**Results**

**Drought affects soil water availability and mineral N pools**

Drought severely affects the soil water availability in all cropping systems (Fig.1: Soil GWC; Kost et al. (2024)). We observed that drought-treated soil has lower gravimetric water content compared to the control over the course of drought treatment, and the water availability recovered at the final sampling time (eleven weeks after rewetting event) (Fig.1 A: Soil GWC). The total mineral N content, including the ammonium (NH4+) and nitrate (NO3+) contents, were strongly affected by drought in the mixed- (CONFYM) and mineral fertilized (CONMIN) conventional systems, but not in the biodynamic (BIODYN) cropping system (Fig. 1 B: Ammonium, nitrate, and total mineral N contents, Table S1: N pools statistical analysis). Drought altered the mineral N pools, where the ammonium and nitrate increased during drought treatment, and recovered back to the control levels after the removal of the rain-out shelter and rewetting event (Fig. 1 B: Ammonium, nitrate, and total mineral N contents).

**The effects of drought on the ammonia-oxidizers diversity and composition were marginal**

Overall, there were no differences of the observed richness and Shannon diversity index between drought and control of the AOB and AOA communities in both bulk soil and rhizosphere (Fig. 2: Alpha diversity, Table S2: alpha diversity statistical analysis). Meanwhile, the observed richness of the Comammox community was marginally affected by drought in the bulk soil, but not in the rhizosphere (Table S2: alpha diversity statistical analysis). In contrast, we found that cropping system was a strong driver of the ammonia-oxidizers alpha diversity, with higher AOA and Comammox, but lower AOB alpha diversity in the BIODYN system than in the CONFYM and CONMIN cropping systems (Fig. 2: alpha diversity, Table S2: alpha diversity statistical analysis).

The unconstrained PCoA plots using Bray-Curtis dissimilarity distances showed distinct separation by cropping system on the first axis, meanwhile, the effect of drought was only apparent within block due to a strong block effect (Supplementary Fig. 2: unconstrained PCoA plots). The results of the whole plot PERMANOVA supported the effect of cropping system in bulk soil and rhizosphere (*P*=0.001), and we could not detect the effect of drought on the ammonia oxidizers composition. However, the restricted permutations PERMANOVA showed the effect of drought on the composition of the AOB (*P*= 0.028, bulk soil; *P*=0.007, rhizosphere) and Comammox (*P*=0.042, bulk soil; *P*=0.001, rhizosphere) communities, but not on the AOA community (*P=*0.08,bulk soil and rhizosphere). To further observe the effect of drought on the beta diversity, we performed constrained analysis using CAP, and the differences on the community composition between drought and control within each cropping system become more evident (Fig. 3: Constrained CAP Plots). The AOA community has the highest compositional differences between drought and control as demonstrated by high overall reclassification rates of 94.2 % and 90.3 % in bulk soil and rhizosphere, respectively. Distinct clustering by the drought treatment were also observed in the Comammox community with overall reclassification rates of 78.8 % and 83.3 % in bulk soil and rhizosphere, respectively. In contrast, the AOB community showed only marginal separations between drought and control within cropping system with lower overall reclassification rates of 60.5 % and 54.2 % in bulk soil and rhizosphere, respectively. Evaluation of the Euclidean distances calculated from the positions provided by the discriminant analysis showed that the highest differences between treatment (drought vs control) were within the BIODYN cropping system, particularly in the AOA and Comammox communities (Fig. 3: The distance boxplot calculated from the discriminant CAP analysis). Meanwhile, for the AOB community in the bulk soil, CONMIN system has the largest distance between drought and control (Fig. 3: The distance boxplot calculated from the discriminant CAP analysis).

