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Resistance and resilience of co-occurring nitrifying microbial guilds to drying-rewetting stress in soil

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ABSTRACT

Nitrification, the oxidation of ammonia via nitrite to nitrate, contributes to nitrogen losses in agricultural soils. When nitrification is a two-step process, it depends on the successful metabolic interaction between ammonia oxidising archaea (AOA) and bacteria (AOB), and nitrite oxidising bacteria primarily within *Nitrobacter* (NIB) and *Nitrospira* (NIS). However, consequences of dry spells caused by climate change on the composition and co-associations of these microbial guilds and the fate of nitrogen remain unclear. Here we subject four distinct soils to either one long or two shorter drought periods (7–11 % water holding capacity) followed by rewetting in a microcosm experiment to evaluate the hypothesis that drying-rewetting stress triggers distinct responses in the functional guilds due to differences in environmental preferences and adaptation strategies. While AOB were highly resistant, AOA were the most sensitive to drying among the four guilds and decreased in relative abundance. This coincided with reduced ammonia oxidation rates in three soils by on average 27 % compared to the control. However, we observed almost full recovery of AOA one week after rewetting. NIS, but not NIB, were strongly affected by rewetting with no recovery during the experiment, showing shifts in community composition and relative abundance with up to 30 % affected ASVs. Network analysis revealed that drying-rewetting affected co-occurrences between ammonia and nitrite oxidisers in a soil-dependant manner, possibly indicating a destabilisation of their metabolic interaction. Overall, this study emphasises the importance to consider weather extremes like drought on soil nitrifier community dynamics and the fate of nitrogen in soils.

1. Introduction

Microorganisms play a crucial role in nitrogen (N) transformations, thereby controlling the bioavailability of soil N and in which form it is present (Kuypers et al., 2018). Nitrification, the process in which ammonia is oxidised to nitrate, contributes both directly and indirectly to N loss by driving nitrate leaching and fuelling denitrification. The latter leads to gaseous N loss, including emissions of the greenhouse gas nitrous oxide. Ammonia is either oxidised to nitrate by complete ammonia oxidisers ('comammox' bacteria) or to nitrite by ammonia-oxidising archaea (AOA) and bacteria (AOB) and then to nitrate by nitrite oxidising bacteria within several genera, primarily *Nitrobacter* (NIB) and *Nitrospira* (NIS). Thus, the two-step nitrification process depends on the successful interaction between two functional guilds. AOA and AOB abundance are generally positively correlated to NIS and NIB, respectively (Placella and Firestone, 2013; Simonin et al., 2015; Stempfhuber et al., 2017), and the associations between specific lineages shape the spatial distribution of nitrifying communities in soil (Jones and Hallin, 2019). These associations are largely explained by differences in niche preferences, e.g. capacity to use different substrates, substrate affinity, pH, and osmotic stress tolerance (Wessén et al., 2011; Nowka et al., 2015; Han et al., 2017; Saghaï et al., 2021; Qin et al., 2024). Several studies indicate that AOA and NIS have an advantage at lower nutrient content than AOB and NIB when grouped as functional guilds, but this is not necessarily the case at the population level (e.g. Simonin et al., 2015; Wertz et al., 2012). Moreover, there is evidence that ammonia and nitrite oxidation may become decoupled under conditions of environmental stress such as drying-rewetting events, leading to nitrite accumulation (Gelfand and Yakir, 2008). This shows that the assembly of nitrifier communities not only depends on physicochemical

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conditions in the soil but also on the stability of associations between different nitrifying guilds, and that responses of such nitrifier assemblages to climate-change induced weather extremes may have implications for soil N fluxes.

The frequency and duration of dry and wet spells are increasing due to climate change and subject soil microorganisms to severe stress (Schimel, 2018). As soil becomes drier, diffusion rates decrease, restricting the availability of resources, and osmotic stress increases. By contrast, rewetting causes a rapid drop in osmolality and an increase in the soil content of carbon compounds, ammonium, and nitrate (Birch, 1958). These conditions can change the composition and activity of microbial communities (Barnard et al., 2013; Priemé and Christensen, 2001), with impacts on the cycling of nutrients in soils (Gordon et al., 2008; Zhang et al., 2020). Previous work has shown that drying-rewetting favours AOB over AOA, possibly because of more efficient adaptation to osmotic pressure during drought and higher ammonium availability following the nutrient flush caused by rewetting (Thion and Prosser, 2014; Kaurin et al., 2018; Zhang et al., 2024; Bintarti et al., 2025), but little is known about the effect of drying-rewetting on nitrite oxidisers (Séneca et al., 2020). Nevertheless, re-occurring drying/rewetting events may have other effects as the resistance (the degree to which a community remains unchanged after a disturbance) and resilience (the capacity to return to the original state or to an alternative stable state) of microbial communities to a contemporary disturbance is influenced by previous disturbance events (Shade et al., 2012; Fuchslueger et al., 2016; Canarini et al., 2021). Understanding the impacts of drying-rewetting cycles on nitrifying guilds and possible implications for nitrogen cycling in agricultural soils is particularly important, as drought has been identified as the main threat to global crop yields (Lesk et al., 2016).

Here, we determined the effects of drying-rewetting cycles on the community composition and co-occurrence of ammonia and nitrite oxidising guilds driven by the cooperation between these guilds, as well as on the ammonia oxidation rates in four contrasting soil types (Table 1). To do this, we set up a microcosm experiment where soils were subjected to either one long drought period or two shorter drought periods with a rewetting event after each drought followed by a final recovery period (Fig. 1). We hypothesised that different nitrifying guilds will display distinct responses to drying-rewetting stress with higher substrate concentrations favouring AOB and NIB over AOA and NIS, respectively, due to differences in environmental preferences. As this will result in shifts in community composition, we further hypothesise that these shifts will modify co-associations between lineages of different nitrifying guilds. As an overall consequence, we expect to see changes in nitrification activity.

Table 1
Geographic origin and properties of the soils.

	Soil B	Soil E	Soil U	Soil S
Origin	Bretenière, France	Ekhaga, Sweden	Ulleråker, Sweden	Schnega, Germany
Coordinates	47.234715, 5.109561	59.830742, 17.808193	59.824883, 17.648267	52.904663, 10.831922
Soil type (USDA)	Silty clay	Silty clay loam	Clay loam	Loamy sand
Soil texture	54 % clay 42 % silt 4 % sand	37 % clay 57 % silt 6 % sand	37 % clay 37 % silt 27 % sand	0 % clay 25 % silt 75 % sand
pH (H_2O)	8.12	6.53	8.01	5.39
Tot-C (%)	2.91	3.80	1.90	1.10
Org-C (%)	2.56	3.78	1.87	1.10
Tot-N (%)	0.24	0.40	0.17	0.09

2. Material and methods

2.1. Soil sampling

Soil samples (5–20 cm depth) were collected in June 2021 in four agricultural fields in France (Bretenière; 47°14′05.0″N, 5°06′34.4″E), Sweden (Ekhaga; 59°49′50.7″N, 17°48′29.5″E, and Ulleråker; 59°49′29.6″N, 17°38′53.8″E), and Germany (Schnega; 52°54′16.8″N, 10°49′54.9″E). The soils were homogenized, sieved (2 mm ∅) and stored at −20 °C until the start of the experiment. Soil water content was estimated in duplicates as the difference in weight before and after drying ~5 g soil at 105 °C for 24 h. The maximum water holding capacity (WHC) was estimated as the gravimetric water content of the soil after overnight soaking in water and draining for 5 h. Soil properties, including pH (in water), total carbon (C_{tot}), organic carbon (C_{org}) and total nitrogen (N_{tot}) were determined at the Soil and Plant Laboratory (SLU, Uppsala, Sweden) and soil texture (PARIO method) at the Soil Physics Laboratory (SLU, Uppsala, Sweden; Table 1). Soils will be referred to as soil B (Bretenière), soil E (Ekhaga), soil U (Ulleråker), and soil S (Schnega).

