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Seed treatment with plant-defense elicitors decreases the abundance of ammonia oxidizers associated with winter wheat roots

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ABSTRACT

Priming with elicitors, including phytohormones, is known to induce physiological changes in plants that affect resource allocation and nutrient uptake strategies. However, effects of these changes on belowground microbial communities, particularly those involved in nitrogen (N) transformations, remain poorly characterized. Here, we tested the hypothesis that treating seeds with elicitors would affect N-transforming communities associated to roots and rhizosphere, influencing soil N availability and thus plant performance. We measured the effects of treating winter wheat seeds with cis-jasmone, methyl jasmonate or methyl salicylate on plant traits and on the genetic potential for ammonia oxidation by ammonia-oxidizing bacteria (AOB) and archaea (AOA), the initial inorganic N transformation process resulting in N loss. Elicitors reduced the genetic potential for ammonia oxidation by AOB in root-associated microbial communities and increased traits reflecting N uptake in winter wheat. Proposed mechanisms include increased exploitative competition for ammonium, limiting substrate availability for microorganisms, and induced biological nitrification inhibition capacity actively suppressing ammonia oxidizers. There were minor effects on the composition of the AOB and AOA communities, but an indicator species analysis showed that several AOB and a few AOA genotypes were indicative of elicitor treatments. Seed treatment with elicitors may represent an overlooked strategy to minimize nitrification to mitigate N losses from arable soils.

1. Introduction

Elicitors, including phytohormones, are increasingly used in plant protection strategies as a way to reduce pesticide use and alleviate effects of adverse environmental conditions on crop yield, while minimizing detrimental effects on non-target organisms and the environment (Maffei et al., 2012; Ahmad et al., 2016; Mauch-Mani et al., 2017). As such, they represent a promising way to enhance resistance in crops that are often neither bred for their inducible responses nor cultivated in environments to which they are adapted (Stout et al., 2002). The exogenous application of elicitors results in physiological priming, an induced state whereby plants are able to deploy their defense more effectively when exposed to a future stress (Martinez-Medina et al., 2016). Priming with phytohormones often targets the jasmonic acid and salicylic acid signaling pathways, two major components of resistance-inducing mechanisms in plants (Balmer et al., 2015), and have been successfully used to enhance resistance against herbivores, pathogens or abiotic stress in cereals (Moraes et al., 2008; Kang et al., 2012; Kalaivani et al., 2016; Kraus and Stout, 2019; Tayyab et al., 2020; Bhavanam and Stout, 2021; Ninkovic et al., 2021) and other plants (Worrall et al., 2012; Gordy et al., 2015; Farooq et al., 2016; Mouden et al., 2020). Physiological changes in resource allocation or nutrient uptake strategies can further affect below-ground microbial communities, as these are partly shaped by root exudation and nitrogen (N) acquisition patterns (Bell et al., 2015; Zhalnina et al., 2018; Lopes et al., 2022). Yet, elicitor-induced effects on soil microorganisms have so far mainly been addressed within a plant protection framework (Sonnemann et al., 2002; Doornbos et al., 2011; Carvalhais et al., 2013; Liu et al., 2017; 2018; Zhang et al., 2022) despite the critical roles that microorganisms are known to play in other aspects of plant performance (Wagner et al., 2014; Panke-Buisse et al., 2015) and in soil nutrient cycling (Philippot et al., 2009).

Since N is the most prominent plant growth-limiting nutrient in terrestrial ecosystems (LeBauer and Treseder, 2008), it is particularly

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relevant to understand whether elicitor-mediated changes in plant physiology can affect microorganisms involved in N transformations, thereby influencing plant available N content, and how this feeds back to the plant. It is well established that the N fluxes between diverse N-transforming microorganisms through the consumption and production of different forms of inorganic N are a major determinant of whether N is retained or lost from soils (Kuypers et al., 2018). One key process is nitrification, where soil-bound ammonium is oxidized into the more mobile nitrate by various lineages of archaea and bacteria (Prosser and Nicol, 2012; Daims et al., 2015). If not assimilated, nitrate can then be lost directly via leaching or fuel processes leading to gaseous N losses, including the potent greenhouse gas nitrous oxide. At the global scale, this contributes to reductions in the N-use efficiency of arable soils, with an average loss of 50% of the N inputs (Lassaletta et al., 2014). As ammonia oxidation is the first step leading to such losses, there is a strong interest in developing strategies to mitigate this process. Current approaches include the use of biological or synthetic nitrification inhibitors and the selection of plant traits related to high rates of N acquisition to reduce substrate availability for ammonia oxidizers, including breeding for crops with biological nitrification inhibition (BNI) capacity (Philippot and Hallin, 2011; Coskun et al., 2017; Subbarao et al., 2021). However, nitrification inhibitors are not approved for use in all countries, the selection of plant traits develops slowly and future BNI crops may be geographically restricted, which emphasizes the need for alternative approaches, and the application of elicitors could be an interesting candidate.

The aim of this study was to determine how treating plants with elicitors affects the composition of root and rhizosphere microbial communities, including their capacity to oxidize ammonium, and the subsequent effects on plant performance. To do this, we set up a pot experiment where winter wheat seeds were treated with the jasmonic and salicylic acid derivatives cis-jasmone (CJ), methyl jasmonate (MeJa) and methyl salicylate (MeSa) prior to germination. The exogenous application of these elicitors has been shown to increase plant resistance against drought, by modulating levels of osmolytes and antioxidant enzymes (Tayyab et al., 2020), and attacks *via* the production of resistance-related metabolites (Thaler et al., 1996; Moraes et al., 2008; Tang et al., 2015) as well as the induction of volatile compounds attracting natural enemies of herbivores (Thaler, 1999; Birkett et al., 2000). Since the elicitors were not added to the plants when they were growing in the soil, any direct effects of elicitors on the soil communities establishing in the root environment were minimized. Plants were grown under two fertilization regimes to assess how N availability influences the plants' response to the elicitor treatments (Verly et al., 2020). The genetic potential for ammonia oxidation and community composition of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), but also of total archaea and bacteria to gain broader insights, were determined, and above- and below-ground plant traits were measured to assess plant performance. We hypothesized that the seed treatments would modify the physiological status of the growing plants and affect both the abundance and composition of the microbial communities associated with roots, with consequences for soil N availability and thereby plant performance.

