Supplementary Information

**Effects of Environmental Variables on Abundance of**

**Ammonia-oxidizing Communities in Sediments of**

**Luotian River, China**

Amjed Ginawi1,2, Lixiao Wang1, Wang Huading1, Yu Bingbing1, Yunjun Yan1,\*

1 Key Lab of Molecular Biophysics of Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan, 430074, China.

2 Faculty of Marine Science and Fisheries, Red Sea University, Port Sudan, Sudan.

 amjedginawi@rsu.edu.sd (A.G.); zdwanglixiao@163.com (L.W.); huadingwang@hust.edu.cn  (H.W.); bingbingyu@hust.edu.cn (B.Y.).

**\*** Correspondence: yanyunjun@hust.edu.cn; Tel.: +86-27-87792213

**Table S1.** The PCR primer sets and thermal profiles

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target gene** | **Primer** | **Thermal program** | **Size (bp)** | **References** |
| 16S rRNA | 27F 5’-AGAGTTTGATCCTGGCTCAG-3’1492R 5’-(S)\*ACGGCTACCTTGTTACGACT-3’ | 5 min at 95°C, one cycle 1min a 95°C, 45s at 57°C, 60s at 72°C, 30 cycles8 min at 72°C, one cycle | 1500 | (Lane 1991) |
| Bacterial 16S rRNA gene- qPCR | 27F 5’-AGAGTTTGATCCTGGCTCAG-3’16S-rtR 5’-GCTGCCTCCCGTAGGAGT-3’ | 10 min at 95°C, one cycle60s at 95°C, 30 s at 60°C, 30 s at 72°C, 40 cycles, 8min at 72°C, one cycle | 310 | (Amann et al. 1990) |
| AOA | Arch *amoA* F: 5’-TAATGGTCTGGCTTAGACG-3’Arch *amoA* R: 5’-GCGGCCATCCATCTGTATGT-3’ | 5 min at 95 °C, 35 cycles of 45 s at 94 °C, 60 s at 53 °C, and 60 s at 72 °C, followed by 15 min at 72 °C. | 635 | (Francis et al. 2005) |
| AOB | *amoA*-1F: 5’-GGGGTTTCTACTGGTGGT-3’*amoA*-2R : 5’-CCCCTCTGGAAAGCCTTCTTC-3’ | 5 min at 95 °C, 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C, and a final cycle consisting of 60 s at 60 °C and 10 min at 72 °C.  | 491 | (Rotthauwe et al. 1997) |

\*S= G or C, PCR: polymerase chain reaction, AOA: ammonia-oxidizing archaea, and AOB: ammonia-oxidizing bacteria.

