An actinomycete strain of **Nocardiopsis lucentensis** reduces arsenic toxicity in barley and maize

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Abstract

Accumulation of arsenic in plant tissues poses a substantial threat to global crop yields. The use of plant growth-promoting bacterial strains to mitigate heavy metal toxicity has been illustrated before. However, its potential to reduce plant arsenic uptake and toxicity has not been investigated to date. Here, we describe the identification and characterization of a *Nocardiopsis lucentensis* strain isolated from heavy metal contaminated soil. Inoculation with this bioactive actinomycete strain decreased arsenic root and shoot bioaccumulation in both C3 and C4 crop species namely barley and maize. Upon arsenate treatment, *N. lucentensis S5* stimulated root citric acid production and the plant's innate detoxification capacity in a species-specific manner. In addition, this specific strain promoted biomass gain, despite substantial tissue arsenic levels. Detoxification (metallothionein, phytochelatin, glutathione-S-transferase levels) was upregulated in arsenate-exposed shoot and roots, and this response was further enhanced upon *S5* supplementation, particularly in barley and maize roots. Compared to barley, maize plants were more tolerant to arsenate-induced oxidative stress (less H_2O_2 and lipid peroxidation levels). However, barley plants invested more in antioxidative capacity induction (ascorbate-glutathione turnover) to mitigate arsenic oxidative stress, which was strongly enhanced by *S5*. We quantify and mechanistically discuss the physiological and biochemical basis of *N. lucentensis*-mediated plant biomass recovery on arsenate polluted soils. Our findings substantiate the potential applicability of a bactoremediation strategy to mitigate arsenic-induced yield loss in crops.

Keywords: arsenic, antioxidants, actinomycetes, bioremediation, oxidative stress

1. Introduction

Soil pollution is one of the main factors limiting agricultural productivity worldwide. The presence of growth- and yield-limiting compounds of the natural or anthropogenic origin in the rhizosphere determines the output of existing farmlands. There is a need to investigate how agricultural systems comprising of different crop species respond and adapt to soil pollutants. C3 and C4 species differ in their metabolic carbon fixation pathways, and this difference often significantly affects their general physiology and susceptibility/resistance to environmental stresses (AbdElgawad et al. 2020). Also, the anatomical differences between C3 plants (e.g., barley) and C4 plants (e.g., maize) are causal for the occurrence of a waterful biochemical process called photorespiration in C3 plants, which results in higher sensitivity of C3 plants to stresses than C4 plants. (Bräutigam et al., 2016). Photorespiration is a major source of reactive oxygen species (ROS), affecting cellular redox homeostasis (Voss et al., 2013). Therefore, there is a need to understand the species-specific (C3 and C4) effects of soil pollution on crop productivity and design future remediation strategies.

Bactoremediation, using the intrinsic capability of specific bacterial strains to accumulate and or/degrade specific pollutants, alter their bioavailability in the soil or induce plant stress tolerance, has the potential to mitigate the effects of pollutants on crop yield (Abhilash et al. 2016). Consequently, an increased effort has gone into the isolation and identification of bacterial strains that potentially promote plant growth in the presence of soil pollutants. As a result, specific bacterial strains which (partially) restored plant growth in e.g. polychlorinated biphenyls (PCB), trinitrotoluene (TNT) and heavy metal contaminated soils have been characterized (Sheng et al. 2008, Thijs et al. 2014, Vergani et al. 2019). Bacterial species which have been isolated from historically metal polluted soils were found to produce high quantities of siderophores (metal-chelating compounds), IAA (auxin; growth-promoting plant hormone), ACC-deaminase (an enzyme which breaks down stress-induced plant hormone ethylene) and/or increase the solubilisation of phosphate (e.g. Rajkumar et al. 2006, Jiang et al. 2008, Yu et al. 2014, Ma et al. 2016). As a result, these isolated bacterial strains enhanced the growth and biomass production of metal-exposed plants. Surprisingly, the physiological and biochemical basis of bacterial mitigation of heavy metal stress is still poorly understood. Moreover, while the potential of bio- or bactoremediation has been

illustrated for enhancing tolerance to heavy metal exposure, it's applicability for mitigating plant metalloid toxicity has so far barely been investigated.

Nevertheless, topsoil arsenic pollution resulting from irrigation with contaminated groundwater poses a major concern to global agricultural crop yields (Kalita et al. 2018). Arsenate (oxygenated arsenic) is easily taken up by plant cells via phosphate transporters, where it can inhibit the cell's energy metabolism and induce oxidative stress (Byers et al. 1979, Finnegan and Chen 2012, Li et al. 2015, Singh et al. 2018). In maize (C4) and heavy-metal tolerant ryegrass (C3), arsenic induces (non)-enzymatic (anti)-oxidative stress markers such as hydrogen peroxide (H_2O_2), catalase (CAT), ascorbate peroxidase (POX), glutathione reductase (GR) and glutathione peroxidase (Anjum et al. 2016, Li et al. 2019). In addition, trivalent arsenic interferes with the cell's redox metabolism by binding and perturbing thiol-containing proteins (Bergquist et al. 2009, Mishra et al. 2019). Consequently, rhizosphere arsenic exposure reduces plant root and shoot size, stature, biomass production and viability (Garg and Singla 2011). The identification of an affordable, practical and highly effective bioremediation strategy to mitigate plant arsenic bioaccumulation and toxicity is therefore of crucial importance.

Here, we investigated how soil arsenate exposure affects economically relevant C_3 (barley) and C_4 (maize) crops, and how a bacterial strain isolated from heavy metal contaminated soil interacts. We hypothesized that concentration-dependent, organ-specific, and species-specific responses would be observed in response to arsenate exposure. At the same time, bacterial inoculation may protect them at the physiological and biochemical levels. Overall, we report on the isolation and identification of a *Nocardiopsis lucentensis* actinomycete strain with the capacity to mitigate arsenic-induced growth reduction in C3 and C4 crops. We also characterized the integrated physiological and biochemical response of both crops to arsenate exposure and elucidated the species-specific growth-promoting effect of the bacterial strain.

2. Material and Methods

2.1 Isolation of the actinomycete strains

The actinomycete strains were isolated from heavy metal contaminated soil collected from local grasslands in Giza (Egypt), using a soil dilution method. One gram of dried

soil was agitated in distilled water (10mL) and heated (50°C) for 30 min. Serial dilutions were prepared and introduced into petri plates containing the isolation medium (0.5% glycerol, 2 g L^{-1} yeast extract, 1 g L^{-1} K_2HPO_4, 50 µg mL^{-1} nystatin, 1.5% agar) for 14 days at 28°C. Different actinomycete colonies (which have chalky textures and different colors of aerial mycelia) were sub-cultured until pure isolates were obtained.

2.2 Morphological and biochemical, characterization of the isolates

The purified isolates were characterized by colony and spore chain morphology (Shirling and Gottlieb 1966), nitrogen and carbon utilization (Williams et al. 1983). The bioactivity and plant growth-promoting potential of the isolated strains were assessed by measuring hormone and siderophore production (Gordon and Weber 1951, Schwyn and Neilands 1987), the total antioxidant capacity (FRAP) and phenol/flavonoid content (quantitatively) (Abu El-Soud et al. 2013). The spore-bearing hyphae, spore chain and spore surface were imaged using scanning electron microscopy (JEOL JSM-6380 LA).