2.2. Experimental design

The microcosm experiment included the four soils B, E, U and S subjected to three treatments: one drying-rewetting cycle, two drying-rewetting cycles, and a control kept at 45–50 % WHC (Fig. 1). In total, 120 microcosms were included to allow destructive sampling of all treatments in triplicate at three timepoints (‘drying’ on day 42, ‘rewetting’ on day 49, and ‘recovery’ on day 77) in addition to triplicate sampling of each soil at day 0. The experiment was set up in a climate chamber with 20 °C, 60 % relative humidity, and continuous darkness throughout the entire experiment.

When establishing the experiment, soils were first thawed at 4 °C for one day followed by 1 day at room temperature. Glass pots with an inner diameter of 12.5 cm were filled with 200 g fresh weight (FW) soil, corresponding to a dry weight (DW) of 138 g (soil B), 138 g (soil E), 149 g (soil U), and 172 g (soil S). The microcosms were covered with sterile cotton cloth and aluminium foil to reduce evaporation while allowing soil aeration. After 7 days of acclimatisation at 45–50 % WHC, soils were either kept at the same conditions or subjected to one long drought period (35 days) or two shorter drought periods (14 days followed by rewetting and 7 days of recovery at 45–50 % WHC in between), with both drought treatments followed by a recovery period of 28 days weeks at 45–50 % WHC (Fig. 1). The microcosms were weighed every second day to monitor WHC and adjust the water content in the control soils. Both watering of the control soils and rewetting after drought were done by carefully pipetting water on the surface of the soil to avoid physical disturbance. The aluminium foil was removed from microcosms undergoing the drought treatment, causing a reduction of the soil moisture to 7 (soil U and S) and 11 % WHC (soil B and E) within 14 days (Fig. 1). At each of the three sampling days (day 42, 49, and 77), the soil from each microcosm was homogenized and stored at −20 °C until further analysis of inorganic N (ammonium and nitrate, section 2.3), potential ammonia oxidation activity (section 2.4), and analysis of the functional guilds (section 2.5).

2.3. Measurement of soil ammonium and nitrate

To determine soil ammonium and nitrate content, soil was extracted with 2 M potassium chloride (1:5 ratio) in 50 mL Falcon tubes and incubated on a horizontal shaker for 1 h at 300 rpm. After centrifuging (5 min, 3500 *g*), the supernatant was filtered through Munktell 00H filter paper (Ahlstrom, Helsinki, Finland) and stored at 4 °C until analysis (max. 4 days). The concentration of ammonium and nitrate was measured on a segmented flow analyser (AutoAnalyzer 500, SEAL Analytical, Inc., Mequon, Wisconsin, US). The ammonium content

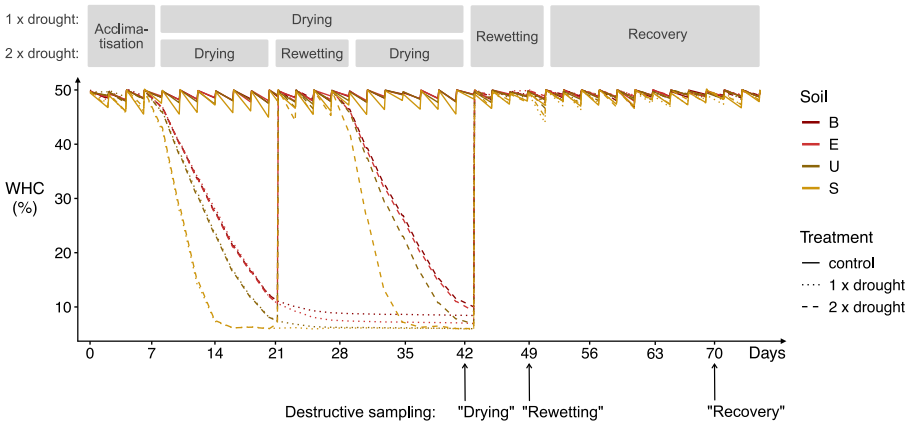

Fig. 1. Experimental set-up and water holding capacity (WHC) in the four soils subjected to either one long (1 x drought) or two shorter droughts (2 x drought) over the course of the experiment. Soil moisture was monitored every two days and adjusted to 45–50 % WHC when necessary. The droughts were imposed after an acclimatisation period of 7 days. Individual microcosms were destructively sampled at the end of the drought treatments (day 42), after rewetting (day 49), and after the recovery phase (day 77) as indicated by arrows. Colours represent different soils and line types indicate treatment.

before subjecting the soils to drought ranged from 0.78 ± 0.25 to 1.33 ± 0.39 mg N kg^{-1} DW soil, whereas the nitrate content ranged from 10.24 ± 10.44 to 32.22 ± 13.19 mg N kg^{-1} DW soil.

2.4. Potential ammonia oxidation assays

Potential ammonia oxidation rates, hereafter ammonia oxidation rates, were measured following the ISO 15685 protocol (2012) with some modifications and with three different sources of ammonium. Soil was thawed at 4–7 °C two days prior to the assay. Soil was mixed in a 1:4 (w:v) ratio with an unbuffered solution containing 1 mM sodium chlorate to inhibit nitrite oxidation (Xu et al., 2010), and either with 198 mg/L diammonium sulphate as per the ISO protocol or with an equivalent amount of nitrogen in the form of urea or yeast extract. These substrates were chosen to account for possible differences in substrate affinities and preferences between and within AOA and AOB (Levičnik-Höfferle et al., 2012; Qin et al., 2024), with the assumption that ammonia concentrations would be lower with urea and yeast extract due to their need to be mineralized to ammonium prior to oxidation. Soil slurries were incubated in loosely capped bottles on an orbital shaker (210 rpm, 25 °C). After 2 h and 8 h, 1 mL of soil slurry was removed and mixed with 1 mL 4 M potassium chloride to stop ammonia oxidation. These times were selected based on preliminary trials showing linearity of ammonia oxidation across all four soils. After centrifugation (2 min, 3000 *g*), nitrite content was measured colorimetrically (Griess test), using a microplate reader (SpectraMax Plus 384, Molecular Devices, LLC, California, US).