2. Materials and methods

2.1. Soil sampling

Soil (0–15 cm depth) was collected in August 2020 at Lövsta Research Station, Uppsala, Sweden (59°49′51.54″N; 17°48′25.77″E), in a fallow field where repeated tillage was performed during the year to control perennial weeds. Field management history and soil texture (Soil physics laboratory, SLU, Uppsala, Sweden), pH and nutrient content (Soil and plant laboratory, SLU, Uppsala, Sweden) are presented in Table S1. The soil was sieved (4 mm) and stored at −20 °C until further processing. The water holding capacity (WHC) and the water content (oven-drying at 105 °C for 24 h) were estimated for three replicates and used to adjust the WHC of the soil to 60% for the current experiment.

2.2. Seed treatment

Winter wheat (*Triticum aestivum*, cv. Julius) seeds were surface sterilized by two successive baths in 70% ethanol (3 min) and 1% sodium hypochlorite (3 min) followed by a thorough rinsing with sterilized water. Seeds (n = 100) were then submerged in gas-tight glass flasks in aqueous solutions of CJ (1 and 5 mM; Tokyo Chemical Industry, Tokyo, Japan), MeJa (1 and 5 mM; Merck KGaA, Darmstadt, Germany) or MeSa (1 and 10 mM; Merck KGaA) and incubated in the dark for 24 h at 25 °C on a shaker (125 RPM). Dose levels were selected based on existing knowledge on the effects of these elicitors on plant physiology (Birkett et al., 2000; Kalaivani et al., 2016; Kraus and Stout, 2019). Seeds treated with 10 mM CJ or MeJa did not germinate (data not shown) and were replaced by 5 mM treatments. The solutions were prepared by mixing sterile water, Tween20 10% (1% v/v) (Bio-Rad, Hercules, CA, USA) and each elicitor to achieve the desired final concentration, following (Kraus and Stout, 2019). For the control treatments, the seeds were either submerged in water only or in a water and Tween20 solution. All treatments corresponded to an application of 5 ml g^{-1} of seed. Seeds were drained, rinsed with sterile water and sown immediately after the 24-h incubation period.

2.3. Experimental design

Effects of the seed treatments were investigated in a pot experiment conducted in a climate-controlled chamber and included four replicates. Each pot (13.7 × 13.7 × 23 cm) contained 1.6 kg of soil and a 2 cm layer of autoclaved clay pellets (Plantagen, Järfälla, Sweden; 8–14 mm) in the bottom. Ten seeds were sown in each pot, and after germination, the pots were thinned to four plants. To prevent effects of other plants, weeds were continuously removed. The soil moisture was monitored throughout the experiment by weighing the pots and sterile water was added when necessary to maintain ca. 60% WHC. The position of each pot in the climate chamber was randomized within each replicated block (n = 4), with day/night temperatures of 20/15 °C and a day length of 18 h. To assess the influence of soil ammonium availability, half of the pots received the equivalent of 25 kg N ha^{-1} in the form of ammonium chloride (adjusted to pH 6.8) four days after germination while the remaining pots were unfertilized. There were no signs of pests or pathogens on the plants throughout the experiment based on ocular inspection.

2.4. Harvesting and measurement of plant traits

Plants were harvested 23 days after germination to allow time for archaeal and bacterial microbiome selection (Edwards et al., 2015; Graf et al., 2022) and to minimize potential confounding factors associated with plant growth in pots (e.g. nutrient depletion in the unfertilized treatments). Rhizosphere, defined as the soil attached to the roots, and root samples were collected. For each pot, roots from two individual plants were pooled in a 50 mL falcon tube containing phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4). Rhizosphere and root-associated communities were separated by vortexing the roots at maximum speed until they became white. The roots were then removed from the falcon tube and the phosphate-buffered saline solution with the rhizosphere soil was centrifuged at 5000 g for 5 min. The pellets (corresponding to the rhizosphere communities) and washed roots (corresponding to the root-associated communities) were stored at −20 °C until DNA extraction. The roots of a third plant in each pot were used to measure root traits. They were first scanned with a flatbed Epson Perfection V800 scanner (Seiko Epson Corporation, Suwa, Nuagano, Japan). Total root length was measured using the SmartRoot plugin (Lobet et al., 2011)

implemented in ImageJ (Schneider et al., 2012). Root dry weight was obtained after oven-drying at 65 °C for 4 days. Specific root length was calculated for each plant as the ratio of total root length to total root dry weight.

Above-ground plant traits were measured on three individual plants in each pot and the median value was used for the statistical analyses. Leaf greenness values were obtained by averaging two measurements on four different leaves per plant using a SPAD-502 meter (Konica Minolta Sensing, Tokyo, Japan). Leaf area was estimated by analyzing leaf pictures taken with a Canon EOS 100D camera (Canon Inc., Tokyo, Japan) using ImageJ. Shoot dry weight was obtained after oven-drying at 65 °C for 4 days. Specific leaf area was calculated for each plant as the ratio of total leaf area to total leaf dry weight. Shoot N content was measured on dry shoot material (one per replicate, i.e. n = 4 per treatment) at the Analysis Laboratory, SLU, Uppsala, Sweden.