2.3 Identification of the potential bioactive strain

The selected bioactive strain was identified to the genus level by 16S ribosomal RNA sequencing. DNA was extracted (DNeasy UltraClean Microbiol Kit, Qiagen), amplified using universal 16S rRNA primers (27F: 5'-AGTTTGATCMTGGCTCAG-3', 1492R: (5'-TACGGYTACCTTGT-TACGACTT-3') and sequenced (Macrogen, South Korea). Obtained sequences were compared to the 16S rRNA GenBank database and multiple sequence alignment was performed using ClustalW available in MEGA X (Kumar et al. 2018). Phylogenetic tree was constructed (Neighbour-joining method, (Saitou and Nei 1987)) and tree topology was evaluated (bootstrap analysis, 1000 resamplings).

2.4 Plant materials and growth conditions

Soil (0.5 kg; Tref EGO substrates, Moerdijk, NL) was pre-incubated with 20 mL of log-phase actinomycete culture (10^8 CFU mL^{-1}) by gently applying the culture to the soil while mixing. As a control, soil was pre-incubated with 20 mL of bacterium-free culture medium. Moist soil was distributed in 25x25cm pots and kept in the dark at 30°C for 1 day prior to sowing. Maize and barley grains were stratified (2 days, 4°C) and sown in actinomycete-treated and untreated (control) soil. Plants were grown in a custom build

climate-controlled chamber at 21/18°C in a 16/8h day/night photoperiod (150 µmol PAR m^{-2} s^{-1}, 60% humidity) and exposed to different arsenate (AsO_4^{3-}) soil concentrations (control: 0 mg kg^{-1}; mild: 25 mg kg^{-1}; severe: 100 mg kg^{-1}). The soil water content was kept at 60% throughout the experiment. After six weeks of growth, the rhizosphere, roots and shoots were collected and aliquoted for further analysis. The fresh and dry weight of roots and shoots was determined.

2.5 Quantification of photosynthetic parameters

Prior to sample collection, the light-saturated photosynthetic rate and stomatal conductance of fully mature leaves were quantified (LI-COR LI-6400, LI-COR Inc., Lincoln, NE, USA) (AbdElgawad et al. 2015). Photochemical efficiency (Fv/Fm) of non-cyclic electron transport in photosystem II was measured on 30 min dark-adapted leaves (leaf number 5 or 6) with a fluorimeter (PAM2000, Walz, Effeltrich, Germany). As a proxy for the degree of photorespiration the glycine/serine ratio was quantified by UPLC (Waters Acquity UPLC-tqd system, Milford, Worcester County MA, USA; BEH amide column; Al Jaouni et al., 2018). Shoots were homogenized in acetone and the supernatant was used to measure the chlorophyll A, chlorophyll B and carotenoid concentrations (AbdElgawad et al. 2015).

2.6 Quantification of the arsenic concentration in soil and plant samples

Arsenic was extracted from soil and whole plant samples by overnight digestion in concentrated HNO_3 and $HClO_4$ and subsequent heating to 120°C until $HClO_4$ fumes were released. The concentrate was resuspended in 10% HCl (v/v) containing 0.4% $NaBH_4$ and analyzed by Flow Injection Hybride Generation Atomic Absorption Spectrophotometry (FI-HG-AAS, Perkin Elmar AAnalyst 400, USA) using external calibration (Welsch 1990).

2.7 Quantification of root exudates

Ten grams of soil were washed with distilled water and filtered. The phenolic content was measured in the aqueous phase by UV-VIS spectrophotometry (Shimadzu UV-VIS 1610 PC, Japan, (Zhang et al. 2006)). Citric acid was extracted in 0.1% phosphoric acid-containing butylated hydroxyanisole. Ribitol was added as an internal standard. After centrifugation, the supernatant was used for quantification by HPLC (LaChom L-7455 diode array, LaChrom, Tokyo, Japan).

2.8 Quantification of detoxification related parameters

Glutathione-S-transferase (GST) activity was extracted in potassium phosphate buffer)50 mM, pH 7.0) and quantified according to the method described by (Mozer et al. 1983). Metallothionein (MTC) content was measured electrochemically using the differential pulse voltammetry Brdicka reaction (Diopan et al. 2008). To measure total phytochelatins, total non-protein thiols in plant samples were extracted in 5% sulfosalicylic acid, mixed with Ellman's reagent and quantified spectrophotometrically at 412nm (De Knecht et al. 1992). The total phytochelatins content was estimated from the difference between the total non-protein thiol and total glutathione (GSH) content.

2.9 Quantification of oxidative damage markers

Malondialdehyde (MDA) content was quantified in root and shoot samples to determine lipid peroxidation. Plant samples were homogenized and extracted in 80% ethanol. The MDA concentration was determined using the thiobarbituric acid assay (Hodges et al. 1999). The hydrogen peroxide (H_2O_2) content was quantified in 0,1% trichloroacetic acid (TCA) root and shoot extracts using the Xylenol orange method, which relies on peroxide-catalysed Fe^{2+} oxidation (Jiang et al. 1990). For each sample, a catalase treated (H_2O_2-free) fraction was used as a negative control.

2.10 Quantification of antioxidative parameters

Root and shoot samples were homogenized in ice-cold 80% ethanol, centrifuged (5000 rpm, 15min) and the supernatant was used for the quantification of the total antioxidative capacity and antioxidative metabolites. The total antioxidative capacity was measured using the 'Ferric Reducing Antioxidant Power' (FRAP) assay and Trolox as a standard (Benzie and Strain 1999). Reduced Ascorbate (ASC) and glutathione (GSH) levels were quantified by HPLC. Total ASC and GSH concentrations were determined after reduction with dithiothreitol (DTT) (Zinta et al. 2014). Total phenolic and flavonoid contents were quantified using the Folin-Ciocalteu and aluminum chloride assays, respectively (AbdElgawad et al. 2016).

For the determination of key enzyme activities related to the plant's antioxidative response, proteins were extracted from 200 mg of frozen plant material in 2 mL extraction buffer (50 mM potassium phosphate buffer, pH 7.0, 10% PVP, 0.25% Triton X-100, 1 mM PMSF, 1 mM ASC). After centrifugation (10 min at 13000 rpm, 4°C),

the supernatants were used to spectrophotometrically evaluate the activities of peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR). SOD activity was determined by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm (Dhindsa et al. 1982). POX activity was determined based on the oxidation of pyrogallol (Kumar and Khan 1982). CAT activity was assayed by monitoring the breakdown of H_2O_2 at 240 nm (Aebi 1984). APX, GR, MDHAR and DHAR activities were measured as previously described (Murshed et al. 2008). GPX activity was assayed by measuring the decrease in NADPH absorbance at 340 nm (Drotar et al. 1985). Enzyme activities were normalized to the total soluble protein concentration, according to the Lowry method (Lowry et al. 1951).

2.11 Statistical analysis

Experiments were carried out following a completely randomized block design using 4 replicates for each treatment (n=4). Data analyses were performed using SPSS (Chicago, IL, USA) and R (R Team 2013). Data normality and homoscedasticity were checked using the Kolmogorov–Smirnov (SPSS)/Shapiro-Wilk (R) and Levene´s test, respectively. All the data were subjected to two-way (SPSS; arsenate and *N. lucentensis* treatment as factors) and four-way (R; species, organ, arsenate treatment and *N. lucentensis* treatment as factors) analysis of variance (ANOVA; α=0.05). A Duncan's (SPSS; following two-way ANOVA) or Tukey HSD test (R; following four-way ANOVA) was applied for subsequent pairwise statistical comparison of means. Heatmap construction and principal components analysis (PCA) were carried out on z-score normalized data using MultiExperiment Viewer (http://mev.tm4.org/) and R respectively. PCA graphs were created showing the distribution of individual samples in the first two PCA dimensions. The parameters and the degree to which they contribute to the total variation explained by the first two PCA dimensions were depicted as arrows.

