**Divergent responses of soil AOA and AOB to drought in drained and rewetted fen peatlands**

**Expanded Methods Section**

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1. **Standard cultivation for 16S, archaeal and bacterial amoA genes**

The purified cultures chosen to create standards for downstream qPCR analysis were the ammonia-oxidizing bacteria *Nitrosopira multiformis* [1], ammonia oxidizing archaea *Nitrosophaera viennensis* [2], and *Escherichia coli* [citation needed?]. First, the target genes were amplified via PCR with the following 25 ul reaction: 0.75 ul GoTaq® DNA Polymerase (Promega, Wisconsin, USA), 5 ul 5x GoTaq® Reaction Buffer (Promega), 2.5 ul MgCl₂, 0.5 ul DNTPs, 13.375 ul H₂O, 0.125 ul each forward and reverse primer, and 1 ul DNA template. The archaeal amoA gene was amplified from *N. multiformis* with the primer pair camoA-19F [3, 4]/tamoA-629R [5], using a PCR cycle with a 5 minute initialization phase at 95°C, followed by 35 rounds of a 45 second denaturation stage at 95°C, a 45 second annealing stage at 55°C, and a 1 minute elongation phase at 72°C, before a final 10 minute finalization stage at 72°C. The bacterial amoA gene was amplified from *N. viennensis* using the primer pair amoA-1F/2R [6], and the 16S rRNA gene was amplified from *E. Coli* using the 515F [7]/806R [8] primers . Both the AOB and 16S rRNA templates were amplified using the following PCR protocol: a 2 minute initialization stage at 95°C, then 35 rounds of a 30s elongation stage at 95°C, a 30s annealing stage at 55°C, and a 45s elongation stage at 72°C, before a 5 minute finalization stage at 72°C.

The lengths of the resulting PCR products of the genes of interest were then verified via gel electrophoresis before being cleaned with the Zymo DNA Clean & Concentrator kit following the standard protocol (California, USA). The purity of the resulting gene fragments were assessed with a NanoDrop Spectrophotometer (ThermoFisher, Massachusetts, USA), with the following 260/230 ratios: 1.94 for the 16S rRNA product, 1.66 for the A-amoA product, and 1.74 for the B-amoA product.

The PCR products were then ligased to prepare for plasmid insertion with 1 ul of the Promega T4 DNA Ligase (California, USA), 1 ul Promega pGEM®-T Easy Vector, and 5 ul of the Promega 2x Rapid Ligation Buffer. Additionally, the following volumes of the cleaned PCR products were added: 2 ul of the B-amoA product with 1 ul H2O, 1 ul of the 16S rRNA product with 2 ul H2O, and 1.5 ul of the A-amoA product with 1.5 ul H2O, resulting in a 10 ul reaction. 2 ul of the ligase solution was then mixed with 50 ul competent *E. Coli* and placed on ice for 20 minutes. The solution was then heat shocked at 42°C for 45-50 seconds before being placed on ice for a further 2 minutes. Then, 950 ul room-temperature SOC medium was added to the solution and incubated at 37°C with 150 rpm shaking for 1.5 hours. Finally, 100 ul of the solution was plated onto an agar medium with 166 mg NaCl, 166 mg yeast, 330 mg Tryptone, 500 mg agar, 0.1 ul ampicillin, 0.04 g X-Gal and 1.67 ml water. The agar cultures were then incubated overnight at 37°C.

Colonies that displayed successful plasmid insertion were picked for each gene (as indicated by the white colony color) and amplified via the same PCR protocol as detailed above for the initial gene amplification. The quality of the plasmids was then visually investigated via gel electrophoresis, and two colonies were selected for each inserted gene to amplify in liquid SOC medium with 8.33 ul ampicillin overnight at 37°C with 150 rpm shaking. At this step, cryostocks for each plasmid gene were frozen at -80°C with a 1:1 glycerin mixture. Two samples for each gene were selected for plasmid extraction and immediate use.

The selected plasmids were extracted using the Zymo plasmid extraction kit, which resulted in the following elution concentrations measured with NanoDrop: 201.955 and 124.844 ng/ul for A-amoA, 118.291 and 108.291 ng/ul for B-amoA, and 92.125 and 72.237 ng/ul for 16S rRNA. A subsample of each extracted plasmid was then amplified via the same PCR protocols as above and visually verified with a gel. The extracted genes were then linearized in 50 ul reactions with 1 ug DNA, 5 ul 10x NE buffer, 1 ul enzyme and the rest of the volume with H2O. The plasmids inserts were linearized at 37°C for 1 hour, and then heat inactivated at 65°C for 20 minutes.

A gel of the linearized plasmid inserts demonstrated that there was still uncut plasmid in the extracted products. Therefore, the products at the correct fragment lengths (635 bp for A-amoA, 491 bp for B-amoA and 253 bp for 16S rRNA, with the 3015 bp plasmid) were cut from the gels. The cut gel products were resuspended with the Zymoclean Gel DNA Recovery Kit and verified with another gel for purity. Then, the copies/ul of each gene was calculated via the following formula:

The ultimate copy numbers of the plasmid products were 3.32e9 for A-amoA, 1.44e9 for B-amoA, and 1.65e8 for 16S rRNA. The A-amoA and B-amoA plasmid products were aliquoted to standards with 1e9 copies/ul of the inserted gene in 5 ul of H2O, and stored at -80°C until use for qPCR applications.