2.5. DNA extraction and quantitative PCR

DNA was extracted from rhizosphere and (washed) root samples cut in 1 cm pieces using the NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions, with 0.25–0.30 and 0.35–0.40 g, respectively. For each sample, two independent extractions were done and pooled prior to further analyses. The extracted DNA was quantified with a Qubit® fluorometer using the Broad Range double stranded DNA kit (Thermo Fisher Scientific, Waltham. Massachusetts, USA), with mean DNA yields per treatment ranging between 66.8 ± 2.5 and 74.7 ± 12.6, and 67.4 ± 1.5 and 85.9 ± 13.2 ng ul^{-1}, for root and rhizosphere soil extracts, respectively (Table S2). There was no difference in DNA extraction yield between treatments (ANOVA, $p(F) > 0.05$).

The abundance of total archaea and bacteria (16S rRNA gene), archaeal (*amoA*) and bacterial (*amoA*) ammonia oxidizers, and complete ammonia oxidizers (comammox; *amoA*) were determined by real-time quantitative PCR (qPCR) using specific primers for each group. The qPCR reactions were performed in two independent runs in a reaction volume of 15 μl containing iQ™ SYBR Green Supermix (Bio-Rad), 0.1% Bovine Serum Albumin, primers and 4 ng of template DNA on a CFX Connect Real-Time System (Bio-Rad). Primer sequences and concentration, qPCR conditions and amplification efficiencies are presented in Table S3. Standard curves were obtained by serial dilutions of linearized plasmids with cloned fragments of the specific genes. The amplifications were validated by melting curve analyses and agarose gel electrophoreses. Potential inhibition of PCR reactions was checked by amplifying a known amount of the pGEM-T plasmid (Promega, Madison, WI, USA) with the plasmid specific M13F/M13R primer set (Table S3) and addition of 4 ng of template or non-template controls. No inhibition was detected with the amount of DNA used. The number of 16S rRNA gene copies was corrected by removing the fraction of chloroplast sequences present in the sequencing dataset (54–61%; see below).

2.6. Sequencing of the 16S rRNA and amoA genes

For the root-associated communities, sequencing libraries of 16S rRNA gene, archaeal *amoA* and bacterial *amoA* amplicons were prepared using a two-step procedure. The fragments were first PCR amplified in two independent runs with the same primers as those used for qPCR. Primer sequences and concentrations, as well as cycling conditions, can be found in Table S4. The PCR products were pooled, checked by agarose gel electrophoresis and purified using Sera-Mag beads (Merck KGaA, Darmstadt, Germany). A single 30 μl reaction was performed for the second PCR, using 0.2 μM of the primers with Nextera adaptor and index sequences, and 3 μl of the pooled PCR product from the first PCR. The amplicon size was validated by gel electrophoresis and the final PCR products were purified using Sera-Mag beads. After quantification using a Qubit fluorometer (Invitrogen, Carlsbad, CA, US), two libraries were created by pooling equal amounts of purified 16S rRNA gene amplicons in one, and archaeal and bacterial *amoA* amplicons in the other. A final quality control was performed on a BioAnalyzer (Agilent, Santa Clara, CA, US). Sequencing was done using an Illumina MiSeq instrument and the 2 × 250 bp and 2 × 300 bp chemistry for the 16S rRNA and AOA + AOB pools, respectively. Two samples, the unfertilized 1 mM CJ for AOA and fertilized 10 mM MeSa for AOB, failed during the sequencing.

2.7. Sequence analysis

Bacterial and archaeal *amoA* gene amplicons were processed with the 'dada2' package v. 1.21.0 (Callahan et al., 2016) in the R software v. 4.1.2 (R Core Team, 2021) to infer amplicon sequence variants (ASVs). Briefly, primer sequences were removed and the reads truncated to 270 and 250 bp for forward and reverse reads (maxEE = c(2,5), maxN = 0, truncQ = 2), respectively. Bacterial *amoA* sequences were merged using default parameters, whereas forward and reverse archaeal *amoA* reads were concatenated, following Aigle et al., (2019). Chimeras were discarded using a *denovo* approach with the removeBimeraDenovo function ('consensus' method). Representative sequences for ASVs of each gene were translated to amino acids (esl-translate command implemented in EASEL v. 0.48) and aligned to respective reference alignments (Alves et al., 2018; Jones and Hallin, 2019) using the hmmscan command in HMMER v. 3.3.2 (Eddy, 1998). Non-specific ASVs were discarded. The 16S rRNA gene amplicons were processed using 'dada2' following the procedure used for the AOB, except for truncLen = c(240,230), maxEE = c(2,2) and removal of chloroplast sequences. After discarding the singletons, we obtained 6,468,309 and 316 16S rRNA gene, AOA and AOB ASVs, respectively.

2.8. Phylogenetic placement of amoA and 16S rRNA sequences

Phylogenetic placement was used for the assignment of the ASVs. First, nucleotide alignments of both archaeal and bacterial *amoA* ASVs were generated in ARB v. 7.0 (Ludwig et al., 2004) by mapping the amino acid alignments to their respective nucleotide sequences. The 16S rRNA gene ASVs were aligned to the reference alignment from the Living Tree Project, release December 2021 (Ludwig et al., 2021), using the hmmalign command in HMMER. EPA-NG v. 0.3.8 (Barbera et al., 2019) was then used to place the reads on reference phylogenies for 16S rRNA (Living Tree Project, release December 2021), archaeal *amoA* (Alves et al., 2018) and bacterial *amoA* (Jones and Hallin, 2019), with default parameters. The 16S rRNA gene ASVs were taxonomically assigned using the 'examine assign' command implemented in GAPPA v. 0.8.1 (Czech et al., 2020), whereas the clade assignation for both archaeal and bacterial *amoA* ASVs was performed using the 'prepare extract' command and the –point-mass argument to only consider the most likely placement. The trees were plotted using iTOL v5 (Letunic and Bork, 2021).