3. Results and Discussion

3.1 Characterization of the Actinomycete strains

With the aim of identifying potent actinomycetes strains that could be used for arsenic bioremediation, we isolated 9 strains from heavy metal polluted grasslands in Giza

(Egypt). The 9 isolates were characterized by their colonial morphology (SI Table 1). All isolates developed aerial mycelia mostly with spiral spore chains. The isolates used different N and C sources and similarly the enzymes produced by each isolate varied greatly (SI Table 1). The observed morphological characters indicated that most of the isolates belong to genus *Streptomyces* and its related filamentous genera. *Streptomyces* is considered to be the most abundant actinomycete genus, and has previously been suggested to have great bioremediation potential (Schütze et al. 2014).

Next, we performed a biochemical characterization of the isolates with the aim of selecting an isolate for inoculation with crops grown on arsenate polluted soils. We quantified the biological activities of the 9 isolates (total antioxidative capacity, plant hormones, phytochelatins, siderophores, flavonoid and phenolic compounds production) (Table 1). We found that isolate 5 and 7 both produced high quantities of flavonoid and phenolic compounds that are involved in plant stress adaptation (Table 1). Whereas strain 7 displayed a high antioxidative capacity, strain 5 produced high concentrations of siderophores (metal-chelating compounds) and the plant growth-promoting hormones auxin (IAA) and gibberellic acid (GA) (Table 1). Since, arsenic binding siderophores have previously been isolated and siderophore production has been shown to facilitate arsenic resistance in actinobacteria, we chose isolate 5 as a primary actinomycete strain for further investigation (Retamal-Morales et al., 2018; Das and Barooah, 2018).

Next, isolate 5 was identified to the species level by 16S rRNA sequencing. With 99.72% sequence similarity, isolate 5 was found to be a strain of the halophilic species *Nocardiopsis lucentensis,* from here on referred to as *'S5'* (SI Fig 1A) (Yassin et al. 1993). In agreement with our species identification, the mycelia produced from isolate 5 displayed variable length spore chains with smooth-surfaced spores originating from the mature aerial mycelium. The substrate mycelium showed branching and fragmentation into mainly rod-shaped and rarely coccoid spores (SI Fig. 1B).

Several *Nocardiopsis* species are ubiquitous plant root endophytes which, like *N. lucentensis*, produce bioactive/antimicrobial compounds (Bennur et al. 2015, Ibrahim et al. 2018). For example, a wheat rhizosphere *Nocardiopsis* isolate promoted plant growth by producing auxin, siderophores and enhancing soil phosphate solubilization

(Jog et al. 2014). Notably, *Nocardiopsis* species have been implicated as candidates for heavy metal bioremediation (El-Gendy and El-Bondkly 2016). Nevertheless, the effect of *Nocardiopsis* strains on plant arsenic uptake and toxicity has not been investigated.

3.2 *N. lucentensis* S5 differentially mitigates arsenic accumulation in barley and maize

To evaluate if our *N. lucentensis S5* isolate could affect arsenic uptake in economically relevant crop species, we supplemented barley (C_3) and maize (C_4) plants with different concentrations of soil arsenate (control: 0 mg kg^{-1}; mild: 25 mg kg^{-1}; severe: 100 mg kg^{-1}), the predominant arsenic species in agricultural soils (Nriagu et al., 2007), in the presence or absence of *S5*. Next, we quantified arsenic bioaccumulation in 6-weeks old roots and shoots.

We found that, for both species, the degree of arsenic accumulation in roots and shoots was proportional to the level of arsenate exposure (Fig. 1), confirming that plants take up arsenic from arsenate-polluted soils (Gulz et al., 2005). Roots consistently accumulated more arsenic ($p=4.10^{-14}$). In the absence of *S5*, barley and maize roots accumulated similar arsenic concentrations (mild: ~23,7 µg gDW^{-1}, severe: ~70,5 µg gDW^{-1}). Maize accumulated less arsenic in the shoot (38±3 µg gDW^{-1}) when exposed to 100 mg arsenate kg^{-1} soil compared to barley (52±2 µg gDW^{-1}), confirming that the degree of root to shoot arsenic transport differs between plant species (Gulz et al., 2005). Together, these data show that arsenate exposure leads to arsenic accumulation in both barley and maize roots and shoots.

Inoculation with *S5* strongly reduced arsenic bioaccumulation in both species ($p<2.10^{-16}$) (Fig. 1). In barley, arsenic concentrations decreased by 47% (mild) and 30% (severe) in roots, and 63% (mild) and 41% (severe) in shoots. In maize plants, the effect of *S5* was markedly more pronounced (p=0.005), inoculation decreased root arsenic levels by 76% (mild) and 63% (severe), and shoot concentrations by 75% (mild) and 49% (severe). These data show that the presence of *S5* strongly but differentially inhibits arsenic uptake in both crop species. In addition, our findings suggest that *S5* might colonize barley and maize roots, providing a basis for future characterization of this *Nocardiopsis* isolate.

To investigate whether the lower plant arsenic bioaccumulation in the presence of *S5* could be due to higher soil retention we quantified arsenic soil concentrations in samples 6 weeks after treatment with high arsenic levels (100 mg kg^{-1}), and investigated how these might relate to the presence of citric acid and phenolic root exudates in the presence or absence of *S5*. Citric acid and phenolic compounds are the primary root exudates produced under stress conditions (e.g. heavy metal exposure), and contribute to plant heavy metal resistance (Pinto et al., 2008; de Sousa et al., 2019). Arsenic uptake by plants significantly lowered arsenic levels in the soil, therefore higher arsenic bioaccumulation corresponded to lower soil arsenic concentrations (SI Table 2). Inoculation with *S5* reduced levels of plant accumulated arsenic and led to higher arsenic retention in the rhizosphere. This effect was more pronounced for maize than for barley, showing that the effect of *S5* plant arsenic uptake is species specific. We found that the increase in soil arsenic levels positively correlated with soil citric acid levels, suggesting that the presence of *S5* in arsenate polluted soils stimulates root citric acid secretion (Fig. 2, SI Table 2). In line with the differential impact of *S5* on plant arsenic uptake, maize plants produced higher citric acid levels compared to barley.

Citric acid can act as a chelating agent and locally lower rhizosphere pH, thereby affecting arsenic bioavailability (Campbell and Nordstrom 2014). Consistent with our results, it was previously shown that C_4 species produce and/or secrete higher quantities of organic acids compared to C_3 species (Vranova et al. 2013). Our data now suggest that citric acid excretion is enhanced by inoculation with *S5*. Hence, soil treatment with this specific *N. lucentensis* strain has the potential to inhibit arsenic uptake in barley (C_3) and, to a greater extent, maize (C_4) crops by stimulating plant citric acid secretion.

