**METHODS**

**Experimental design and soil sampling**

The rain-out study was conducted in 2022 at the DOK (bio-Dynamic, bio-Organic, and “Konventionell”) experimental field at Therwill, Switzerland. The field has been investigated long-term under five different fertilization and pesticide management systems (Hartmann et al., 2015; Maeder et al., 2002). For this study, three fertilization and pesticide management methods were chosen from the DOK trial: biodynamic (D), conventional (K), and mineral-fertilized (M) plots, due to their contrasting treatments (Table 1). The study was performed using a strip-split-plot design, with the three different fertilization methods as the main plot. The rain shelters were installed in each plot to exclude the rainfall to simulate the drought effect, while the control plots had no rain shelter installed. The rain shelters were made from…and size.. to exclude this percentage of rainwater (Elena, more detail). There were four replicates for each treatment combination (2 fertilization and 3 irrigation treatments); in the end, there were 24 plots in total. The field was planted with winter wheat crops before the rain shelter installment and the shelters were set up when the crop was at the early plant growth stage in April to start the drought stress treatment. The wheat crops were maintained until harvesting at the ripening stage in July. How was the irrigation management of the crops in the control and drought plots? Were there any fertilizers/pesticides added during the experiment?

Bulk soils and rhizosphere soils were sampled gradually over the course of the experiment. The first sampling was at the stem extension stage on April 18th (stage 6, the first node of stem visible; *n* = 24 bulk soil, *n* = 24 rhizosphere). The second was at the heading stage on June 1st (stage 10.5, flowering; *n* = 24 bulk soil, *n* = 24 rhizosphere). The third was at the harvesting/ripening stage on July 5th (stage 11; *n* = 24 bulk soil, *n* = 24 rhizosphere) before the rain shelters were removed from the plots and rewetting was performed. The fourth (*n* = 24) and fifth (*n* = 24) samplings were conducted on July 20th and September 13th, respectively, after the removal of the rain shelters and the rewetting process by collecting only the bulk soils. In total, we collected 120 of bulk soil and 72 of rhizosphere soil samples. Bulk soils were sampled from….using soil core (how many samples per plot, how much, how deep and poll all samples together?) or were they just soils that unattached from the plant roots? Bulk soils were sieved through what size of sieves (4mm)? to remove any plant debris and to achieve more homogenous soil particles. Soil samples were stored at -80C? for DNA extraction. Rhizosphere soils were collected by pulling the plants (how many plants per plot?), then carefully removing the loosely-attached soils from the roots by gently shaking the roots? The remaining, tightly-attached rhizosphere soils were then collected and stored at -80C for further analyses.

Soil physiochemistry analyses were performed for how many grams of each bulk soil sample at the…. The measured soil parameters including soil water content, soil dry matter, pH, mineral nitrogen content (NO3, NH4), total soil nitrogen and carbon, and plant available potassium (K), magnesium (Mg), and phosphorus (P) content (need detailed procedures in supplementary?).

**Amplicon libraries preparation and sequencing of *amoA* genes**

Soil DNA extractions of the total 192 bulk soil and rhizosphere samples were performed using the soil KIT (…) following the manufacturer’s protocol? (Elena). The extracted DNA was quantified using…The analysis of ammonia-oxidizing communities was conducted by sequencing of *amoA* genes of AOB, AOA, and comammox. The sequencing libraries were performed using two-step polymerase chain reaction (PCR) amplification approach. The first step PCR amplification of *amoA* genes of AOB and AOA were conducted using *amoA*-1F (5’-GGGGTTTCTACTGGTGGT-3’) and *amoA*-2R (5’-CCCCTCKGSAAAGCCTTCTTC-3’) primer pair (Rotthauwe et al., 1997); and CrenamoA23f (5’- ATGGTCTGGCTWAGACG-3’) and CrenamoA616r (5’-GCCATCCATCTGTATGTCCA-3’) primer pairs (Tourna et al., 2008), respectively. The PCR conditions used to amplify the *amoA* genes of AOB and AOA as follows: 3 min at 94 °C; 25 cycles consisting of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C; and a final cycle of 10 min at 72 °C. Amplifications were performed in 15 µL total mixtures in a 96-well 0.2 mL PCR plate containing 1x Phusion High-Fidelity (HF) Master Mix (Thermo Scientific™, Waltham, MA, USA),), 250 ng T4 Gene 32 Protein (T4gp32) (QIAGEN, Hilden, Germany), 0.5 µM of each primer, and 6 ng/µL of template DNA. Each sample was conducted in duplicate and were pooled to be used as template for the second-step PCR. The second-step PCR (barcoding) was performed to construct amplicon libraries by introducing multiplex index-sequences (barcode) to the overhang adapters using multiplex primer pair specific for each sample.