2.9. Statistical analyses

All statistical analyses and figures ('ggplot2' package v. 3.3.5; Wickham, 2016) were done using the R software. Gene abundance and plant traits were initially tested for normality and homogeneity of variance using Shapiro-Wilk and Levene tests, respectively ('rstatix' package v. 0.7.0; Kassambara, 2021); and no extreme outliers were identified ('rstatix' package). Shoot N content values were arcsine-transformed to account for the proportional nature of the data. Pairwise comparisons between each elicitor treatment and the corresponding control were performed using t-tests ('base' package). No statistically significant difference was observed between the two controls for any of the plant traits (Fig. S1) or abundance of genes (Fig. S2), and the control with Tween20 was used in subsequent pairwise comparisons. The correlation matrix including the plant traits and the gene abundances was generated using the 'Hmisc' package v. 4.6–0 (Harrell, 2022) and plotted using the 'corrplot' package v. 0.92 (Wei and Simko,

2021). Statistical differences in days before germination between the different treatments were tested using the Kruskal-Wallis test and the false discovery rate correction available in the 'agricolae' package v. 1.3.5 (de Mendiburu, 2019).

Rarefaction curves of species richness were generated for each gene from the corresponding raw ASV table using the 'vegan' package v. 2.5–7 (Oksanen et al., 2018). They showed that the sequencing depth was sufficient to capture most of the diversity present in the samples (Fig. S3). Rarefied tables were obtained by averaging the ASV counts over 1000 computations using 'vegan'. After rarefaction, 3,861, 204 and 313 16S rRNA gene, AOA and AOB ASVs were retained, respectively. Alpha-diversity analyses were conducted on the rarefied ASV tables with Pielou's evenness calculated using 'vegan' and Faith's phylogenetic diversity (Faith, 1992) using the phylogenetic placement files (-fdp command in the GUPPY suite of tools v. 1.1). Both indices were checked for normality, homogeneity of variance and the presence of extreme outliers as described above. Comparisons of treatment were performed using ANOVA or, when underlying assumptions for ANOVA were violated, Kruskal-Wallis tests ('agricolae' package).

Beta-diversity analyses were conducted on centered log-ratio (clr)-transformed rarefied ASV tables to account for the compositional nature of sequencing data (Gloor et al., 2017). Zero counts were replaced using a Bayesian-multiplicative replacement ('zCompositions' package v. 1.4.0 (Martín-Fernández et al., 2015); and the zero-replaced ASV tables were clr transformed using the 'compositions' package v. 2.0–4 (van den Boogaart et al., 2022). The transformed datasets were used in Principal Component Analyses (PCA) to visualize differences in community composition and structure ('vegan' package). Potential differences in dispersion between the different treatments were explored using the betadisp function ('vegan' package) and their significance assessed using a permutation test. No significant differences were detected and permutational multivariate analyses of variance (PERMANOVA) were conducted on the β-diversity to assess the effects of elicitor treatment and fertilization using the adonis function in 'vegan'.

Indicator ASVs, corresponding to ASVs with a frequency in a given elicitor treatment differing from that of both controls, were identified using the signassoc function and Sidak's correction for multiple testing ('indicspecies' package v. 1.7.9; De Caceres and Legendre, 2009). This approach allows the detection of both positive (i.e. greater frequency compared to the controls) and negative (i.e. lower frequency compared to the controls) associations. Only indicator ASVs absent (in the case of negative association) or with a median frequency $\geq$0.001% in the elicitor treatment were retained.

3. Results

3.1. Effects of elicitors on plant functional traits

The effects of elicitor treatments on plant traits were mainly positive, and CJ displayed the strongest influence. Specific leaf area (SLA) was the trait the most affected, particularly at the highest doses (5 mM for CJ and MeJa, 10 mM for MeSa; Fig. 1a and S4), whereas effects on shoot biomass and greenness were detected only in the 5 mM CJ treatments. Although not significant for any of the individual elicitor treatments, we also observed a trend towards higher shoot N content in treated plants compared to the control. Regarding root traits, there was a general increase in biomass (Fig. 1b and S5) but the difference was only significant in the fertilized 5 mM CJ treatment. Effects on specific root length (SRL) were more variable, with small negative and positive changes in fertilized and unfertilized pots, respectively.

Shoot and root biomass were positively correlated, and SRL and root biomass were negatively correlated, in both fertilized and unfertilized pots. In fertilized plants, shoot biomass was negatively correlated with both SLA and SRL but, together with root biomass, positively correlated with greenness (Fig. 2). Compared to the controls, the elicitor treatment delayed germination by one day in the 1- and 5-mM treatments and by