3.3 *N. lucentensis S5* treatment reduces plant biomass loss in arsenate polluted soils

Previous studies showed that arsenic inhibits plant growth in a species-specific manner (e.g. Anjum et al. 2016, Li et al. 2019). Consequently, we were interested to know whether the *S5*-induced reduction in arsenic uptake could benefit maize and barley biomass production. We therefor quantified root and shoot biomass production (Fig. 3). In line with previous reports, we found that arsenate inhibits root and shoot biomass

production in a species-specific manner (Anjum et al. 2016, Li et al. 2019). The barley root fresh weight (FW) and dry weight (DW) decreased by ~42% and ~50% respectively when exposed to mild and severe soil arsenate levels (Fig. 3). Similarly, the barley shoot FW decreased by ~44% upon arsenate exposure (Fig. 3A, SI Fig. 2). The shoot DW remained unaffected, suggesting that, in barley, arsenate exposure strongly reduces shoot water content (Fig. 3B). In maize root and shoot biomass production responded markedly different. Maize roots dry matter was increased (50%), whereas their FW remained unaffected upon arsenate exposure (Fig. 3). Conversely, the maize shoot DW decreased by ~52% in mild and severe arsenate treatments, while the shoot FW also remained unaffected. The latter suggests that, contrary to barley, maize plants invest in root biomass production upon arsenate exposure. Together, these results illustrate the differential response and sensitivity of these C_3 and C_4 species to arsenate exposure.

Under control conditions the presence of *S5* stimulated barley (root: 31%, shoot: 31%) and maize FW (root: 25%, shoot: 33%), and shoot DW (barley: 42%, maize: 35%), illustrating a strong overall growth-promoting effect of this *N. lucentensis* strain. In line with *S5* decreasing arsenic uptake, inoculation fully restored biomass production in the presence of As compared to control conditions in both plant species (Fig. 3). When grown on arsenate-polluted soil, *S5* treatment led to a full recovery of the barley root and shoot FW and DW (Fig. 3). Maize shoot FW was enhanced (49%), independent of the soil arsenate concentration (Fig. 3A), resembling previous observations of an arsenic-resistant maize variety (Anjum et al. 2016).

Although *N. lucentensis S5* inoculation reduces As uptake, it's stimulation of growth even in control conditions suggest that partial inhibition of arsenic uptake (through e.g. stimulation of root citric acid secretion) is not the only mode of action through which this bacterial strain mitigates the effects of arsenic.

3.4 Arsenate and *N. lucentensis S5* treatment affect barley and maize biochemistry

Our data differential arsenic uptake and biomass data show that a species-specific mechanism might regulate the plant's response to arsenate exposure in the presence of *S5*. We thus compared the physiological/biochemical response of arsenate exposed barley and maize plants upon *S5* treatment by quantifying key parameters related to

405 photosynthesis (Total chlorophyll, carotenoid, chlorophyll A and B content,
406 photosynthetic activity, photosystem II activity, stomatal conductance), detoxification
407 (metallothionein, glutathione-S-transferase activity, phytochelatin content) and
408 oxidative stress/redox homeostasis (total antioxidative capacity, hydrogen peroxide,
409 malondialdehyde, reduced and total glutathione/ascorbate, flavonoid and tocopherol
410 content, enzyme activities related to the glutathione-ascorbate cycle), in roots and
411 shoots. The selection of these parameters was based on their importance in the plant's
412 response to heavy metal exposure (Vinit-Dunand et al. 2002, Burzyński and Żurek
413 2007, Vernay et al. 2007, Anjum et al. 2016, AbdElgawad et al. 2020).
414
415 To get a global overview of which of these parameters explained the general response
416 to arsenate exposure and *S5* inoculation we performed a Principal Component Analysis
417 (PCA) analysis combining all data. We found that irrespective of the treatment or
418 species, the root and shoot displayed very different responses (SI Fig. 3). Consequently,
419 we constructed separate PCAs for the root and shoot data.
420 Consistent with our previous results, we identified a clear arsenate concentration-
421 dependent response (illustrated by the separate clustering of plants exposed to control,
422 mild and severe arsenate levels along principal component 1) representing 43% and
423 37% of the total root (SI Fig. 4) and shoot (Fig. 4) variation respectively. Generally,
424 higher arsenate exposure correlated with an increase in oxidative stress (lipid
425 peroxidation; MDA, H_2O_2), antioxidative response (Total Antioxidative Capacity;
426 TAC, Catalase; CAT, Ascorbate Peroxidase; APX, Superoxide Dismutase; SOD) and
427 detoxification (metallothionein; MTC, phytochelatins; PHCHEL), illustrated by their
428 vectors pointing towards the right half of the PCA plot (positive correlation with
429 arsenate exposure severity). In addition, higher arsenate exposure correlated with a
430 decrease in photosynthesis-related parameters (SI Fig. 4, Fig. 4), shown by their vectors
431 pointing towards the left half of the PCA plot (negative correlation with arsenate
432 exposure severity).
433
434 The variation described along principal component 2 (root: 15%; shoot: 17%)
435 represented differences in basal barley and maize physiology/biochemistry (barley and
436 maize samples cluster independently from each other, independent of their treatment;
437 maize in bottom half of PCA; Barley in top half of PCA), and the general effect of *N.*
438 *lucentensis S5* inoculation (relative to their mock treatments, *S5* treated samples of the

same species exposed to the same arsenate loading mostly move along principal component 2). In barley roots exposed to severe arsenate stress (SI Fig. 4; right half of PCA plot), *S5* led to an increase in the overall root defence system (the vectors corresponding to the majority of biochemical parameters largely colocalize with barley samples exposed to severe arsenate and inoculated with *S5*, indicating positive correlation). In line with our biomass observations, the effect of *N. lucentensis S5* treatment was more pronounced in shoots, illustrated by separate clustering of *S5* treated maize (bottom of PCA) and barley (centre of PCA plot) samples relative to their respective arsenate-exposed mock treatments (Fig. 4). Arsenate-treated barley shoots contained higher MDA (lipid peroxidation) levels, indicative of oxidative membrane damage. Nevertheless, *S5* supplementation mitigated the latter (*S5* treated samples move downward along the MDA vector, indicative of lower MDA levels), which is consistent with the observed biomass recovery to control levels (Fig. 3 & 4). Generally, maize plants were characterized by higher carotenoid, ascorbate and glutathione levels (their vectors point towards the lower half of the PCA plot, where maize samples are situated). In both species, *S5* treatment further increased these metabolites (*S5* treated samples move along these metabolites vectors, relative to their mock treatments). Together, these data illustrate that the effect *N. lucentensis S5* inoculation and arsenate treatment is reflected on the biochemical level in both barley and maize, but that both species show a differential response.

3.5 *N. lucentensis S5* differentially affects arsenic detoxification in barley and maize

Next, we aimed to gain a more in-depth understanding of the biochemical processes that lie at the basis of the response to arsenate exposure in the presence/absence of *N. lucentensis S5*. Various studies have shown that plant arsenic and heavy metal detoxification mechanisms both involve metallothioneins (MTC; metal-binding proteins regulating plant metal transport and sequestration), phytochelatins (PHCHEL; glutathione oligomers which bind metals and sequester them to the vacuole) and glutathione-S-transferase (GST; regulates glutathione-metal conjugation) (Schmöger et al. 2000, Cobbett and Goldsbrough 2002, Zimeri et al. 2005, Sharma et al. 2017, Awasthi et al. 2018, Kumar and Trivedi 2018). Our PCA analysis suggests that *S5* inoculation could affect these parameters in both plant species (Fig. 4). Indeed, when investigating these parameters individually, we observed a concentration-dependent

increase in root MTC and PHCHEL levels in both species upon arsenate exposure (Fig. 5A, B). These levels were further enhanced by *N. lucentensis S5* treatment. Hence, barley and maize root MTC and PHCHEL levels increased by 21% (barley MTC), 29% (maize MTC) and 25% (barley PHCHEL), 20% (maize PHCHEL) respectively in the presence of *S5* (Fig. 5A, B). Moreover, under severe arsenate loading, *S5* treatment led to a 31,6% and 61,5% increase in barley and maize root GST activity respectively, illustrating that the entire root detoxification system is addressed upon toxic arsenate exposure (Fig. 5C). These data are in line with previous observations, which showed that an upregulation of GST and phytochelatin levels is key to the plant's response to arsenic exposure (Schmöger et al. 2000, Hartley-Whitaker et al. 2001, Zimeri et al. 2005, Kumar and Trivedi 2018). Our findings illustrate that *S5* treatment enhances the plant's detoxification mechanisms, which might allow the plant to promote root biomass production, even in the presence of root arsenic.