2.5. DNA extraction and libraries for sequencing of amoA and nxrB

DNA was extracted from 0.4 g FW soil using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. DNA quality was validated by agarose gel electrophoresis and measurements on a NanoDrop™ (Thermo Fisher Scientific, Waltham, Massachusetts, US), before quantification with a Qubit® fluorometer (Thermo Fisher Scientific). Sequencing libraries were prepared for AOA, AOB, NIB and NIS by using guild specific *amoA* (encoding the ammonia monooxygenase) and *nxrB* (encoding the nitrite oxidoreductase) primers, following a two-step PCR procedure. The first PCR was done in duplicates in 25 μL reaction volume, using 0.5 μM of the respective primers, 1 × Phusion PCR Mastermix (Thermo Fisher Scientific), 1 μg μL^{-1} bovine serum albumin and 5 ng template DNA for AOA, 15 ng for AOB and NIS, and 25 ng for NIB. Primer sequences and thermal cycling conditions are found in Table S1. PCR product size was verified by gel electrophoresis and the duplicates were pooled before purification with Sera-Mag beads (Merck KGaA, Darmstadt, Germany). The second PCR was done in a single 30 μL reaction using the same concentrations of Mastermix and bovine serum albumin as in the first PCR, 0.2 μM primers with Nextera adaptor and index sequences, and 4 μL purified PCR product as template. PCR products were verified and purified as described above and quantified using a Qubit® fluorometer. Two libraries were created by pooling equimolar amounts of *amoA* and *nxrB* amplicons, respectively. After a final quality control using a Bio-Analyzer (Agilent, Santa Clara, CA, US), sequencing was performed by SciLifeLab in Uppsala on an Illumina MiSeq instrument using the 2 x 300 bp chemistry.

2.6. Sequence analyses

Sequence analysis was performed using the R software, version 4.1.1 (R Core Team, 2021). Demultiplexed *amoA* and *nxrB* gene amplicons were processed using the 'dada2' package, version 1.16.0 (Callahan et al., 2016) to infer amplicon sequence variants (ASVs). Forward and reverse reads of all four genes were truncated with the 'filterAndTrim' command using default settings, except for maxEE = c(2,2), based on quality score (AOA *amoA*: 248, 200; AOB *amoA*: 275, 240; NIB *nxrB*: 270, 190; NIS *nxrB*: 290, 220). Forward and reverse sequences were either concatenated (AOA *amoA*) or merged (AOB *amoA* and NIS and NIB *nxrB*). Chimeras ('removeBimeraDenovo' function, 'consensus' method) and singletons were discarded. To identify non-specific ASVs, the representative sequence of each ASV was translated into amino acids ('esl-translate' command implemented in EASEL, version 0.48) and aligned to the corresponding reference alignment using the 'hmmalign' command in HMMER, version 3.3.2 (Eddy, 2011). After back translation to nucleotide sequences in ARB, version 7.0.1 (Ludwig et al., 2004), query ASV sequences were placed on the corresponding reference phylogeny using EPA-NG (Barbera et al., 2019) and sequences falling in the outgroup were discarded. For reference phylogenies, we used published databases for AOA (Alves et al., 2018) and AOB (Jones and Hallin, 2019) and updated the phylogenies for *nxrB* from Jones and Hallin (2019), following the approach described in Saghaï et al. (2023). The resulting ASV tables were rarefied using the 'vegan' package version 2.6–4 (Oksanen et al., 2013).

2.7. Statistical analyses

Statistical analyses were performed using the R Software, version 4.3.3. Based on the rarefied ASV tables for each community, Pielou's evenness was computed in the 'vegan' package, and Faith's phylogenetic diversity (PD) (Faith, 1992) was obtained using the phylogenetic

placements and the 'fdp' command within Guppy, version 1.1 (Matsen and Gallagher, 2011). Rarefied ASV tables were filtered per gene before performing β-diversity analyses to remove low abundant ASVs (i.e. those with abundance <0.001 % in the overall dataset and present in less than 15 % of the samples; Table S3). Zero counts were replaced by Bayesian multiplicative replacement using the 'zCompositions' package, version 1.4.0-1 (Martín-Fernández et al., 2015) and ASV tables were centred log-ratio transformed, to account for the compositionality of the dataset (Gloor et al., 2017). Differences in community composition and structure were visualized with principal component analysis (PCA) using the rda function in 'vegan'. The homogeneity of dispersion between groups was tested using the betadisp function in 'vegan' and their significance assessed using a permutation test. Permutational multivariate analyses of variance (PERMANOVA) were conducted to assess treatment and timepoint effects on community composition using the adonis function in 'vegan'.

Substrate, soil, and timepoint effect on ammonia oxidation rates was assessed using analysis of variance (ANOVA). Variables not following a normal error distribution were transformed by Box-Cox transformation before analysis. For the percent change between control and treatment, the combined standard deviation *SD* of both groups was calculated by error propagation (Taylor, 2022) as follows:

$$SD_{percentchange} = \left|\frac{100}{control}\right| * \sqrt{SD^2_{treatment} + \left(\frac{treatment}{control}\right)^2 SD^2_{control}}$$

The standard error and 95 % confidence interval *CI* were calculated with $n = 6$ data points and a confidence level of $\alpha = 0.05$ using the *t*-distribution. Confidence intervals that did not span over zero were considered significant percentage changes of treatment from control.

2.8. Differential abundance and network analysis

Differential abundance and network analysis were performed using the R Software, version 4.3.3. On non-rarefied ASV tables, a filter was applied to reduce sparsity in the data causing a risk for spurious correlations. Per gene and soil, ASVs with an abundance below 0.001 % and a presence below 45 % were removed (Table S2).

The effect of drying-rewetting cycles on relative abundance of ASVs was estimated per soil and timepoint using a generalised linear mixed model, computed with the glmer function in the 'lme4' package, version 1.1–35.2 (Bates et al., 2015). Generalised linear mixed models allow to infer linear regressions from Poisson distributed count data and support the inclusion of fixed effects (treatment) and random effects (sample ID). Differences in sequencing depth were accounted for by adding an "offset" factor, the $\log_2$ of the read sum per sample. Following Huet et al. (2023), we considered that an ASV of abundance *Y*, in any *k* replicates of any *i* treatment follows a Poisson law of parameter Λ as $Y \sim P(\Lambda)$:

$$log(\Lambda_{ik}) = o_{ik} + \mu + \alpha_i + Z_{ik1 \le j \le 3} idd\, N\left(0, \sigma^2\right)$$

where $i = \{1, \ldots, 3\}$ represents the treatments, $k = \{1, \ldots, 3\}$ the replicates, *o* the "offset", *α*.

The treatment effect, and *Z* the random sampling effect modelling data overdispersion. Multiple pairwise comparisons between treatments were performed with a post-hoc Tukey test using the emmeans function of the 'emmeans' package, version 1.10.0 (Lenth, 2024). After p-value adjustment using the false discovery rate method (Benjamini and Hochberg, 1995), ASVs with $p \le 0.01$ were considered significantly affected.

