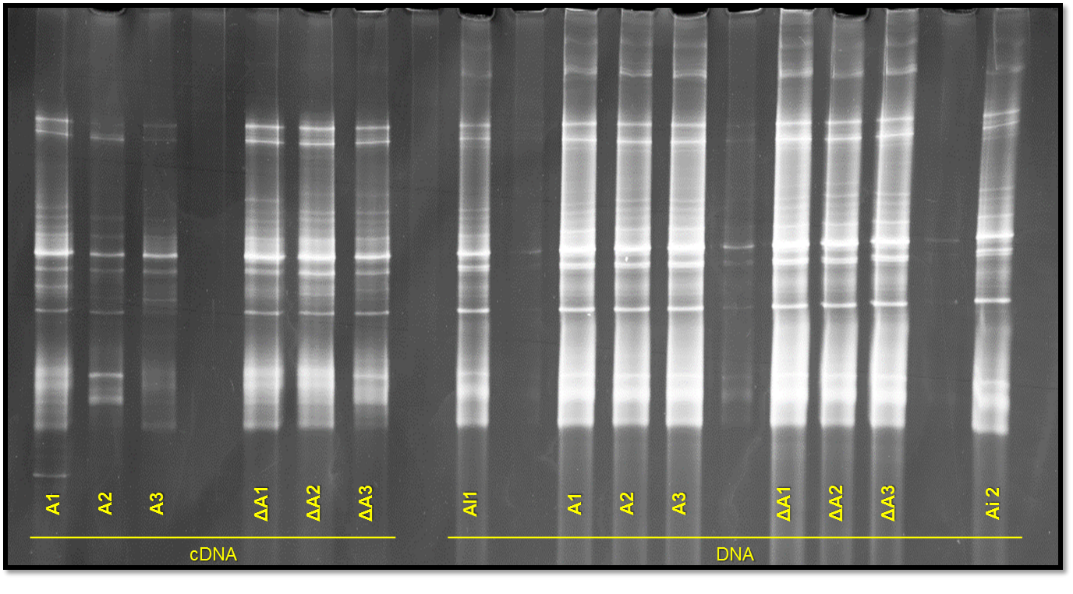
**AF\_Δ1**

**AF\_Δ2**

**AF\_Δ3**

**AF\_i1**

**Figure S4.** Image of the DGGE gel of *amoA* gene fragments and transcripts amplified from ammonia-oxidizing archaea on Afurada sediment samples exposed to different salinity conditions (constant salinity (AF\_C1, AF\_C2 and AF\_C3) and fluctuation salinity (AF\_ Δ1, AF\_Δ2 and AF\_Δ3)) as well initial Afurada sediment (AF\_i1 and AF\_i2).



**AF\_C1**

**AF\_C2**

**AF\_C3**

**AF\_ Δ 1**

**AF\_ Δ 2**

**AF\_ Δ 3**

**AF\_i1**

**AF\_ Δ 1**

**AF\_ Δ 2**

**AF\_ Δ3**

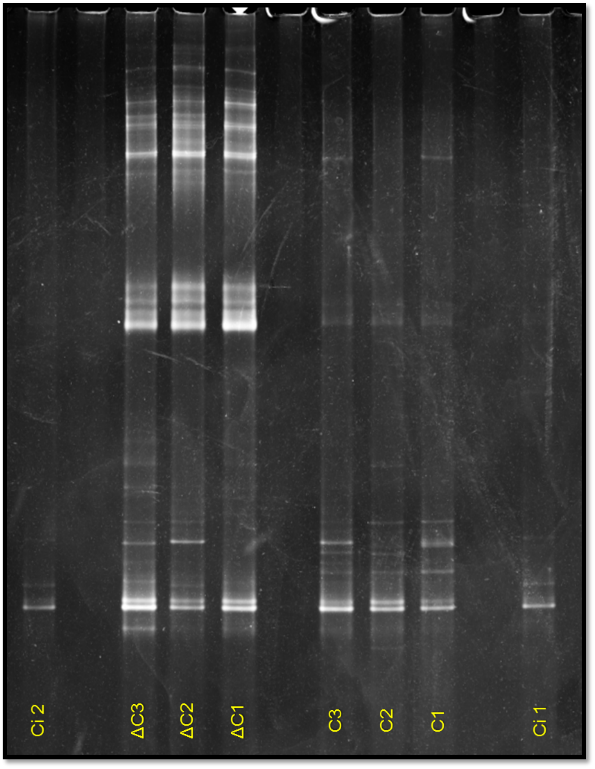
**AF\_C3**

**AF\_C2**

**AF\_C1**

**AF\_i2**

**Figure S5.** Image of the DGGE gel of *amoA* gene fragments and transcripts amplified from ammonia-oxidizing bacteria on Afurada sediment samples exposed to different salinity conditions (constant salinity (AF\_C1, AF\_C2 and AF\_C3) and fluctuation salinity (AF\_Δ1, AF\_Δ2 and AF\_Δ3)) as well initial Afurada sediment (AF\_i1 and AF\_i2).