In the maize shoot, all three detoxification parameters were elevated in severe arsenate conditions (Fig. 5). However, in parallel with the overall lower shoot arsenic bioaccumulation, maize shoot MTC, PHCHEL levels and GST activity remained largely unaffected upon *S5* treatment (except for a 26,1% shoot PHCHEL increase at 100mg kg^{-1} arsenate). This might suggest that, in the absence of *S5*, innate maize shoot detoxification is sufficient to cope with the observed levels of bioaccumulated arsenic. Contrastingly, detoxification was upregulated in arsenate-exposed barley shoots, and this response was further enhanced upon *S5* supplementation (MTC mild: 22,3%, MTC severe: 28,3%, PHCHEL mild: 22,0%, PHCHEL severe: 29,0%). Compared to maize, this again illustrates a higher sensitivity of barley to arsenic exposure (Fig. 5A,B). Hence, similar arsenate exposure levels lead to an upregulation of the detoxification mechanisms in barley, but not in maize.

Together, these data show that *S5* treatment stimulates plant arsenic detoxification in an arsenate concentration-dependent, species- and organ-specific manner. Importantly, in the absence of arsenate, the presence of *N. lucentensis S5* does not affect MTC and PHCHEL levels, illustrating that, independent of its general growth-promoting effect, this actinomycete strain can dynamically enhance plant stress tolerance.

3.6 *N. lucentensis S5* does not affect photosynthesis in arsenate exposed barley and maize

S5-inoculated plants still accumulate significant arsenic, in spite of decreased As uptake and increase As detoxification (Fig. 1). To investigate how *S5* is able to stimulate biomass production in the presence of substantial arsenic bioaccumulation we quantified photosynthesis, the core process directing the plant's ability to produce biomass (i.e. the generation of ATP for the production of physiologically active and structural carbohydrates). We found that, in line with previous reports (Stoeva et al. 2005) and the loss in shoot biomass (FW in Barley, DW in maize) upon arsenate exposure, photosynthesis was significantly downregulated in both species. Hence, arsenic bioaccumulation strongly affected the plant's energy metabolism (Fig. 6A). A decrease of 60,9% and 78,3% in photosynthetic activity was observed for barley shoots exposed to mild and severe soil arsenate respectively. In maize, an overall decrease of ~45,0% was apparent in both conditions, supporting a significant yet lower arsenic susceptibility compared to barley (Fig. 6A). Arsenate (aka pentavalent arsenic; As[V]) is chemically similar to phosphate, causing it to compete with root phosphate uptake (Tripathi et al. 2007, Srivastava and Sharma 2014). Following uptake, arsenic binds to ADP, the ATP-synthase substrate for photosynthesis-mediated ATP (the chemical energy source for subsequent carbohydrate production in the Calvin cycle) production (Tripathi et al. 2007, Srivastava and Sharma 2014). As a result, and in support of our data, arsenic toxicity can directly interfere with photosynthetic efficiency. This significant decrease in photosynthetic activity upon arsenate exposure could lie at the basis of the loss in shoot biomass.

Surprisingly, *S5* treatment promoted photosynthesis in maize plants exposed to mild arsenate concentrations only (Fig. 6). This suggests that changes in the photosynthetic activity do not explain the observed *S5*-mediated biomass increase. This is also reflected by the fact that leaf gas exchange (stomatal conductance), photosystem II efficiency (PSII; chlorophyll fluorescence), chlorophyll A, B and total chlorophyll content were largely irresponsive ($p>0.05$) to the presence of *S5* (SI Fig. 5). Together, these data suggest that *S5*-stimulated biomass production occurs independent of photosynthesis (through e.g. usage of carbohydrate reserves and/or enhanced soil nutrient uptake).

Importantly, however, we observed a strong species-specific response of shoot carotenoid levels upon *S5* treatment ($p=1.10^{-4}$) (Fig. 6B). Whereas in the absence of *S5*

carotenoid concentrations remained unaffected in arsenate exposed barley and maize plants, subsequent *S5* treatment led to a 60,6% carotenoid increase in barley (and to a lesser extent in maize: 32,0% under mild arsenate stress) (Fig. 6B). Carotenoids are photosynthetically active pigments with antioxidative properties. Notably, interruption of the electron transport chain during photosynthesis (e.g. by limiting ADP levels due to ADP-arsenic complexation) can cause the production of reactive oxygen species, leading to oxidative stress (Tripathy and Oelmüller 2012). Here, our data show that *S5* treatment stimulates the production of carotenoids in barley shoots, possibly allowing the plant to cope with higher ROS levels due to photosynthetic inhibition. Basal carotenoid levels were by default higher in maize ($p=2.10^{-8}$), suggesting a native photoprotection and antioxidative capacity (Fig. 6B). Together, these results indicate that (1) arsenate exposure strongly inhibits plant photosynthesis and (2) *N. lucentensis S5* treatment stimulates carotenoid production in a species-specific manner.

3.7 *N. lucentensis S5* affects the oxidative stress response to arsenate in a species-specific manner

Our data suggest that the regulation of oxidative stress could be an important aspect of the plant's response to arsenic toxicity and *S5* treatment. Consequently, we investigated individual parameters related to oxidative damage and antioxidative response (SI table 3-4). Environmental stressors can induce the production of reactive oxygen species (ROS) which in turn leads to an upregulation of the plant's innate antioxidative system (Naudts et al., 2014). When the plant is unable to cope with the amount of ROS that is produced, free radicals can damage biomolecules and disturb general plant homeostasis (Versieren et al., 2017). The relative sensitivity to stress-induced ROS production and the plant's antioxidative capacity have previously been shown to be species-specific (AbdElgawad et al., 2015). Similarly, we found that arsenate exposure led to a species-dependent increase in root and shoot H_2O_2 and MDA levels, indicative of ROS production and ROS-induced membrane damage (lipid peroxidation) respectively (Fig. 7A-B, SI table 2-3). In the shoot, H_2O_2 levels were elevated in severe As conditions in both species, which led to significant lipid peroxidation in barley but not in maize (Fig. 7B). Similarly, H_2O_2 levels were strongly induced in barley and maize roots, but at severe arsenate stress, barley roots experienced higher oxidative membrane damage (Fig. 7A-B). These data show that oxidative stress characterizes plant arsenic toxicity, supporting previous reports (Requejo and Tena 2005, Ahsan et al. 2008, Finnegan and

Chen 2012, Sharma et al. 2017, Kalita et al. 2018). Moreover, they suggest that compared to maize, barley plants are more prone to arsenate-induced oxidative stress.