For the network analyses, only soil B and U could be used due to seven missing samples in soil E (n = 24) and soil S (n = 23). The missing samples cause incomplete replication of the functional guilds in the data set which would result in an incorrect introduction of zero counts that pose a risk for spurious correlations in the networks. For soil B and U (n = 27), networks were inferred using a Poisson log-normal model with a latent Gaussian layer and an observed Poisson layer (Chiquet et al., 2019, 2021), developed to handle sparse count data, using the 'PLNmodels' package, version 1.2.0 (Ibid.). Differences in sequencing depth were accounted for by adding the $\log_2$ of the read sum as an "offset" factor per sample. The most robust network per model was selected using a Stability Approach to Regularization Selection (Liu et al., 2010). Due to the experimental set up, treatment and timepoint could not be separated since treatment phases covaried with timepoints. Per soil, two networks M0 and M1 were constructed. The M0 model was constructed without covariates, which means that all possible effects are included. For the M1 model, treatment and timepoint were added as covariates, which causes the removal of the effect of treatment and timepoint from the generated network. Thus, edges in the resulting M1 network were the ones not affected by the covariates. By subtracting these nodes and edges (i.e. nodes and edges of the M1 network) from the M0 network, only nodes and edges related to treatment and timepoint should remain.

3. Results

3.1. Potential ammonia oxidation rates

The capacity for ammonia oxidation differed between soils, as shown by the rates detected in the control (Table S3), where the highest potential was observed in soil E with values up to 0.67 mg NO_2–N g^{-1} DW soil h^{-1}, followed by soil B with 0.59, soil U with 0.35, and soil S with 0.04 mg NO_2–N g^{-1} DW soil h^{-1} ($F_{(3,\ 72)} = 2986.67$, $p < 0.001$, Table S4). The timepoint had a small significant effect ($F_{(2,\ 72)} = 4.9$, $p < 0.01$), whereas the type of substrate provided during the assay did not ($F_{(2,\ 72)} = 1.17$, $p > 0.05$). The single, long drought treatment resulted in significantly lower ammonia oxidation rates compared to the control in soil B, E, and U with all three substrates, as indicated by a 95 % confidence interval, whereas the rates in soil S were unaffected by the drying-rewetting treatments (Fig. 2). A significant negative effect of the two shorter drying-rewetting cycles was only observed in soil B with yeast extract as substrate. After rewetting, the rates did not differ from the control except for a small increase in soil B (with ammonia) and a decrease in soil E (with yeast extract). After the recovery period, the rates were significantly higher in the drought treated soil U.

3.2. Inorganic N

In line with the observed effects on ammonia oxidation rates, there was a tendency for lower ammonium and nitrate content during drying, with significant effects in soil B, E, and U (Fig. S1). However, there were small but consistent differences between the two drought treatments during drying. The nitrate content was higher in relation to the control with two drying-rewetting cycles compared to the treatment with one cycle, whereas ammonium content showed the opposite pattern (Fig. S1). Thus, nitrate content and ammonia oxidation rates were positively correlated in all soils (Spearman's $\rho = 0.31$–0.42, $p < 0.01$; Fig. 2 and Fig. S1) except in soil S, which had very low ammonia oxidation activity. Correlations between ammonia oxidation rates and ammonium content were only significant in soil B (Spearman's $\rho = 0.41$, $p < 0.001$).

3.3. Diversity and composition of nitrifier communities

Phylogenetic diversity varied between the four guilds, with AOA having the highest and NIB the lowest PD in all soils, whereas evenness was similar in all soils and guilds, except for the lower evenness of NIB (Table S3). Drying-rewetting did not affect the evenness or PD of ammonia oxidisers or nitrite oxidisers in any of the soils (Fig. S2 and S3). By contrast, the treatments affected the community composition of the guilds across all soils, with NIS communities displaying the strongest shifts in *β*-diversity (Fig. 3; Table S5). During drying, communities in the single drought treatment were similar to the control communities, but

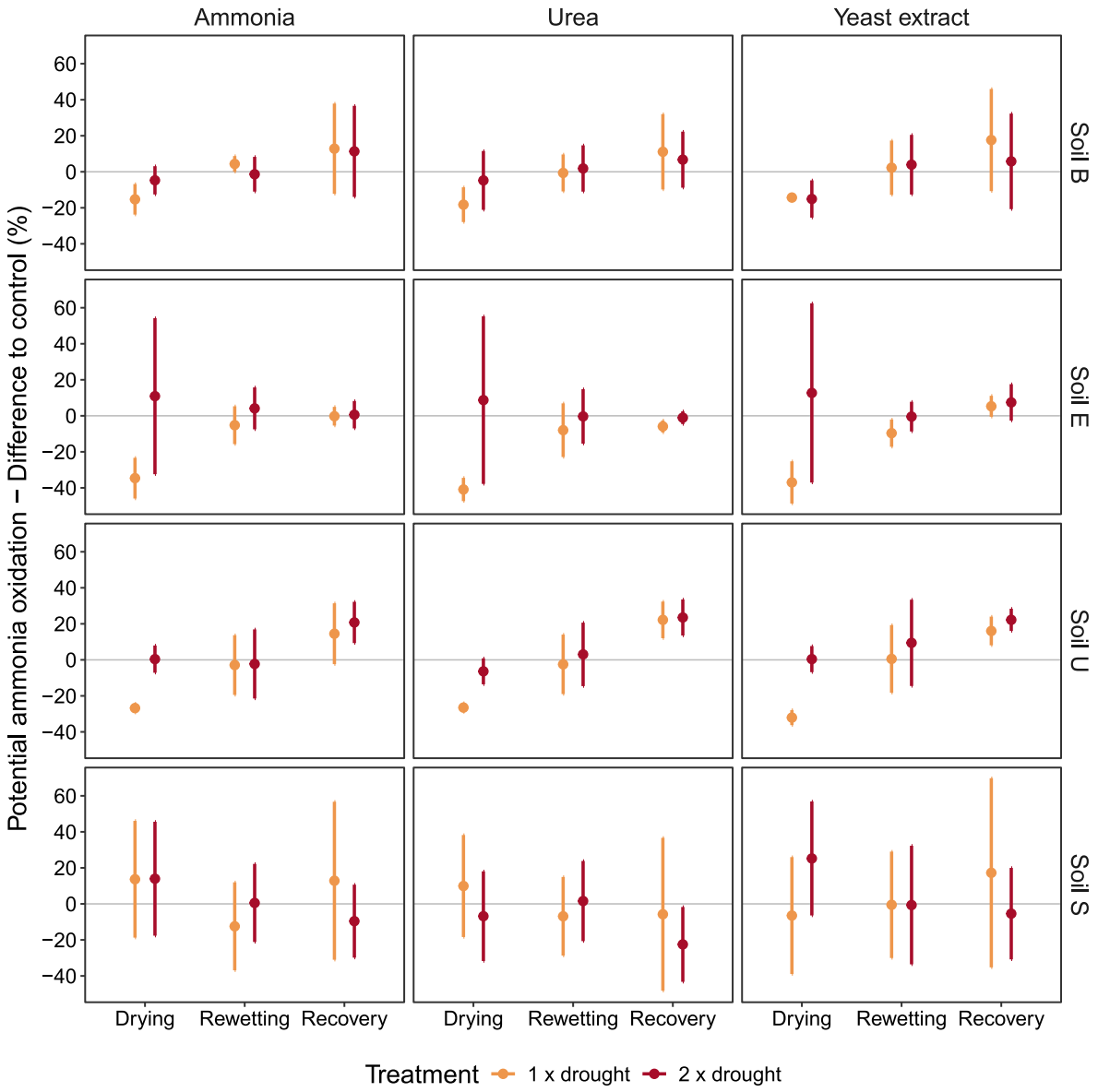

Fig. 2. Potential ammonia oxidation rates with three different nitrogen sources in the four soils. Activity was determined at the end of the two drought treatments (day 42), after rewetting (day 49), and after the recovery phase (day 77) and is presented as percent change in comparison to the control. Bars represent 95 % confidence intervals (hidden behind symbol in some cases). When confidence intervals did not span over zero, the percentage change of treatment from control was considered significant. Colours indicate drought treatments.

differed after rewetting in soil B, E, and U. Communities in soils subject two drying-rewetting cycles did not show this pattern. In two of the soils in which NIS was strongly affected (soil B and U), the AOA community composition or its dispersion was also significantly affected by time and treatment (Fig. 3; Table S5), with communities sampled during drying being most dissimilar to the communities in the control. AOB and NIB communities were unaffected by drying and rewetting (Fig. S5).