**B**

**CR\_i1**

**CR\_i2**

**CR\_C1**

**CR\_C2**

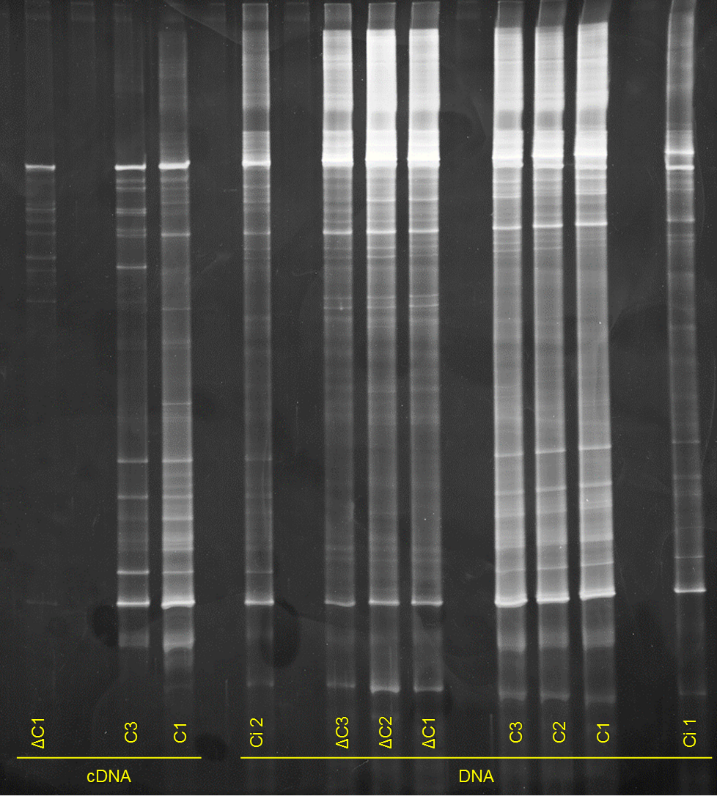
**CR\_C3**

**CR\_ Δ3**

**CR\_ Δ2**

**CR\_ Δ1**

**DNA**



**CR\_C1**

**CR\_C3**

**CR\_i2**

**CR\_C1**

**CR\_C2**

**CR\_C3**

**CR\_i1**

**CR\_ Δ 2**

**CR\_ Δ3**

**CR\_ Δ1**

**CR\_ Δ1**

**A**

**Figure S6.** Image of the DGGE gel of *amoA* gene fragments and transcripts (**Figure S6-A**) amplified from ammonia-oxidizing archaea on Crestuma sediment samples exposed to different salinity conditions (constant salinity (CR\_1, CR\_2 and CR\_3) and fluctuation salinity (CR\_Δ1, CR\_Δ2 and CR\_Δ3)) as well initial Crestuma sediment (CR\_i1 and CR\_i2). Image of the DGGE gel of *amoA* gene fragments (**Figure S6-B**) amplified from ammonia-oxidizing bacteria on Crestuma sediment samples exposed to different salinity conditions (constant salinity (CR\_1, CR\_2 and CR\_3) and fluctuation salinity (CR\_Δ1, CR\_Δ2 and CR\_Δ3)) as well initial Crestuma sediment (CR\_i1 and CR\_i2).



**Figure S7.** Box plot representing the average relative abundance of the different ammonia-oxidizing genera identified in the different salinity treatments (constant – AF\_C and CR\_C, and fluctuation – AF\_Δ and CR\_Δ; n=2).