Consistent with the mitigating effect of *S5* treatment on arsenate uptake (Fig. 1), we found that *S5* inoculation led to a species-specific decrease in MDA levels, in both roots and shoots (Fig. 7B). Importantly, *S5* treatment did not alter MDA levels in control conditions (Fig. 7B), showing that *S5* might specifically increase the plant's antioxidative capacity when exposed to arsenate. Consequently, we investigated the levels and activities of key antioxidative metabolites (total phenolics, flavonoids, polyphenols) and enzymes (related to the ascorbate-glutathione cycle) respectively (Fig. 7C). The concentration of phenolic antioxidative compounds increased upon arsenate exposure in roots and shoots of both species (Fig. 7C, SI table 3-4). Whereas barley and maize root concentrations were similar ($p>0.05$), maize shoots displayed higher polyphenol (~31,5%, $p=1.10^{-7}$) and flavonoid (~33,0%, $p=1.10^{-6}$) contents. In line with higher carotenoid levels (Fig. 6B), this again suggests that maize maintains a higher innate shoot antioxidative capacity. Interestingly, barley shoot tocopherol levels (indicative of antioxidative membrane protection) were ~27,8% higher compared to maize ($p=6.10^{-7}$) (Fig. 7C, SI table 4). Importantly, however, *S5* treatment did not cause marked changes in these parameters across species and As levels, showing that upregulation of phenol-mediated antioxidative protection does not explain the positive effect of *S5* treatment.

The ascorbate-glutathione cycle lies at the core of the plant's ability to metabolize reactive oxygen species. Whereas the redox-state of ascorbate and glutathione are interconnected, they are not mutually inclusive. Hence, the relative extent to which plants rely on ascorbate and/or glutathione signaling can reflect key differences in species-specific stress tolerance physiology. Compared to barley, we found that maize roots and shoots contain consistently lower levels of reduced ascorbate ($p<2.10^{-16}$), whereas both plant species displayed a strong upregulation of the total root and shoot glutathione pool in response to arsenate exposure ($p<2.10^{-16}$), especially in the presence of *S5* ($p=1.10^{-8}$) (Fig. 7, SI Table 3-4). These data indicate that (1) barley and maize rely on ascorbate and/or glutathione antioxidative signaling to a different extent and (2) *S5* treatment enhances glutathione production in arsenate exposed plants.

608

609 Consistent with this we found that the enzyme which functions at the ascorbate-
610 glutathione interface (dehydro-ascorbate reductase; DHAR) displayed strong arsenic-
611 ($p=9.10^{-9}$), species- ($p=1.10^{-7}$) and organ-specific ($p=3.10^{-3}$) response to *S5*
612 supplementation (Fig. 7C, SI Table 3-4). Hence, only in barley the DHAR activity
613 strongly increased in an arsenate-dependent manner in roots (severe: 78,7%) and shoots
614 (severe: 69,2%). Upon *S5* treatment this increase became less pronounced (p=0.01),
615 especially in roots (42,6%) and shoots (46,2%) exposed to severe soil arsenate loading
616 (Fig. 7C, SI Table 3-4). In contrast, maize root and shoot DHAR activities were
617 consistently lower and remained unaffected throughout all treatments, in line with the
618 observed lower reduced ascorbate levels (Fig. 7C, SI Table 3-4). In support of our
619 previous findings (which are indicative of barley arsenic hypersensitivity), these data
620 suggest that compared to maize, barley roots and shoot rely on a higher
621 ascorbate/glutathione turnover upon arsenic bioaccumulation. Crucially, *S5*-mediated
622 downregulation of DHAR activity in severely arsenic-exposed barley roots and shoots
623 could increase the overall reduced-glutathione pool allowing the plant to more
624 efficiently detoxify arsenic (phytochelatins, GST-mediated glutathione conjugation)
625 and/or metabolize ROS (glutathione oxidation).
626

627 These findings are substantiated by the observation that the enzymes which affect or
628 co-regulate the cyclic nature of glutathione-independent ascorbate redox signaling
629 displayed an arsenate-, species-, organ- and/or *S5*-independent response. More
630 specifically, we found that CAT and POX showed an arsenic-dependent upregulation
631 ($p_{cat}=2.10^{-10}$; $p_{pox}=8.10^{-8}$) which was species-, organ- and actinomycete-independent
632 ($p>0.05$) (Fig. 7C, SI Table 3-4). Hence, these H_2O_2-metabolizing enzymes seem to
633 represent a conserved and robust stress response to arsenate exposure. The SOD and
634 MDHAR activity on the other hand displayed an organ- ($p_{sod}=1.10^{-9}$; $p_{mdhar}=2.10^{-3}$) but
635 species- and *S5*-independent response ($p>0.05$) to arsenate exposure. Similarly, the
636 APX activity exhibited a species- ($p=3.10^{-5}$) and organ-dependent ($p=8.10^{-4}$) but *S5*-
637 independent (p=0.78) response (Fig. 7C, SI Table 3-4). Together, these data suggest
638 that glutathione-independent ascorbate signaling is not involved in species-specific *S5*-
639 mediated arsenic stress mitigation. These observations place glutathione-related
640 signaling at the core of the barley and maize-specific physiological response to arsenic
641 toxicity in the presence of *N. lucentensis S5*.

4.Conclusions

Soil arsenate pollution poses a substantial threat to crop yields. However, the biological response of C3 and C4 crops to arsenic accumulation is poorly understood and effective bioremediation strategies are currently non-existent. To address this, we investigated the responses of barley (C3) and maize (C4) plants to soil arsenate exposure in the presence of a siderophore-producing *N. lucentensis* actinomycete strain isolated from heavy metal polluted soils. We found that barley showed a higher sensitivity to arsenate exposure, reflecting the higher sensitivity of C3 species to arsenic stress. Conversely, we also observed differential susceptibility of C3 and C4 species to soil supplementation with *N. lucentensis* (S5 isolate). The differential sensitivity of both species to arsenate pollution and *N. lucentensis S5* treatment highlights the importance of future studies to incorporate both C3 and C4 species in their experimental design.

Given that arsenic-binding siderophores have previously been isolated from bacteria, we hypothesized that *N. lucentensis S5* treatment could induce arsenic soil retention (Das and Barooah, 2018). This was indeed supported by a reduction of arsenic uptake in both species and higher soil arsenic levels. Interestingly however, relative to barley plants, maize plants heavily relied on citric acid-mediated soil arsenic retention, a feature that was strongly induced by *N. lucentensis S5*. Contrastingly, barley plants invested more in arsenic detoxification and oxidative stress mitigation, which were also strongly enhanced by *S5*. Overall, (1) barley and maize plants differentially accumulate arsenic, (2) Maize plants appear to be more resistant to arsenic exposure, (3) the physiological response to arsenate exposure is species- and organ-specific and (4) *N. lucentensis* treatment induces species-specific physiological changes which allow the plants to mitigate arsenic uptake and arsenic toxicity. These findings further emphasize the striking differences in C3 and C4 metabolism in the context of environmental toxicity. The recurring differences in C3/C4 physiology seem to dictate the mode of action through which *N. lucentensis* reduced arsenic toxicity in both plant species. Whether these mechanisms apply for other C3/C4 species remains to be investigated.

Taken together, mechanistic insights provided in this study can help to develop bioremediation strategies for the cultivation of crops on arsenic polluted agricultural land. We argue that future research efforts should (1) explore the biochemical and

physiological responses of other key crop species to soil arsenate exposure and (2) the mitigating potential of other bacterial strains. In addition, follow-up studies are needed to assess the applicability of bacterial bioremediation to alleviate arsenic-induced crop losses in an outdoor agricultural setting.