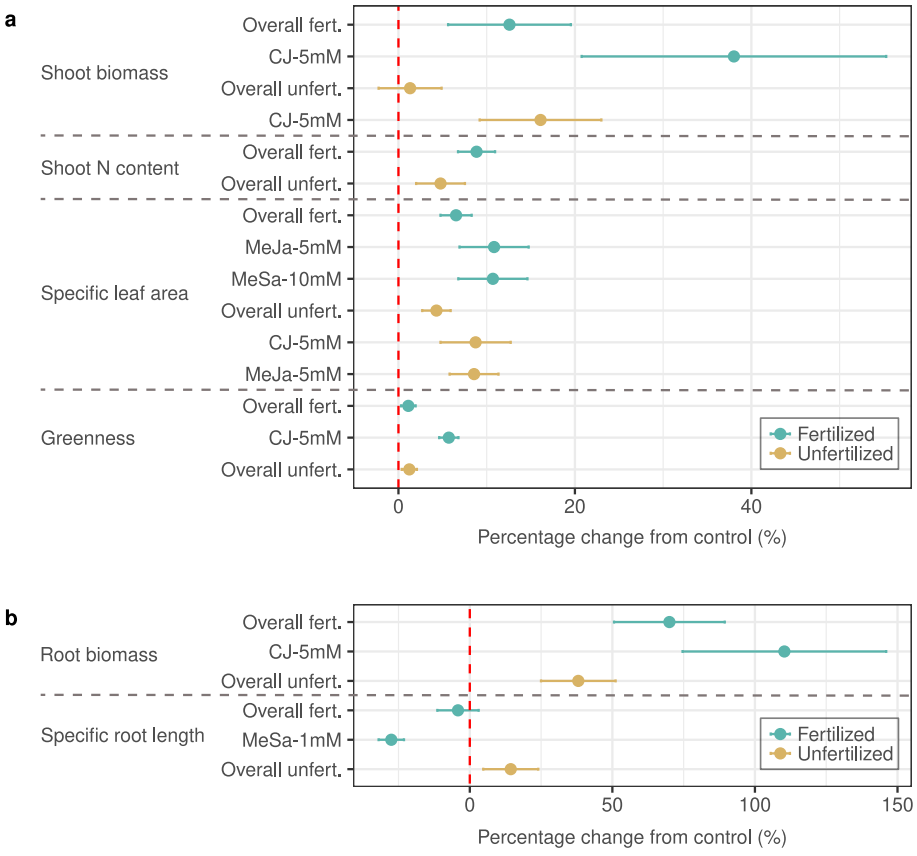

Fig. 1. Percentage change in (a) above- and (b) below-ground plant traits between elicitor-treated and control plants, in unfertilized (yellow) and fertilized (cyan) pots (mean ± s.e.). The dashed red line indicates no change compared to the control. Individual treatments are only shown if they are significantly different from their control (*t*-test, $p < 0.05$, $n = 4$). The data for all treatments and plant traits is presented in Figs. S4 and S5. CJ: cis-jasmone; MeJa: methyl jasmonate; MeSa: methyl salicylate.

two days in the 10 mM treatment (Fig. S6).

3.2. Effects of elicitors on microbial abundance and genetic potential for ammonia oxidation

Archaeal *amoA* genes were more abundant than their bacterial counterparts in rhizosphere and root compartments across all treatments, indicating a higher genetic potential for archaeal than bacterial driven ammonia oxidation (Fig. S2). The comammox *amoA* genes were below detection limits in both compartments and were not investigated further.

Significant effects of elicitor treatments on microbial abundance were only observed among the root-associated communities, with MeJa and MeSa showing the largest impact (Fig. 3 and S7). The influence of elicitors on the abundance of the total archaeal and bacterial community was limited, except in the fertilized 1 mM MeSa treatment where a ca. 30% increase was observed. Nevertheless, the genetic potential of root-associated AOB was overall lower in elicitor-treated plants compared to the control by an average of 8–19% in unfertilized and fertilized pots, respectively. The effect was particularly pronounced in fertilized 1 mM and 5 mM MeJa and 10 mM MeSa treatments, with mean decreases ranging between 32 and 39%. The genetic potential of root-associated AOA communities followed the same trend (Fig. S7), and both AOA and AOB were negatively correlated with SLA, in both fertilized and unfertilized soils (Fig. 2).

Although no significant effects of the elicitors were detected in the rhizosphere, there was a trend towards lower genetic potential for ammonia oxidation by AOA in 5 mM CJ and in fertilized MeJa and MeSa treatments, and for AOB in fertilized 1 mM CJ, 1 and 5 mM MeJa and 10 mM MeSa treatements (Fig. S8). In MeJa and MeSa, positive and negative changes in the genetic potential of both AOA and AOB were associated with unfertilized and fertilized plants, respectively. Finally, the archaeal *amoA* abundance in the rhizosphere was positively correlated with shoot biomass (Fig. 2).

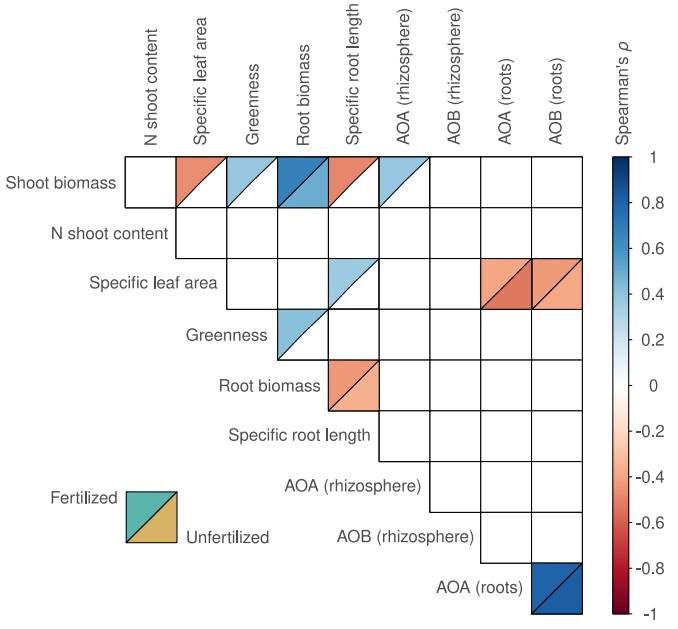

Fig. 2. Correlation matrix based on spearman correlations between plant traits and the genetic potential for ammonia oxidation by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in root-associated and rhizosphere communities, in fertilized and unfertilized pots. Only significant correlations ($p < 0.05$) are indicated.