Supplementary Tables

**Table S1.** Salinity and temperature mean values from 36 days of daily monitoring in constant salinity treatments reactors from Afurada (AF\_C1, AF\_C2 and AF\_C3) and Crestuma (CR\_C1, CR\_C2 and CR\_C3) and in fluctuation salinity reactors from Afurada (AF\_Δ1, AF\_Δ2 and AF\_Δ3) and Crestuma (CR\_Δ1, CR\_Δ2 and CR\_Δ3).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Reactor** | **Number of water  renewal’s** | **Salinity (psu)** | | | | **Temperature (°C)** | |
| **Mean** | **Min** | **Max** | **SD** | **Mean** | **SD** |
| AF\_C1 | 69 | 15.3 | 15.0 | 16.1 | 0.2 | 18.8 | 2.2 |
| AF\_C2 | 69 | 15.3 | 15.0 | 16.1 | 0.2 | 18.8 | 2.2 |
| AF\_C3 | 69 | 15.3 | 15.0 | 16.1 | 0.2 | 18.8 | 2.2 |
| AF\_∆1 | 69 | 15.5 | 1.5 | 29.2 | 9.4 | 18.8 | 2.2 |
| AF\_∆2 | 69 | 15.5 | 1.5 | 29.3 | 9.5 | 18.8 | 2.2 |
| AF\_∆3 | 69 | 15.5 | 1.5 | 29.1 | 9.4 | 18.8 | 2.2 |
| CR\_C1 | 69 | 0.2 | 0.1 | 1.7 | 0.2 | 18.8 | 2.2 |
| CR\_C2 | 69 | 0.2 | 0.1 | 1.4 | 0.2 | 18.8 | 2.2 |
| CR\_C3 | 69 | 0.1 | 0.1 | 1.3 | 0.2 | 18.8 | 2.2 |
| CR\_∆1 | 69 | 15.5 | 1.4 | 29.3 | 9.5 | 18.8 | 2.2 |
| CR\_∆2 | 69 | 15.4 | 1.3 | 29.3 | 9.5 | 18.8 | 2.2 |
| CR\_∆3 | 69 | 15.5 | 1.2 | 29.3 | 9.6 | 18.8 | 2.2 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Site** | **Incubation condition** | **ID** | **µmol N mL-1 cm-2 h-1** |
| Afurada | Constant salinity | AF\_C1 | 0.0048 |
| AF\_C2 | 0.0036 |
| AF\_C3 | 0.0036 |
| Salinity fluctuation | AF\_Δ1 | 0.0033 |
| AF\_Δ2 | 0.0035 |
| AF\_Δ3 | 0.0040 |
| Crestuma | Constant salinity | CR\_C1 | 0.0048 |
| CR\_C2 | 0.0062 |
| CR\_C3 | 0.0055 |
| Salinity fluctuation | CR\_Δ1 | 0.0014 |
| CR\_Δ2 | N/A |
| CR\_Δ3 | 0.0013 |

**Table S2**. Potential nitrification rates ([15N]O2- + [15N]O3- produced) in triplicate treatments after the addition of 20 µM of [15N]H4+ in Afurada (AF\_C and and AF\_Δ) and Crestuma (CF\_C and and CR\_Δ) reactors. N/A – not available.

**Table S3**. Potential nitrification rates ([15N]O2- + [15N]O3- produced) measured in triplicate slurries after the addition of 100 µM of [15N]H4+ in Afurada (AF\_C and and AF\_Δ) and Crestuma (CF\_C and and CR\_Δ) salinity treatments.

|  |  |  |  |
| --- | --- | --- | --- |
| **Site** | **Incubation condition** | **ID** | **µmol N mL-1 g-1 h-1** |
| Afurada | Constant salinity | AF\_C1 | 0.0027 |
| AF\_C2 | 0.0029 |
| AF\_C3 | 0.0030 |
| Salinity fluctuation | AF\_Δ1 | 0.0051 |
| AF\_Δ2 | 0.0029 |
| AF\_Δ3 | 0.0024 |
| Crestuma | Constant salinity | CR\_C1 | 0.0035 |
| CR\_C2 | 0.0027 |
| CR\_C3 | 0.0028 |
| Salinity fluctuation | CR\_Δ1 | 0.0014 |
| CR\_Δ2 | 0.0012 |
| CR\_Δ3 | 0.0013 |