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Declaration Statement

The authors declare that they have no conflict of interest.

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Legends

Table 1: Characterization of nine bacterial isolates extracted from heavy metal contaminated soil. The concentrations of flavonoids, phenolic compounds, auxin (IAA), gibberellic acid (GA) and siderophores, the total antioxidative capacity (FRAP and DPPH) are depicted. Values are averages ± SEM (n=4). Different letters indicate statistical significance (α=0.05).

Figure 1: **The effect of inoculation with *N. lucentensis* S5 on arsenic bioaccumulation in barley and maize.** Concentrations of arsenic in roots and shoots of control (C) and arsenate (M: mild, S: severe) treated plants, in the absence (grey: -S5) and presence (yellow: +S5) of the actinomycete *N. lucentensis* S5. Different letters indicate statistical significance between samples from the same organ and species (α=0.05). Values are averages ± SEM (n=4).

Figure 2: **The effect of inoculation with *N. lucentensis* S5 on arsenic accumulation in plants and rhizosphere biochemistry.** Principal component analysis of rhizosphere citric acid (soilCitricAcid), phenolic compound levels (soilPhenol) and arsenic levels in the roots (As_plant) and rhizosphere (As_soil) of barley (B) and maize (M) plants exposed to severe soil arsenate loading. The PCA is based on Z-score normalized data. Green and blue clusters represent maize and barley samples respectively. Cluster with

dashed and unbroken outlines represent samples grown in the absence (mock) or presence of *N. lucentensis* S5 (S5) respectively.

Figure 3: **The effect of inoculation with *N. lucentensis* S5 on plant biomass accumulation.** Shoot and root fresh weight (A) and dry weight (B) of 6 week-old barley and maize plants exposed to control (C; 0 mg kg^{-1}), mild (M; 25 mg kg^{-1}) and severe (S; 100 mg kg^{-1}) soil arsenate in the absence (grey; -S5) or presence (yellow; +S5) of *N. lucentensis* S5. Different letters indicate statistical significance between samples from the same organ and species (α=0.05). Values are averages ± SEM (n=4).

Figure 4: **The physiological/biochemical effect of As and inoculation with S5 on maize and barley shoots.** Principal component analysis of barley (B, black outlines) and maize (M, blue outlines) shoot samples exposed to control (0; white clusters), mild (M, yellow clusters) and severe (S, red clusters) soil arsenate concentrations in the absence (mock, dashed cluster outlines) or presence (Act, non-dashed cluster outlines) of *N. lucentensis*. E.g. the sample nomenclature B_M_Act refers to Barley exposed to Mild arsenate in the presence of the Actinomycete *N. lucentensis*. Vectors indicate the relative degree to which individual parameters contribute to the separation of the samples in the total variation explained by the first two principal components. The PCA is based on Z-score normalized data.

Figure 5: **The effect of As and S5 inoculation on plant detoxification.** Plant detoxification parameters of 6 week-old barley and maize plants exposed to control (C; 0 mg kg^{-1}), mild (M; 25 mg kg^{-1}) and severe (S; 100 mg kg^{-1}) soil arsenate in the absence (grey; -S5) or presence (yellow; +S5) of *N. lucentensis*. (A) total metallothionein content (MTC), (B) total phytochelatines content, (C) Glutathione-S-Transferase (GST) activity. Different letters indicate pairwise statistical significance between samples from the same organ and species (α=0.05).

Figure 6: **The effect of As and S5 on photosynthesis and carotenoid levels.** Photosynthetic activity (A) and carotenoid production (B) of 6 week-old barley and maize plants exposed to control (C; 0 mg kg^{-1}), mild (M; 25 mg kg^{-1}) and severe (S; 100 mg kg^{-1}) soil arsenate in the absence (grey; -S5) or presence (yellow; +S5) of *N.*

lucentensis. Different letters indicate pairwise statistical significance between samples from the same organ and species ($\alpha \leq 0.05$).

Figure 7: **The effect of As and S5 inoculation on oxidative stress/antioxidative response parameters.** Oxidative stress/antioxidative parameters of 6 week-old barley and maize roots and shoots exposed to control (C; 0 mg kg^{-1}), mild (M; 25 mg kg^{-1}) and severe (S; 100 mg kg^{-1}) soil arsenate in the absence or presence of *N. lucentensis* S5. (A) hydrogen peroxide (H_2O_2) and (B) malondialdehyde (MDA) concentration (lipid peroxidation). (C) heatmap of antioxidative parameters. The colour scale indicates higher (red) or lower (blue) concentrations or activities. Abbreviations: TAC; total antioxidative capacity, ASC; ascorbate, GSH; glutathione, POX; peroxidases, CAT; catalase, SOD; superoxide dismutase, APX; ascorbate peroxidase, DHAR; dehydroascorbate reductase, MDHAR; monodehydroascorbate reductase, GR; glutathione reductase, GPX; glutathione peroxidase.

Supplemental Information

Supplemental Table 1: Characterization of bacterial isolates extracted from heavy metal contaminated soil. "+" and "–"reflect the presence and absence of a specific property, respectively. The colour gradient reflects the relative intensity of the response for each individual parameter (white = low response, red = high response).

Supplemental Table 2: Arsenic, phenolic compound and citric acid contents of the barley and maize rhizospheres 6 weeks after control, mild (M) and severe (S) soil arsenate supplementation in the absence or presence (Act) of *N. lucentensis*. Data are reported as the mean ± SE (n=4). Different letters indicate statistical significance between samples from the same species and organ (α=0.05).

Supplemental Table 3: An overview of the arsenic concentration and biotic parameters quantified in barley and maize roots of control and arsenate (M: mild, S: severe) treated plants, in the absence and presence (Act) of the actinomycete *N. lucentensis*. Data are reported as the mean ± SE (n=4). Different letters indicate statistical significance between samples from the same organ and species (α=0.05). As;

Arsenic concentration, FW; Fresh Weight, DW; Dry Weight, GSH; reduced glutathione, MTC; metallothioneins, GST; glutathione-S-Transferase, H2O2; hydrogen peroxide, MDA; malondialdehyde, TAC; Total Antioxidative Capacity, Pphenol; polyphenols, Flav; flavonoids, ASC; reduced ascorbate, TASC; total ascorbate, TGSH; Total Glutathione, Toco; tocopherols, POX; peroxidase, CAT; catalase, SOD; superoxide dismutase, APX; ascorbate peroxidase, DHAR; dehydroascorbate reductase, MDHAR; monodehydroascorbate reductase, GR; glutathione reductase, GPX; glutathione peroxidase

Supplemental Table 4: An overview of the arsenic concentration and biotic parameters quantified in barley and maize shoots of control and arsenate (M: mild, S: severe) treated plants, in the absence and presence (Act) of the actinomycete *N. lucentensis*. Data is reported as the mean ± SE (n=4). Different letters indicate statistical significance between samples from the same organ and species (α=0.05). As; Arsenic concentration, FW; Fresh Weight, DW; Dry Weight, GSH; reduced glutathione, MTC; metallothioneins, GST; glutathione-S-Transferase, H2O2; hydrogen peroxide, MDA; malondialdehyde, TAC; Total Antioxidative Capacity, Pphenol; polyphenols, Flav; flavonoids, ASC; reduced ascorbate, TASC; total ascorbate, TGSH; Total Glutathione, Toco; tocopherols, POX; peroxidase, CAT; catalase, SOD; superoxide dismutase, APX; ascorbate peroxidase, DHAR; dehydroascorbate reductase, MDHAR; monodehydroascorbate reductase, GR; glutathione reductase, GPX; glutathione peroxidase

Supplemental Figure 1: Molecular and morphological characterization and identification of isolate 5 (cfr. Table 1). (A) Phylogenetic neighbour-joining tree of the strain corresponding to isolate 5 (4-P n2) showing its taxonomic position within genus Nocardiopsis. (B) Electron micrograph of *N. lucentensis* showing the fragmented substrate mycelium

Supplemental Figure 2: Representative maize and barley plants 6 weeks after exposure to 0 mg (C; control), 25 mg (M; mild) and 100 mg (S; severe) soil arsenate in the absence (grey; mock) or presence (orange) of the actinomycete *N. lucentensis*.

