**Results**

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

Drought severely affects the soil water availability in all cropping systems, with a decrease ranging between X to X% in gravimetric water content compared to the control (Fig.1: Soil GWC; Kost et al. (2024)). the water availability recovered at the final sampling time (eleven weeks after rewetting event) (Fig.1 A: Soil GWC). Large differences in ammonium and nitrate were observed between cropping systems….please expend and provide numbers when possible. 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). Thus, the ammonium and nitrate significantly 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**

The lso aIn contrast, 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). However, a subtle yet significant effect of drought was observed on the observed richness of the Comammox community in the bulk soil only (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). To further investigate the effect of drought on the beta diversity of ammonia oxidizers, we performed constrained analysis using CAP..By accounting for the block effects, 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. The effect of drought on the community structure was also influenced by the cropping system with a better clustering by the drought treatment in the BIODYN and CONFYN cropping system than in the CONMIN cropping system. 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. To further quantify the impact of drought, we calculatedthe Euclidean distances between the drought and control treatments based on the discriminant analysis. The response to drought was dependent both on the ammonia-oxidizing community and on the cropping system. Thus,the highest differences in community structure between treatment (drought vs control) were observed for the AOA and Comammox communities within the BIODYN cropping system (Fig. 3: The distance boxplot calculated from the discriminant CAP analysis).

I miss something about the sampling time (was the last date -after rewetting excluded- from these analyses?)

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

We performed a differential abundance analysis to identify ammonia-oxidizing ASVs exhibiting differences in relative abundances between drought and control in each cropping system. In overall, we detected a relatively small number of ASVs that were significantly affected by drought, representing between X and Y % of the dominant OTUs (Fig.4: DAA). Among the three ammonia-oxidizing groups, the AOB community has the largest number of affected 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 with 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). Only 10 AOA and Comammox were identified as sensitive to drought, all of them belonging to the dominant lineages of *Nitrososphaerales* and *Ca. Nitrosotaleales*, and Comammox *Nitrospira* clade B, respectively (Fig.4: DAA).

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

The abundance of ammonia-oxidizing communities measured by *amoA* genes quantification were 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**

Correlation analysis showed that in general, all of ammonia-oxidizers beta diversity in bulk soil positively correlated with ammonium (NH4+) content, soil pH, total C and N, as well as magnesium (Mg) content (Fig. 6: correlation figure; Table S4: correlation statistical analysis), where the communities had strongest correlation with soil pH. Meanwhile, we could not identify any correlations between ammonia-oxidizers diversity with nitrate (NO3+) content or soil water content (GWC). The AOB and Comammox communities also demonstrated to have marginal correlation with phosphorus (P) and potassium (K) content, respectively (Table S4: correlation statistical analysis). Moreover, we found that *amoA* gene abundance of all ammonia-oxidizing groups negatively correlated with NH4+ with different magnitudes, with NH4+ being the strongest driver for the AOA abundance. By contrast, there were no significant correlations between NO3+ content with ammonia-oxidizers abundance. While soil pH, and total C and N primarily connected with *amoA* gene abundances, soil pH was not a significant driver for the AOB abundance. Importantly, we found a positive correlation between the community abundance with soil water content, which supported the previous findings of the observed drought effect on the *amoA* gene abundance. Furthermore, correlation analysis indicated that the abundance of all ammonia-oxidizing groups was negatively correlated with soil dry matter (TS) (Fig. 6: correlation figure; Table S4: correlation statistical analysis).