**Table S4.** Relative abundance (%) of the top 20 dominant phyla in downstream (AF) and upstream (CR) *in situ* sediments (AF\_i, CR\_i) and laboratory experiments (AF\_C, AF\_Δ, CR\_C and CR\_Δ). The values correspond to the relative abundance of the two replicates (1 and 2) analyzed per each salinity treatment (CR\_C, CR\_Δ, AF\_C and AF\_Δ).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Phylum** | **Relative abundance (%) in the different treatments** | | | | | | | | | |
| **AF\_i** | **AF\_C1** | **AF\_C2** | **CR\_i** | **CR\_C2** | **CR\_C3** | **AF\_Δ1** | **AF\_ Δ2** | **CR\_ Δ1** | **CR\_Δ2** |
| *Proteobacteria* | 39.5 | 47.2 | 50.1 | 41.4 | 45.9 | 46.3 | 45.9 | 53.4 | 70.8 | 61.9 |
| *Bacteroidetes* | 31.0 | 12.5 | 14.5 | 23.5 | 15.3 | 13.8 | 12.8 | 13.5 | 14.3 | 15.7 |
| *Planctomycetes* | 3.7 | 12.5 | 8.5 | 7.6 | 9.6 | 9.2 | 13.6 | 7.2 | 6.1 | 7.6 |
| *Cyanobacteria* | 14.8 | 7.2 | 6.7 | 5.7 | 1.0 | 0.7 | 14.9 | 9.1 | 0.8 | 0.6 |
| *Acidobacteria* | 1.7 | 5.2 | 4.7 | 5.7 | 7.9 | 7.6 | 2.0 | 3.8 | 1.5 | 3.1 |
| *Nitrospirae* | 1.0 | 1.8 | 2.1 | 3.0 | 7.1 | 10.7 | 1.5 | 1.5 | 1.0 | 1.9 |
| *Verrucomicrobia* | 2.0 | 0.7 | 0.8 | 5.0 | 3.8 | 3.2 | 0.5 | 0.8 | 0.7 | 1.6 |
| *Thaumarchaeota* | 2.3 | 3.7 | 3.2 | 0.4 | 0.4 | 0.7 | 3.0 | 2.4 | 0.5 | 0.4 |
| *Gemmatimonadetes* | 0.3 | 1.3 | 1.1 | 2.7 | 3.0 | 2.1 | 0.6 | 1.0 | 2.2 | 3.3 |
| *Chloroflexi* | 1.4 | 2.1 | 2.1 | 1.1 | 1.7 | 1.9 | 1.9 | 2.4 | 0.3 | 0.9 |
| *Actinobacteria* | 0.9 | 1.4 | 1.3 | 1.0 | 1.0 | 0.8 | 1.0 | 1.5 | 0.4 | 1.2 |
| *Latescibacteria* | 0.3 | 1.1 | 1.0 | 0.3 | 1.0 | 1.0 | 0.4 | 0.8 | 0.2 | 0.3 |
| *Armatimonadetes* | 0.1 | 0.0 | 0.0 | 0.9 | 0.5 | 0.5 | 0.0 | 0.0 | 0.1 | 0.2 |
| *Firmicutes* | 0.2 | 0.4 | 0.5 | 0.1 | 0.1 | 0.0 | 0.2 | 0.3 | 0.0 | 0.0 |
| *Spirochaetes* | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.0 | 0.0 |
| *Lentisphaerae* | 0.1 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 | 0.1 | 0.1 | 0.3 | 0.3 |
| *Kiritimatiellaeota* | 0.1 | 0.3 | 0.4 | 0.1 | 0.0 | 0.0 | 0.2 | 0.3 | 0.0 | 0.0 |
| *Deinococcus-Thermus* | 0.1 | 0.1 | 0.1 | 0.3 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 | 0.1 |
| *Rokubacteria* | 0.0 | 0.0 | 0.0 | 0.2 | 0.1 | 0.2 | 0.0 | 0.0 | 0.1 | 0.2 |
| *Omnitrophicaeota* | 0.0 | 0.1 | 0.1 | 0.1 | 0.3 | 0.2 | 0.0 | 0.1 | 0.0 | 0.0 |
| Other | 0.5 | 2.1 | 2.4 | 0.7 | 1.3 | 1.1 | 1.2 | 1.5 | 0.3 | 0.6 |

**Table S5.** Relative abundance (%) of the nitrifying genera found in downstream (AF) and upstream (CR) *in situ* sediments (AF\_i, CR\_i) and laboratory experiments (AF\_C, AF\_Δ, CR\_C and CR\_Δ). The values correspond to the relative abundance of the two replicates (1 and 2) analyzed per each salinity treatment (CR\_C, CR\_Δ, AF\_C and AF\_Δ).

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Group** | **Family** | **Genus** | **Relative abundance (%) in the different treatments** | | | | | | | | | |
| **AF\_i** | **AF\_C1** | **AF\_C2** | **CR\_i** | **CR\_C2** | **CR\_C3** | **AF\_Δ1** | **AF\_Δ2** | **CR\_C1** | **CR\_C2** |
| **AOB** | Nitrosomonadaceae | *Nitrosomonas* | 0.2 | 0.3 | 0.4 | 0.0 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 |
| **AOA** | Nitrosopumilaceae | "*Ca*. Nitrosotenuis" | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 0.3 | 8.8 | 0.0 | 0.0 | 0.0 |
| "*Ca*. Nitrosopumilus" | 0.8 | 2.1 | 1.9 | 0.0 | 0.0 | 0.0 | 1.5 | 1.3 | 0.1 | 0.0 |
| "*Ca*. Nitrosoarchaeum" | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.3 | 0.1 | 0.1 | 0.4 | 0.3 |
| **NOB** | Nitrospiraceae | *Nitrospina* | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Nitrospira* | 1.0 | 1.8 | 2.1 | 3.0 | 7.1 | 10.7 | 1.5 | 1.5 | 1.0 | 1.9 |