In line with the β-diversity patterns, differential abundance analysis showed that the relative abundance of ASVs was most affected in NIS communities (Fig. 4). Effects of drying and rewetting on NIS were detected in all soils and were particularly strong in soil U, which displayed both the highest proportion of positively affected ASVs, ranging from 7.35 to 29.72 %, and the highest proportion of negatively affected ASVs, ranging from 2.45 to 19.23 %. The lowest proportion of combined positively and negatively affected ASVs in soil U was observed in the single drought treatment during drying, and the highest in the double drought treatment after the recovery period. For all soils, large differences in relative abundance of NIS ASVs between control and droughted soil remained after the recovery period. The relative abundance of AOA ASVs was mainly affected by drought and there were only small differences between control and droughted soils after rewetting and at the end of the recovery period. Only in soil S the relative abundance was most impacted after rewetting. Less than 5 % of AOB and NIB ASVs were affected at any of the time points.

3.4. Network analysis

Associations between ammonia and nitrite oxidisers examined by network analysis in soil B and U were dominated by positive associations (Fig. S6) and showed similar patterns across soils when edge numbers were adjusted to the average number of ASVs within each guild in the networks with and without covariates (Fig. 5A). When considering the overall networks, patterns of associations within ammonia or nitrite oxidisers differed more between soils and likely indicate shared niche (Fig. S7). Based on Fig. S7, 20 % of all edges and 4.4 % of all nodes were specifically related to drying-rewetting in soil B, whereas in soil U it was

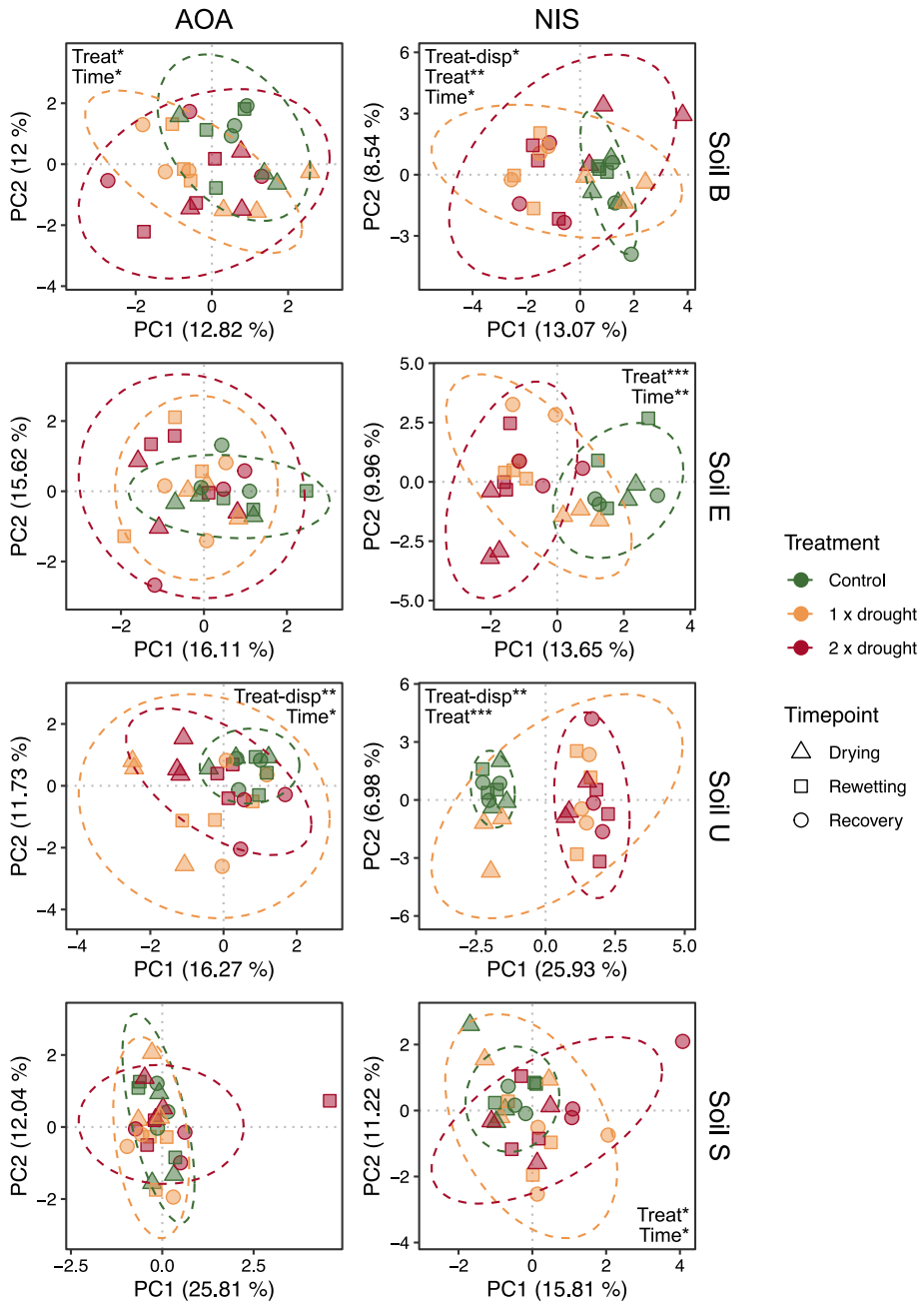

Fig. 3. Principal component analysis (PCA) of ammonia oxidising archaeal (AOA; left panels) and nitrite oxidising bacterial *Nitrospira* (NIS; right panels) communities in the four soils. The composition of the communities was determined at the end of the two drought treatments (day 42), after rewetting (day 49), and after the recovery phase (day 77). Significant differences in community structure and beta-dispersal ('-disp') across treatments and timepoints are indicated inside the plot ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***). Colours represent treatments, shapes time points, and dashed ellipses 95 % confidence intervals of the respective treatment.

38 % of all edges and 8.3 % of all nodes. A major difference between the soils was that treatment-affected edges indicating associations between ammonia and nitrite oxidisers were dominated by AOA and NIS in soil B and by AOB and NIS in soil U (Fig. 5B and C). The taxa involved differed substantially between soils for ammonia oxidisers, whereas the nitrite oxidiser clades largely overlapped (Fig. 5B and C).