**Table S2.** The gene copy numbers of 16S rRNA, AOA, and AOB in sampling sites. Values and standard deviations were estimated from triplicate an analysis within a triplicate qPCR.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **May** | **August** | **October** |
| **Sampling Sites** | **16S rRNA (copies/μg of DNA extract)** | **AOA (copies/μg of DNA extract)** | **AOB (copies/μg of DNA extract)** | **16S rRNA (copies/μg of DNA extract)** | **AOA (copies/μg of DNA extract)** | **AOB (copies/μg of DNA extract)** | **16S rRNA (copies/μg of DNA extract)** | **AOA (copies/μg of DNA extract)** | **AOB (copies/μg of DNA extract)** |
| S1 | 1.56E+09 | 5.23E+08 | 4.64E+08 | 1.19E+09 | 4.79E+08 | 2.03E+08 | 1.98E+09 | 3.85E+08 | 4.63E+08 |
| S2 | 6.79E+08 | 5.03E+08 | 2.55E+08 | 6.79E+08 | 5.23E+08 | 3.24E+08 | 6.09E+08 | 5.21E+08 | 3.83E+08 |
| S3 | 6.17E+08 | 3.22E+08 | 3.36E+08 | 6.89E+08 | 2.23E+08 | 3.45E+08 | 6.04E+08 | 3.69E+08 | 3.25E+08 |
| S4 | 3.83E+08 | 3.19E+08 | 3.30E+07 | 3.87E+08 | 2.83E+08 | 3.60E+08 | 3.08E+08 | 3.92E+08 | 2.66E+08 |
| S5 | 7.42E+08 | 4.41E+08 | 5.45E+08 | 7.62E+08 | 4.25E+08 | 5.05E+08 | 7.40E+08 | 4.08E+08 | 4.97E+08 |
| S6 | 1.01E+09 | 5.27E+08 | 1.23E+08 | 1.25E+09 | 5.13E+08 | 1.08E+08 | 1.31E+09 | 5.22E+08 | 1.25E+08 |
| S7 | 6.51E+08 | 5.28E+08 | 4.79E+08 | 6.68E+08 | 5.02E+08 | 4.53E+08 | 6.07E+08 | 5.24E+08 | 4.49E+08 |
| S8 | 7.17E+08 | 4.00E+08 | 5.09E+08 | 7.79E+08 | 4.43E+08 | 5.05E+08 | 7.17E+08 | 4.68E+08 | 5.11E+08 |
| S9 | 5.85E+08 | 3.78E+08 | 4.56E+08 | 4.91E+08 | 3.82E+08 | 4.60E+08 | 5.85E+08 | 4.74E+08 | 4.58E+08 |

Table S3. Comparison of AOA and AOB diversities as well as coverage estimates in sampling sites base on 95% amino acid residue cutoff.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **AOA** |  | **AOB** |
| **Sampling sites** | **No. of clone sequenced** | **No. of OTUs\*** | **No. of clone sequenced** | **No. of OTUs\*** |
| S1 | 4 | 4 | 8 | - |
| S2 | 5 | 4 | 6 | - |
| S3 | 5 | 1 | 5 | - |
| S4 | 5 | 1 | 4 | - |
| S5 | 6 | 4 | 9 | - |
| S6 | 5 | 1 | 5 | - |
| S7 | 5 | 4 | 7 | 1 |
| S8 | 14 | 12 | 9 | 1 |
| S9 | 5 | 3 | 8 | 1 |
| Total | 54 | 34 | 61 | 3 |

–, undetected

\*Unique OTUs of the *amoA* sequences were determined using the DOTUR program.

Table S4. T-RF fragments and their corresponding clones of AOA

|  |  |  |
| --- | --- | --- |
| T-RF | Clone | Genus |
| 108 | S6, S5, S9 | *Nitrososphaera* (3 clones) |
| 116 | S7, S8, S7S8, S7, S8, S1 | *Nitrososphaera* (3 clones)*Nitrosopumilus* (4 clones) |
| 237 | S1, S8, S2 | *Nitrososphaera* (3 clones) |
| 291 | S8, S8 | *Nitrososphaera* (2 clones) |
| 382 | S4, S4 | *Nitrosotalea* (2 clones) |
| 461 | S2, S8 | *Nitrososphaera* (2 clones) |
| 486 | S5, S6, S7, S9, S9, S8S3, S5, S6, S5, S9 | *Nitrososphaera (6* clones)*Nitrosopumilus* (5 clones) |
| Other | S2 | Uncultured ammonia oxidizing (1 clone) |

**References**

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, and Stahl DA. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology* 56:1919-1925.

Francis CA, Roberts KJ, Beman JM, Santoro AE, and Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. *Proceedings of the National Academy of Sciences of the United States of America* 102:14683-14688. 10.1073/pnas.0506625102

Lane DJ. 1991. 16S/23S rRNA sequencing*.* In: Stackebrandt E , Goodfellow M (eds) Nucleic Acid Techniques in Bacterial Systematics. *Wiley,* New York, pp115–175.

Rotthauwe JH, Witzel KP, and Liesack W. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. *Applied and Environmental Microbiology* 63:4704-4712.