Comammox *amoA* genes were amplified using comamoA-F (5’-AGGNGAYTGGGAYTTCTGG-3’) and comamoA-R (5’-CGGACAWABRTGAABCCCAT-3’) primer pair (Zhao et al., 2019). The PCR amplifications were set up in duplicate following the conditions: 3 min at 94 °C; 40 cycles consisting of 30 s at 94 °C, 30 s at 52 °C, and 30 s at 72 °C; and a final cycle of 10 min at 72 °C. The PCR reaction solutions were prepared in a total volume of 15 µL in a 96-well 0.2 mL PCR plate containing 1x Phusion Green Hot Start II High-Fidelity Master Mix (Thermo Scientific™, Waltham, MA, USA), 250 ng T4gp32, 0.5 µM of each primer, and 6 ng/µL of template DNA. For comammox, the first-step PCR products were cleaned up using the SequelPrep™ Normalization Plate (96) Kit (Invitrogen™, Waltham, MA, USA) before being used as template for the second-step PCR. The amplified *amoA* genes were verified by visualizing the PCR products of all samples, including the negative controls, on a 2 % agarose gel. Final PCR products of AOB, AOA, and comammox were purified and normalized according to the manufacturer’s protocol of the SequelPrep™ Normalization Plate (96) Kit. Barcoded, purified, and normalized *amoA* gene amplicons of AOB, AOA, and comammox were sequenced at the GenoScreen sequencing facility in Lille, France, using Illumina MiSeq platform with reagent kit v2 and paired-end reads sequencing format (2 x 250 bp).

***amoA* gene amplicon sequence analysis**

The raw *amoA* gene sequence data of AOB, AOA, and comammox were analyzed using the AMOA-SEQ sequence pipeline (https://github.com/miasungeunlee/AMOA-SEQ/tree/main) developed by Lee et al. 2023 (not yet published). The AMOA-SEQ pipeline implements the DADA2 tool (Callahan et al., 2016) to perform filtering and correcting sequence errors to generate Amplicon Sequence Variant (ASVs). The demultiplexed sequences were processed by removing primers and ambiguous bases, followed by quality filtering using the DADA2 standard filtering parameters (maxN = 0, truncQ = 2, rm.phix = TRUE, and maxEE = 2). To ensure the quality of the data, we discarded any reads that did not meet the minimum length requirements (200 bp for AOB and AOA, and 204 bp for comammox) and truncated the reads to a specific length (200 bp for AOB and AOA, and 210 bp for comammox). Dereplication was performed to identify unique sequences. Full denoised sequences were then generated by either merging the forward and reverse reads for comammox or simply concatenating the non-overlapping forward and reverse reads for AOB and AOA. Furthermore, an ASV table was constructed and any chimeric sequences were eliminated from the table.

The next step in the AMOA-SEQ pipeline was selecting the DADA2 generated ASV sequences that match the expected amplicon size (452, 410, and 396 bp for AOB, AOA, and comammox, respectively) using SeqKit (Shen et al., 2016). These sequences were then translated against the AMOA sequence database to generate correct ASV sequences using DIAMOND blastx (Buchfink et al., 2021). The AMOA database implemented in the AMOA-SEQ pipeline is constructed from different resources (NCBI and IMG-JGI databases) and previous studies (Aigle et al., 2019; Alves et al., 2018; Palomo et al., 2022). Additionally, the AMOA database includes data from Lee et al. (2023) own study, which has yet to be published. Taxonomic assignment of these curated ASV sequences to the reference data sets of the AMOA database was performed using DIAMOND tool (Buchfink et al., 2021).

**Quantificationof total microbial and ammonia-oxidizing communities**

Real-time quantitative PCR (qPCR) assays of 16S rRNA and *amoA* genes were performed to quantify the abundances of total bacterial and ammonia-oxidizing communities, respectively. Prior to qPCR, we tested the presence of PCR inhibitors in the DNA samples by adding known copies of standard plasmid DNA (pGEM®-T Easy Vector Systems) (Promega, Madison, WI, USA) into the diluted DNA extracts (10-fold dilution), and also into nuclease-free water as controls. The specific T7 and SP6 primers were used for the inhibition test. To investigate any PCR inhibition occurrences, we assessed the qPCR cycle-threshold (Ct) values of the samples and controls.

Quantification of total bacterial communities was carried out using 341F and 534R primer pair (Muyzer et al., 1993), which amplifies the V3 region of the 16S rRNA gene. Ammonia-oxidizing bacterial and archaeal abundances were determined using the *amoA* gene-targeted primers as described previously (Bru et al., 2011; Leininger et al., 2006; Tourna et al., 2008). Whereas, the abundances of comammox *amoA* genes were assessed using two primer sets targeting comammox *Nitrospira* clade A (comaA-244F and comaA-659R) and B (comaB-244F and comaB-659R) (Pjevac et al., 2017). Two independent qPCR runs were performed for each gene quantification. Quantitative PCR was performed in a 15 µL reaction mix containing the Takyon™ low ROX SYBR 2X MasterMix blue dTTP (Eurogentec), 250 ng T4gp32, 1 µM of each primer, and 3 ng/µL of DNA.

The AOA and CAOB amoA gene were amplified under the same condition: an initial denaturation step at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 1 min.

according to the previous study

Standard curves were obtained using serial dilutions of linearized plasmids (pGEM-T) containing cloned amoA genes. Controls without templates resulted in values lower than the detection limit or negligible values. Inhibitory effects on PCR performance was tested for all samples by mixing a known amount of the pGEM-T plasmid with the soil DNA extracts or water before running a qPCR with the plasmid specific T7 and SP6 primers. The measured cycle threshold (Ct) values obtained for the different DNA extracts and the controls with water were not significantly different indicating that no inhibition occurred.

**Ammonia-oxidizing community analysis**

**Data and code availability**

The computational workflows for sequence processing and ecological statistics are available on GitHub(..). Raw sequence data of amoA gene of AOB, AOA, and comammox have been deposited in the Sequence Read Archive NCBI database under Bioproject accession number …..