3.3. Effects of elicitors on diversity and composition of root-associated communities

None of the elicitors affected the α-diversity of the total archaeal and bacterial communities or the AOA or AOB root-associated communities, and there was no difference in α-diversity between the two fertilization regimes (Table S5). As expected, PD was highest in the total archaeal and bacterial communities (38.4 ± 1.1, mean ± s.d.), followed by AOA (2.4 ± 0.1) and AOB (0.9 ± 0.1). A gradient of evenness was also observed; the total archaeal and bacterial communities being the most even (0.87 ± 0.01), followed by AOB (0.75 ± 0.01) and AOA (0.61 ± 0.01).

Both seed treatment and fertilization significantly altered the β-diversity of the total archaeal and bacterial communities associated with the roots of the growing plants (PERMANOVA, $R^2 = 0.26$ and 0.03, respectively; $P < 0.01$), with more variation observed in the controls (Fig. S9a). In contrast, only minor changes were observed in the case of AOA and AOB communities (PERMANOVA: $P > 0.05$; Fig. 4, S9b, c). The indicator species analysis further confirmed these results, with more ASVs associated with elicitor treatments in the 16S rRNA gene dataset ($n = 151$) compared to AOA ($n = 12$) and AOB ($n = 29$), although they represented a comparable fraction of the total number of ASVs in all three sequencing datasets (5–8%). The elicitor effect on the total archaeal and bacterial communities was mainly driven by various members of the Actinomycetota, Bacteroidota, Chloroflexiota and Pseudomonadota phyla in the unfertilized 5 mM CJ and 5 mM MeJa treatments (Fig. S10). Some bacterial classes, including Acidobacteria, Betaproteobacteria and Gammaproteobacteria, were affected across most treatments. The majority of the indicators among the AOA ASVs were found in CJ-treated plants (Fig. 5). Regarding AOB, ASVs within clade Nitrosospira 3a2, and to a lesser extent Nitrosospira 2a, were almost consistently impacted, either positively or negatively. About one third of the ammonia oxidizer ASVs that varied significantly between elicitor and control treatments were affected by more than one elicitor (Fig. S11).

4. Discussion

Seed treatment with the elicitors MeSa and MeJa decreased the abundance of the root-associated ammonia-oxidizing communities and the plants displayed a trend towards higher SLA and shoot N content. Since these two plant traits reflect plant N uptake strategies (Wright and Reich, 2004; Grassein et al., 2015), our results suggest stronger competition for ammonium between wheat and root-associated ammonia oxidizers in these particular treatments, resulting in a lower genetic potential for ammonia oxidation. These observations may be explained either by high exploitative competition limiting substrate availability for ammonia oxidizers (Thion et al., 2016) or active suppression of ammonia oxidizers through the release of biological nitrification inhibitors in root exudates (Subbarao et al., 2015; Kaur-Bhambra et al., 2021; Bozal-Leorri et al., 2022), which are two possible mechanisms leading to lower nitrification activity (Subbarao et al., 2009; Cantarel et al., 2015). Competitive interference is plausible as all three elicitors are known to activate plant metabolic pathways linked to the production of BNI compounds, including phenyl propanoids, glucosinolates and benzoxazinoids (Moraes et al., 2008; Schreiner et al., 2011; Neal et al., 2012; Subbarao et al., 2013). It is not unlikely that this trait can be induced in winter wheat since BNI activity has been demonstrated in several wheat cultivars (Subbarao et al., 2007; 2021; O'Sullivan et al., 2016). Furthermore, the trend of decreasing genetic potential for ammonia oxidation in fertilized pots aligns with the release of BNI compounds being triggered by root exposure to ammonium (Subbarao et al., 2009). The exploitative competition and competitive interference hypotheses are mutually non-exclusive and are further supported by the finding that the negative effects on the genetic potential for ammonia oxidation were more pronounced in the roots than

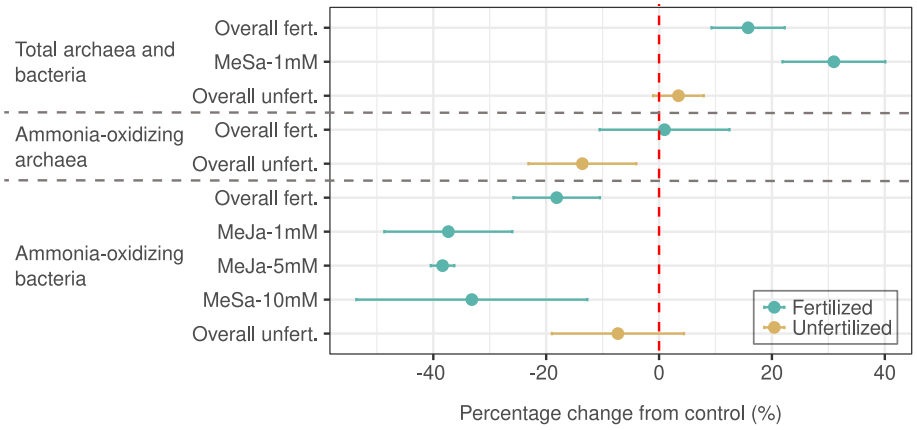

Fig. 3. Percentage change in the abundance of root-associated archaea and bacteria, and genetic potential for ammonia oxidation by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) between elicitor-treated and control plants, in unfertilized (yellow) and fertilized (cyan) pots (mean ± s.e.). The dashed red line indicates no change compared to the control. Individual treatments are only shown if they are significantly different from their control (*t*-test, $p < 0.05$, $n = 4$). The data for all treatments is presented in Fig. S7. CJ: cis-jasmone; MeJa: methyl jasmonate; MeSa: methyl salicylate.

in the rhizosphere, indicating that the elicitor-driven effects were strongest in the immediate vicinity of the roots.