**Most of the ammonia-oxidizer ASVs were resistant to drought**

Mean relative abundance of the ammonia-oxidizing taxa revealed that AOB, AOA, and Comammox communities were dominated by genus *Nitrosospira* (bulk soil: 84.56%, rhizosphere: 83.38%), lineage *Nitrososphaerales* clade Delta (NS-Delta) (bulk soil: 73.51%, rhizosphere: 71.14%), and *Nitrospira* clade B (bulk soil: 97.43%, rhizosphere: 96.85%), respectively. We found that there were no notable shifts of taxonomic composition of the ammonia-oxidizing communities in response to drought, although the community compositions were largely shifted among cropping systems (Supplementary Fig.1: Relative abundance bar plot). We performed differential abundance analysis to investigate ammonia-oxidizing ASVs exhibiting differences in abundances between drought and control in each cropping system. We detected a relatively small number of ASVs that were altered by drought, while most of the ammonia-oxidizing ASVs remained unchanged in response to drought (Fig.4: DAA). Among the three ammonia-oxidizing groups, the AOB community has the largest number of altered ASVs in all samples (17 ASVs in total), and most of them belonged to the dominant genera of *Nitrosospira* and *Nitrosolobus*. The majority of the drought-altered AOB ASVs were negatively impacted, indicating that these ASVs decreased in abundances in response to drought, and these ASVs responded to drought toward the end of sampling time (the last day of drought period or the third sampling, one week (fourth sampling), and eleven weeks (fifth sampling) after rewetting). Another notable observation was that differences in AOB ASVs abundance due to the effects of drought were mainly found in bulk soil samples, rather than rhizosphere, in the conventional cropping systems (CONFYM and CONMIN) (Fig.4: DAA). On the other hand, only a few AOA and Comammox ASVs (less than ten) were identified as sensitive to drought, and they also belonged to the dominant lineages of *Nitrososphaerales* and *Ca. Nitrosotaleales*, and Comammox *Nitrospira* clade B, respectively (Fig.4: DAA).

**Shifts in the abundance of *amoA* genes of ammonia-oxidizers in response to drought**

Even though the effect of drought on the ammonia-oxidizers diversity and composition was relatively marginal, the abundance of ammonia-oxidizing communities measured by *amoA* genes quantification were largely affected by drought (Table S3: ammonia-oxidizers abundance statistical analysis). However, the effects of drought were different depending on the ammonia-oxidizing group, cropping system, as well as sampling date. Drought affected the AOB *amoA* gene abundance within the total microbial community which tended to decrease, and the drought effect was found in bulk soil, specifically in the BIODYN and CONMIN systems (Fig.5: AOB/16S Ratio). The Comammox clade B *amoA* gene abundance in bulk soil was also negatively affected by drought with an overall decrease in *amoA*/16S rRNA gene ratio in all cropping systems (Fig.5: Comammox B/16S Ratio). On the contrary, we could not detect any effect of drought on the AOB and Comammox *amoA* gene abundances in rhizosphere (Table S3: ammonia-oxidizers abundance statistical analysis). The effect of drought on the AOA *amoA* gene abundance was more pronounced in the rhizosphere than bulk soil, and interestingly, the drought-treated rhizosphere soils tended to have increased AOA *amoA* gene abundance (Table S3: ammonia-oxidizers abundance statistical analysis; Fig.5: AOA/16S Ratio). While the drought effect on the AOA and Comammox clade A *amoA*/16S rRNA gene abundance ratio was not significant, we detected a significant interaction effect of drought and cropping system in bulk soil of both groups, as well as the Comammox clade B (Table S3: ammonia-oxidizers abundance statistical analysis). Further pairwise comparisons revealed that drought decreased the AOA and Comammox clade A *amoA*/16S rRNA gene abundance ratio in the BIODYN system, whereas it tended to increase in the CONFYM system (Fig.5: AOA/16S Ratio; Comammox A/16S Ratio). Shifts in *amoA* gene abundances in response to drought were mainly observed either in the beginning of drought period (first sampling) or after rewetting event (fourth and fifth sampling). Statistical analysis showed that sampling time was also found to be associated with drought and cropping system as indicated by significant interaction effects, specifically within bulk soil samples of the AOA and Comammox clade B (Table S3: ammonia-oxidizers abundance statistical analysis).

**Relationship between environmental factors and ammonia-oxidizing communities**