4. Discussion

Drought significantly reduced both the ammonia oxidation rates and soil nitrate content, indicating a negative impact on both ammonia and nitrite oxidation, i.e. an overall lower nitrification activity. The effect was stronger in soils subjected to a single, long drought compared to two shorter drought periods. This either suggests that the effect of a long drought on nitrifiers is more severe or that previous exposure to stress, here the first drying-rewetting event, was enough to increase the resistance of these communities to future stress. It is possible that the first drying-rewetting cycle shifted the microbial communities towards an alternative state that was better at coping with additional drying-rewetting events. This aligns with the ecological theory on 'catastrophic shifts in ecosystems' (Scheffer et al., 2001). Ammonia oxidation activity in soil B, E and U was also resilient as there was no difference between soils subjected to drought and control soils seven days after rewetting. Soil U, however, showed higher activity in droughted soils after the recovery period, possibly triggered by increased nutrient availability after rewetting. By contrast, soil S was unaffected,

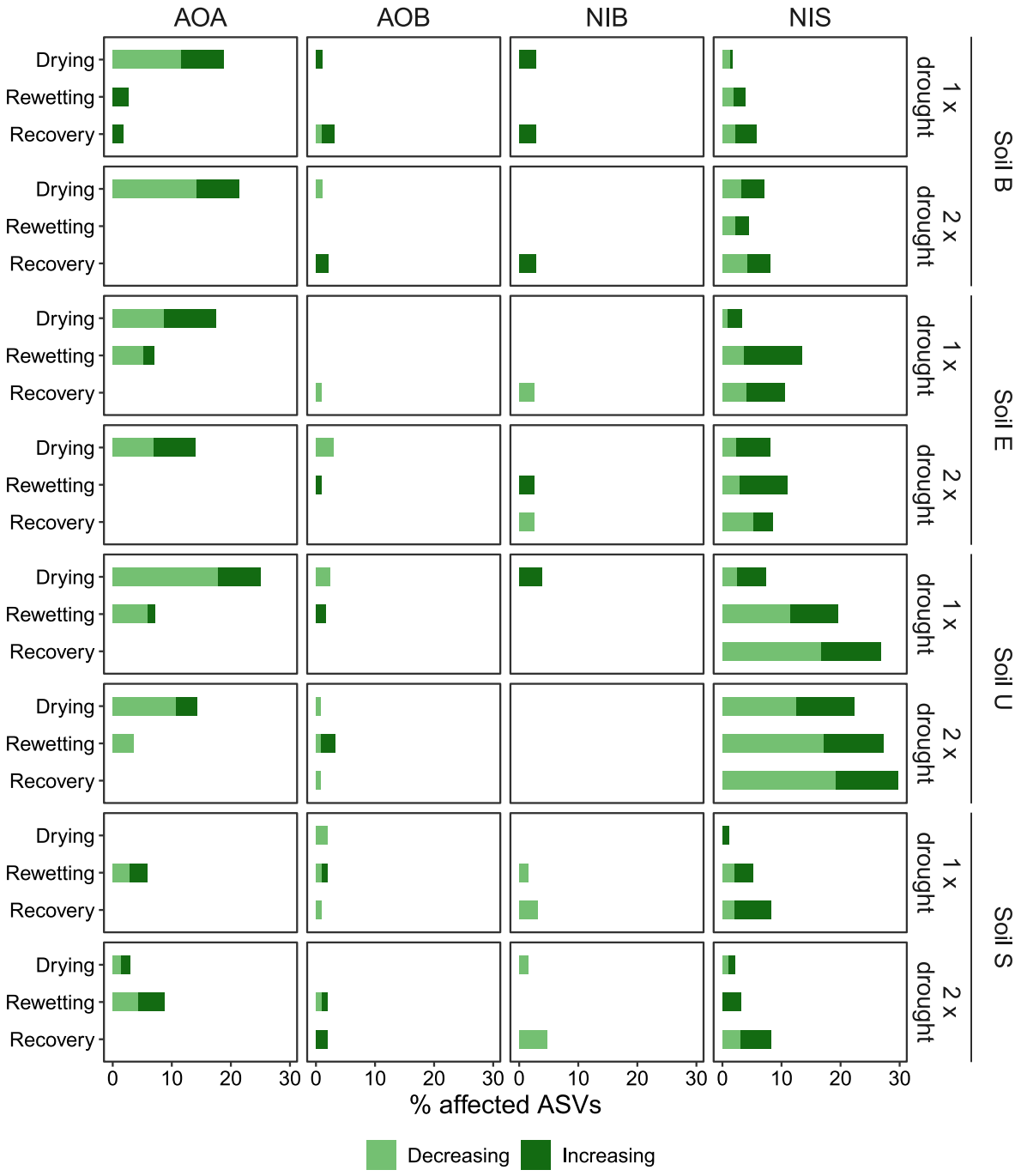

Fig. 4. Percentage of ASVs significantly decreasing or increasing after one long or two shorter droughts in comparison to the control in the four functional groups and across the four soils. Differential abundances were determined at the end of the drought treatments (day 42), after rewetting (day 49) and after the recovery phase (day 77). AOA: ammonia oxidising archaea; AOB: ammonia oxidising bacteria; NIB: *Nitrobacter* type nitrite oxidisers; NIS: *Nitrospira* type nitrite oxidisers.

potentially because sandy soils may be subjected to more regular moisture fluctuations and thus harbour communities better acclimated to drying-rewetting stress than those found in more clayey soils (Peralta et al., 2013; Placella and Firestone, 2013). However, it is more likely that the communities did not respond due to inactivity, as the lower total N and ammonium levels combined with low pH suggest that the ammonia oxidisers are more substrate limited in soil S compared to the other soils. This was supported by the low ammonia oxidation rates observed in the control treatment in soil S. The treatment effects on ammonia oxidation were consistent across substrates of varying complexity (ammonium < urea < yeast extract) and nature (organic vs inorganic) that were used to account for differences in substrate preferences and affinities between and within AOA and AOB (Levičnik-Höfferle et al., 2012; Qin et al., 2024). Since we do not know which community members were active during the assay, we cannot exclude known biases associated with this assay, where the choice of substrate can promote or inhibit specific AOA or AOB clades (Hazard et al., 2021). Likewise, this assay does not enable to estimate the contribution of autotrophic versus heterotrophic nitrification (Gao et al., 2023) to the observed ammonia oxidation rates or increased in nitrate pools.