Several ASVs, especially within Nitrosospira, were indicative of elicitor treatment, although the overall composition of the root-associated AOA and AOB communities was little affected across all treatments. While changes in the relative abundance of a small number of ASVs may not cause statistical differences in overall community composition between control and treated plants, they could affect ammonia oxidation rates if those indicator ASVs correspond to particularly active community members. As AOB ammonia oxidation is associated with higher nitrous oxide yields than that of AOA (Hink et al., 2018), the fact that AOB were more affected than AOA, both in terms of overall abundance and number of indicator ASVs, implies that treatment with elicitors could contribute to mitigate direct and indirect nitrous oxide production from crop-associated microbial communities, which have a major impact on greenhouse gas emissions at the global scale (Philippot et al., 2009). Stronger negative effects on root-associated AOB than AOA in elicitor-treated plants are consistent with the higher exploitative competition hypothesis, where AOA would be generally less affected by the lower N availability since they in general display higher affinity for ammonia compared to AOB (Martens-Habbena et al., 2009; Jung et al., 2022). This could also explain why AOA and AOB communities displayed little intra variation, despite the use of ASVs for the analyses and the growing evidence of the existence of fine phylogenetic-scale niche differentiation within both groups (Alves et al., 2018; Aigle et al., 2019; Saghaï et al., 2022). It aslo aligns with the predominance of AOA over AOB in both plant compartments, since low ammonium availability is expected in the immediate root surroundings of exploitative plants such as grasses, particularly during early growth when N uptake is high (Thion et al., 2016). Ammonia oxidizers, being autotrophs, are likely not directly influenced by potential elicitors-induced changes in (non-BNI) exudates although the ability for mixotrophic/heterotrophic growth in this group has not been ruled out (Prosser and Nicol, 2012). However, changes in exudate profiles can alter the abundance and composition of the heterotrophic communities (Hartmann et al., 2009). Accordingly, we found that the composition of the root-associated total archaeal and bacterial communities was affected by the treatments with elicitors and the indicator species analysis subsequently identified several ASVs related to bacterial phyla known to harbor heterotrophic taxa, including Acidobacteriota, Bacteroidota, Chloroflexi and Verrucomicrobiota. This could impact not only the microbial competition for ammonium between autotrophs and heterotrophs but also the priming of soil organic matter degradation resulting in N mineralization, which ultimately controls the release of ammonium that can be used as substrate by ammonia oxidizers.

The three elicitors differently affected plant functional traits and the root-associated microbial communities. For instance, among jasmonates, we observed a stronger positive effect on plant growth in CJ treatments, whereas both the abundance and composition of root-associated communities were less affected than in MeJa-treated plants. This aligns with previous work showing that differences in lipophilic properties between CJ and MeJa (Yang et al., 2018) cause distinct plant gene expression patterns (Birkett et al., 2000; Liu et al., 2017), leading to disparities in the composition of exudates and, potentially, in their effect on microbial communities (Lopes et al., 2022). However, both dose level and plant nutrition status also influenced the outcome of the seed treatment for a given elicitor in our experiment. The fact that treated plants had a similar or improved growth and nutrition status than control plants 23 days after germination, despite a strong inhibitory effect on germination, is important because a major limitation to the use of exogenous elicitors is the possibility that the benefits are counterbalanced by reductions in plant growth and yield (Cipollini et al., 2003). Such reductions could be caused by trade-offs in resource allocation between growth and defense (Guo et al., 2018), increased susceptibility to untargeted pests (Stout et al., 2002), or the upregulation of, or cross talk between, major metabolic pathways (Hou et al., 2013). Yet, seed priming with elicitors is expected to have a positive cost-benefit balance as it does not generally activate direct defense responses and thus only has a marginal fitness cost in the absence of stress (Martinez-Medina et al., 2016), while providing additional protection to seedlings and minimizing non-target effects. Moreover, seed treatments are also often presented as being more economically and industrially applicable than foliar applications, which require spraying of large volumes of elicitors during the growing season (Haas et al., 2018). Although crop-pest or crop-pathogen interactions were not specifically addressed in this study, we detected several bacterial indicator ASVs enriched in the elicitor treatments that were assigned to lineages beneficial to plant health, such as Actinobacteria, Bacilli, Burkholderiales and Pseudomonadales (Raaijmakers et al., 2009; Mendes et al., 2011), in line with the involvement of jasmonates and salicylic acid in the activation of diverse below-ground defenses (Carvalhais et al., 2013; Lebeis et al., 2015; Liu et al., 2017). In the field, the evaluation of elicitors is complicated by the interplay between crop and cultivar identity, plant developmental stage, pest and pathogen intensity, and environmental conditions (Walters et al., 2005). All these aspects are important, as plants can adapt their resource-use strategies according to developmental stage and environmental conditions (Masclaux-Daubresse et al., 2010; Chaparro et al., 2013; Verly et al., 2020), which can affect the composition of the root-associated microbial communities (Zhalnina et al., 2018; Chen et al., 2019) and influence the genetic potential for ammonia oxidation (Thion et al., 2016).

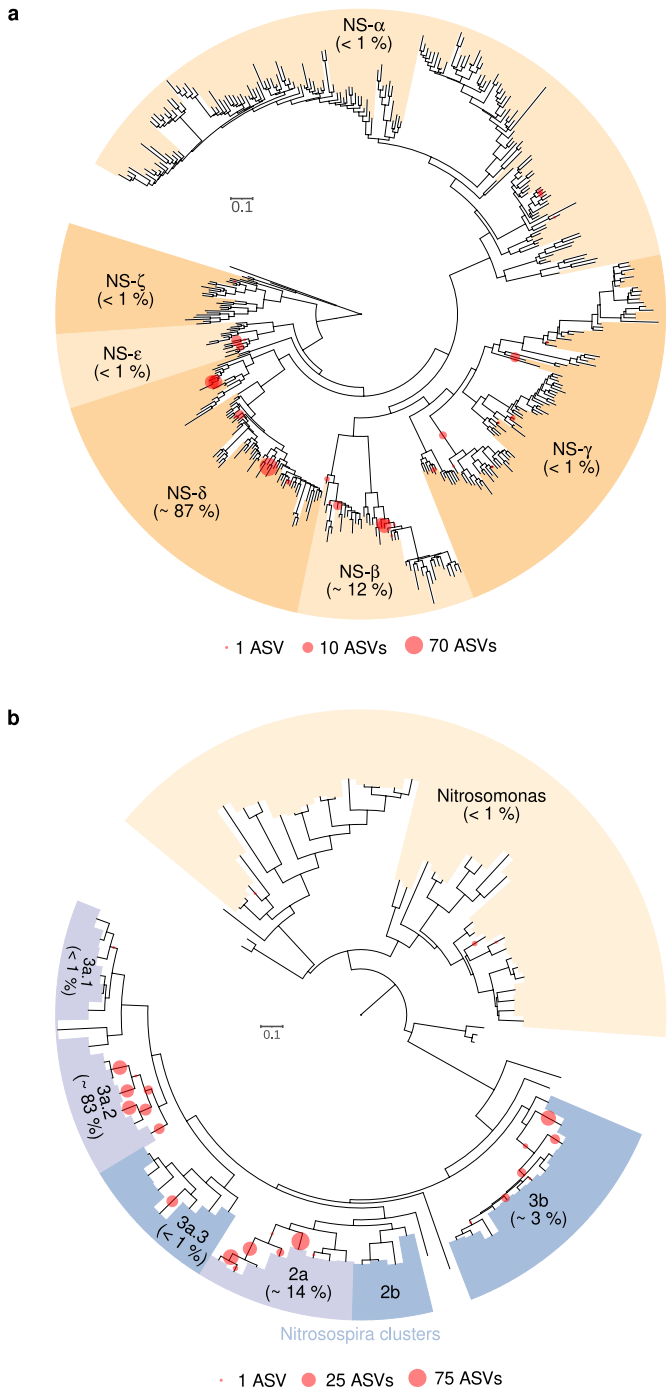

Fig. 4. Phylogenetic placement of (a) ammonia-oxidizing archaeal (AOA) and (b) ammonia-oxidizing bacteria (AOB) ASVs within the reference phylogeny of AOA and AOB *amoA* sequences determined by Alves et al. (2018) and Jones and Hallin (2019), respectively. Circles show location of ASV placement within the reference tree, and circle size is proportional to the number of ASVs. The relative abundance of each clade in terms of reads is indicated next to the clade name. Scale bar denotes estimated nucleotide substitution rate. For clarity, only the region of the tree corresponding to Nitrososphaerales (NS) is shown in (a) and tip names have been excluded.

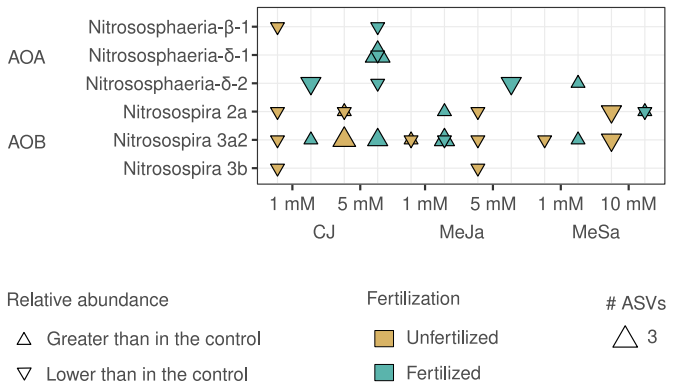

Fig. 5. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) ASV indicators of elicitor treatment based on *amoA* amplicons. Indicator ASVs significantly more (▲) or less (▼) abundant in the root-associated communities of elicitor-treated plants compared to both controls (water and Tween20), in unfertilized (yellow) and fertilized (cyan) pots are shown. The size of the triangles is proportional to the number of ASVs. CJ: cis-jasmone; MeJa: methyl jasmonate; MeSa: methyl salicylate.

5. Conclusions

Our findings suggest that seed treatments with MeJa and MeSa have the potential to improve N-use efficiency in arable soils by suppressing ammonia oxidizer populations and increasing plant N uptake. This calls for further investigation under field conditions to examine the long-term effects of elicitor treatments on root-associated communities and crop yield, which will ultimately determine in which contexts elicitors can be used to simultaneously enhance plant protection, resistance to abiotic stresses and N-use efficiency in arable soils, thus contributing to increases in yield stability.

Author contributions

R.G and S.H. obtained the funding for the study. A.S., R.G. and S.H. designed the experiment. A.S. and E.A. collected the data, and A.S. performed the analyses. A.S., R.G. and S.H. interpreted the results and drafted the manuscript. All authors commented on and approved the final manuscript.

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.

Data availability

The scripts and associated data used to conduct this study are available at Zenodo (https://doi.org/10.5281/zenodo.7374271).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2023.109016.

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