The decrease in ammonia oxidation rates during drought coincided with changes in the relative abundance within AOA but not within AOB, indicating an important role of AOA for ammonia oxidation in the

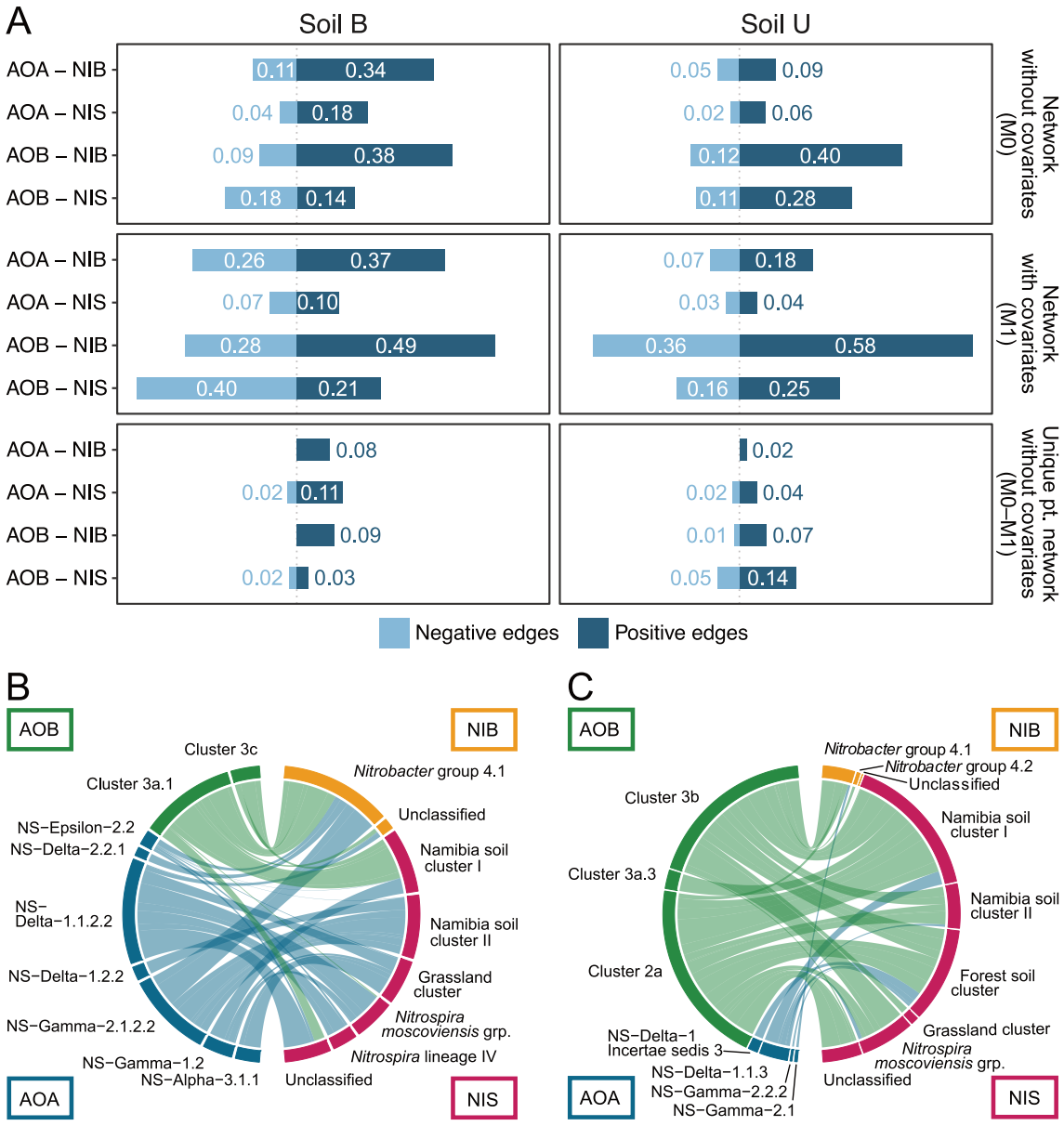

Fig. 5. Network analyses of ammonia and nitrite oxidising microorganisms in soil B and U. **A)** Proportion of negative and positive edges per node in the networks. Upper panels: networks built without covariates (M0). Middle panels: networks where the effects of treatment and timepoint on nodes and edges were removed by including treatment and timepoint as covariates (M1). Lower panels: networks containing only the nodes and edges affected by treatment and timepoint, obtained by subtracting the nodes and edges not affected by the covariates from the network without covariates (M0–M1). Edges per node were calculated by dividing the edge count by the average number of ASVs of each functional group to which the two connected nodes belong (AOA: ammonia oxidising archaea; AOB: ammonia oxidising bacteria; NIB: *Nitrobacter* type nitrite oxidisers; NIS: *Nitrospira* type nitrite oxidisers). Colours represent negative and positive edges. **B, C)** Chord diagrams showing unique (M0–M1) positive network edges between clades of ammonia oxidisers (AOA and AOB) and nitrite oxidisers (NIB and NIS), i.e. edges affected by treatment and timepoint, in soil B (**B**, 42 edges) and soil U (**C**, 43 edges). Edge width denotes edge degree and colours in the ring denote the functional guild.

affected soils. This is supported by observations that ammonia oxidation in soils most often seems to be driven by AOA rather than AOB, which are typically favoured when N levels are higher (e.g. Sterngren et al., 2015; Verhamme et al., 2011), in combination with the low ammonium levels in the soils used in this study. This would imply a general deceleration of N cycling during times of drought, which should be more pronounced in soils where AOA drive ammonia oxidation. The changes in AOA but not AOB community composition also indicate a higher resistance to drought stress among AOB, which aligns with work showing higher sensitivity to osmotic stress of AOA compared to AOB in pure culture (Bello et al., 2019), as well as higher sensitivity to drought as shown in both microcosm (Thion and Prosser, 2014; Bello et al., 2019) and field experiments (Fuchslueger et al., 2014; Séneca et al., 2020; Bintarti et al., 2025). Differential abundance analysis further indicated that although the relative abundance of the majority of affected AOA decreased, a substantial fraction also increased, especially in soil E. This illustrates the large variation in niche preferences that exists within this guild (Saghaï et al., 2021; Wright and Lehtovirta-Morley, 2023; Qin et al., 2024). Notably, effects on the relative abundance of AOA between the droughted and the control soils started to decrease after rewetting and were minimal or absent after the recovery period, indicating high resilience among AOA, with the exception of AOA communities in soil S.

Despite the decrease in ammonia oxidation rates and soil nitrate content during drought, both NIS and NIB community composition were little affected by drought. Possible strategies include mixotrophic

growth (e.g. Daims et al., 2001; Starkenburg et al., 2008), dormancy (Roszak and Colwell, 1987) and adaptation to osmotic pressure, although osmoadaptation does not appear to be widespread among soil NOB (Wu et al., 2024). Instead, NIS communities were significantly affected by rewetting and did not recover within the time frame of the experiment. This coincided with a decrease in soil nitrate content, indicating a decreased nitrification activity in soils B and E. Rewetting events are characterised by a nutrient flush in combination with re-established diffusion (Birch, 1958; Moyano et al., 2013), offering favourable substrate conditions for ammonia oxidation and subsequently increasing resource levels for nitrite oxidisers. At the same time, soil rewetting rapidly changes the osmotic pressure and can limit oxygen diffusion, which would supress ammonia oxidation. Effects of rewetting differed between the two drought treatments, with the largest shift in community composition observed after rewetting of the single drought treatment. In contrast to AOA, NIS communities did not appear to be resilient as the relative abundance of 5–30 % of the ASVs still differed from that of the control at the end of the recovery period, except in soil E. In fact, the fraction of affected NIS ASVs had increased after the recovery period. This challenges the assumption that NIS in comparison to NIB prefer low nitrite conditions (Wertz et al., 2012; Nowka et al., 2015; Simonin et al., 2015) and are more sensitive to changes in osmotic pressure (Li et al., 2021). Instead, these findings confirm niche differentiation at fine phylogenetic scale within this poorly characterized group (Maixner et al., 2006; Gruber-Dorninger et al., 2015; Jones and Hallin, 2019).

In addition to canonical nitrite oxidisers, NIS can also include comammox bacteria that can perform both ammonia and nitrite oxidation, and it is not possible to disentangle these two groups based on *Nitrospira*-type *nxrB* gene sequences (Daims et al., 2016), meaning that comammox sequences could be present in our NIS dataset. However, quantitative analysis in agricultural soil have shown that comammox bacteria have a lower abundance than AOA and AOB (Bintarti et al., 2025) and comammox specific *amoA* genes are typically found at lower abundance compared to *nxrB*, ranging from about three times (Wang et al., 2023) over ten times (Li et al., 2020; Xu et al., 2020) to more than one hundred times lower abundance (Li et al., 2024). Moreover, the total abundance of nitrite-oxidising communities has been shown to be comparable to that of AOA and AOB (Jones and Hallin, 2019). Altogether, these findings suggest comammox constitute a minor fraction of the NIS communities in agricultural soils.

The network analysis of nitrifying communities in soil B and U revealed that positive edges, i.e. co-occurrences between ammonia oxidisers and nitrite oxidisers, generally dominated over negative edges. Further, co-occurrences were more affected by drying-rewetting than negative associations. Co-occurrences could signal shared niche, but since canonical ammonia and nitrite oxidisers are involved in mutualistic relationships, our results could also indicate that drying-rewetting can destabilise associations between these functional guilds. Such decoupling between ammonia and nitrite oxidation may affect N fluxes

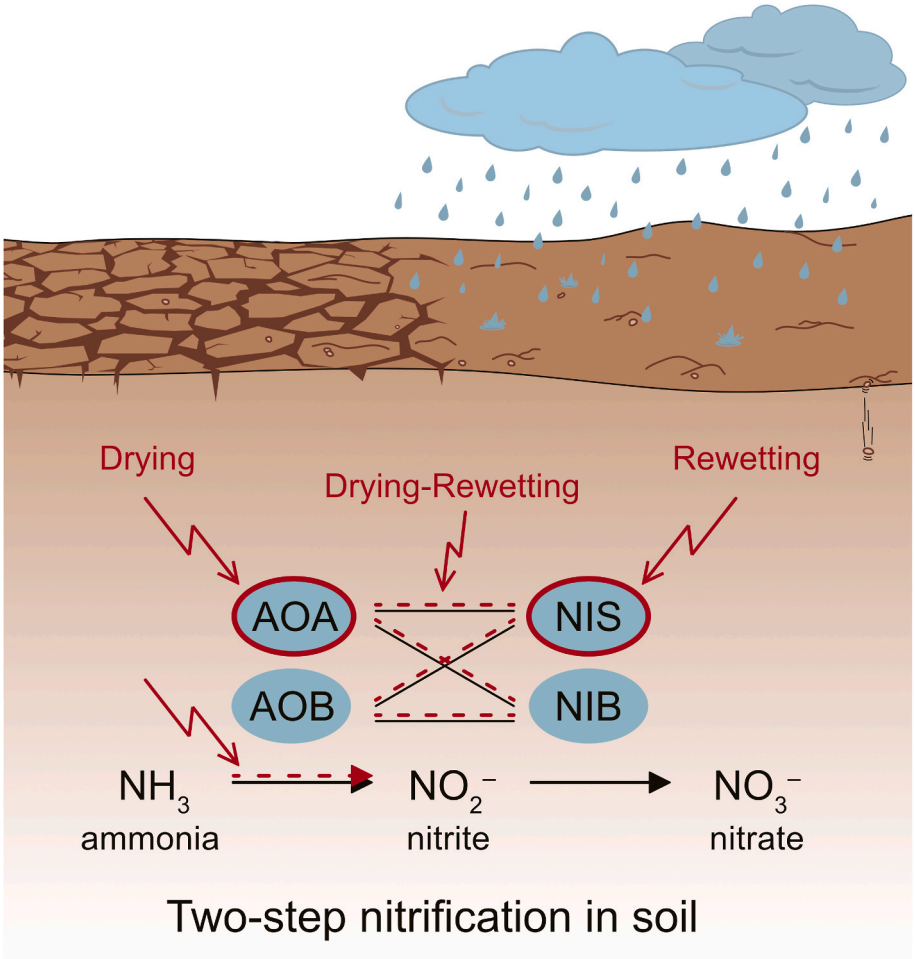

Fig. 6. Conceptual model of observed effects of drying-rewetting stress on nitrifier community composition, ammonia oxidation rates and co-associations between ammonia oxidisers and nitrite oxidisers. Black lines indicate processes and co-associations under control conditions (45–50 % water holding capacity), whereas dashed red lines indicate reduced process rates and weakened co-associations. Red elbow arrows indicate significant effects of drying and/or rewetting on community composition, ammonia oxidation rates or co-associations. AOA: ammonia oxidising archaea; AOB: ammonia oxidising bacteria; NIB: *Nitrobacter* type nitrite oxidisers; NIS: *Nitrospira* type nitrite oxidisers.

in soil and result in accumulation of nitrite (Gelfand and Yakir, 2008). Contrary to previous work suggesting that NIB tend be more often associated with AOB and NIS with AOA (Simonin et al., 2015; Stempfhuber et al., 2017; Jones and Hallin, 2019), we found that NIS ASVs co-occurred with both AOB and AOA ASVs in a soil-dependent manner, even after normalizing the number of edges for each functional group and soil. This likely reflects that NIS is a highly diverse functional group that can fill multiple nonoverlapping niches (Daims et al., 2016). NIB displayed similar patterns, although to a smaller extent due to the lower phylogenetic diversity in this group.

5. Conclusions

Our results show that drought has a strong but short-term impact on ammonia oxidation rates and the relative abundance of AOA ASVs, indicating low resistance but high resilience in this group (Fig. 6). This led to a temporary decelerated nitrogen turnover in dry soil. Effects of rewetting on NIS were more pronounced, as shown by shifts in community composition and in the relative abundance of individual ASVs, without recovery by the end of the experiment, indicating low resilience. By contrast, AOB and NIB communities seemed largely unaffected by drying-rewetting events. Nevertheless, drying-rewetting events affected the co-occurrences of ammonia and nitrite oxidisers, possibly leading to a destabilisation of metabolic interactions among the functional guilds completing nitrification. This study helps to understand the impact of weather extremes on soil nitrifiers and calls for further investigation of the effects of climate change related impacts on soil nitrifier community dynamics and the fate of N in soils.

CRediT authorship contribution statement

Laura J. Müller: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Mara Alicke:** Investigation, Formal analysis. **Sana Romdhane:** Supervision, Formal analysis. **Grace Pold:** Writing – review & editing, Supervision. **Christopher M. Jones:** Writing – review & editing, Supervision. **Aurélien Saghaï:** Writing – review & editing, Writing – original draft, Supervision. **Sara Hallin:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability

Data from this experiment is shared in the supplementary material and amplicon sequencing data has been deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA1208997.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109846.

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