Supplemental Figure 3: principal component analysis of barley and maize samples exposed to control (0), mild (M) and severe (S) soil arsenate concentrations in the absence (-) or presence (+) of *N. lucentensis*. Clusters indicate root- and shoot specific data points. Vectors indicate the relative degree to which individual parameters contribute to the separation of the samples in the total variation explained by the first two principal components. The PCA is based on Z-score normalized data. E.g. sample nomenclature: BRoM- refers to Barley Roots under Mild arsenate exposure in the absence ("-') of *N. lucentensis.* MShS+ refers to Maize Shoots under Severe arsenate exposure in the presence ("+") of *N. lucentensis*.

Supplemental Figure 4: principal component analysis of barley (B, black outlines) and maize (M, blue outlines) root samples exposed to control (0; white clusters), mild (M, yellow clusters) and severe (S, red clusters) soil arsenate concentrations in the absence (mock, dashed cluster outlines) or presence (Act, non-dashed cluster outlines) of *N. lucentensis*. Vectors indicate the relative degree to which individual parameters contribute to the separation of the samples in the total variation explained by the first two principal components. The PCA is based on Z-score normalized data. E.g. the sample nomenclature B_M_Act refers to Barley exposed to Mild arsenate in the presence of the Actinomycete *N. lucentensis*.

Supplemental Figure 5: Chlorophyll A (A), chlorophyll B (B), total chlorophyll (C), chlorophyll fluorescence (D) and stomatal conductance (E) of 6-week-old barley and maize shoots exposed to control (C; 0 mg kg^{-1}), mild (M; 25 mg kg^{-1}) and severe (S; 100 mg kg^{-1}) soil arsenate in the absence (grey; -Act) or presence (yellow; +Act) of *N. lucentensis*. Different letters indicate statistical significance between samples from the same species and organ ($\alpha \leq 0.05$).

Table 1: Relative production of antioxidative, metal-chelating and plant growth promoting compounds by the 9 bacterial isolates.

	bacterial isolate number								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Flavonoids (mg/g extract)	8.7±0.5 ^{a}	8.4±0.3 ^{a}	7.6±0.3 ^{a}	9.2±0.4 ^{a}	13.9±0.6 ^{b}	8.7±0.5 ^{a}	13.1±0.9 ^{b}	7.6±0.3 ^{a}	9.2±0.4 ^{a}
Phenolics (mg/g extract)	38.8±1.6 ^{b}	46.4±5.1 ^{b}	28.3±3.6 ^{a}	42.0±1.7 ^{b}	57.7±3.3 ^{c}	38.8±1.6 ^{b}	63.1±4.3 ^{c}	38.3±3.0 ^{b}	25.3±1.8 ^{a}
FRAP (µmole trolox/g extract)	16.7±1.1 ^{bcd}	15.5±0.7 ^{bcd}	13.7±0.8 ^{ab}	17.2±0.7 ^{cd}	14.6±1.2 ^{bc}	16.7±1.1 ^{bcd}	23.4±1.3 ^{e}	10.8±1.0 ^{a}	18.2±1.3 ^{d}
DPPH (% inhibition)	45.7±0.5 ^{cd}	37.9±1.5 ^{ab}	38.3±2.8 ^{ab}	48.1±1.9 ^{de}	46.8±2.6 ^{d}	39.0±3.1 ^{bc}	54.5±3.1 ^{e}	31.4±2.0 ^{a}	47.4±1.6 ^{de}
IAA (mg/g extract)	3.0±0.2 ^{b}	2.8±0.1 ^{ab}	2.1±0.1 ^{a}	3.6±0.4 ^{bc}	4.8±0.3 ^{d}	3.0±0.2 ^{b}	4.3±0.5 ^{cd}	2.1±0.1 ^{a}	3.1±0.1 ^{b}
GA (mg/g extract)	0.6±0.03 ^{a}	0.6±0.02 ^{a}	0.6±0.02 ^{a}	0.7±0.03 ^{a}	1.0±0.04 ^{b}	0.6±0.03 ^{a}	0.6±0.02 ^{a}	0.6±0.02 ^{a}	0.7±0.03 ^{a}
Siderophores (mg/g extract)	16.7±1.6 ^{b}	14.1±0.8 ^{b}	8.0±0.7 ^{a}	16.5±0.8 ^{b}	21.9±1.2 ^{c}	16.7±1.6 ^{b}	14.1±0.8 ^{b}	8.0±0.7 ^{a}	16.5±0.8 ^{b}

Figure 1: **The effect of inoculation with *N. lucentensis* S5 on arsenic bioaccumulation in barley and maize.**

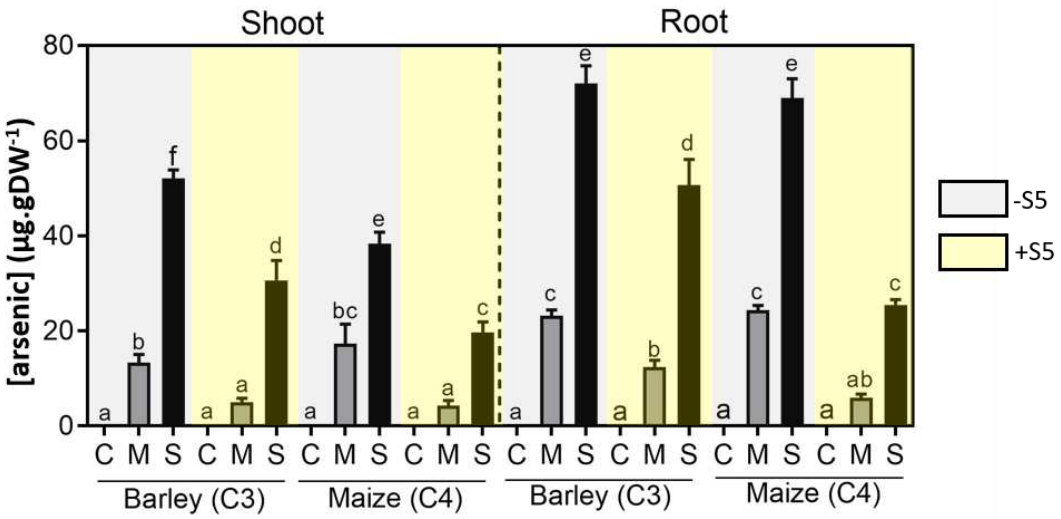

Figure 2: **The effect of inoculation with *N. lucentensis* S5 on arsenic accumulation in plants and rhizosphere biochemistry.**

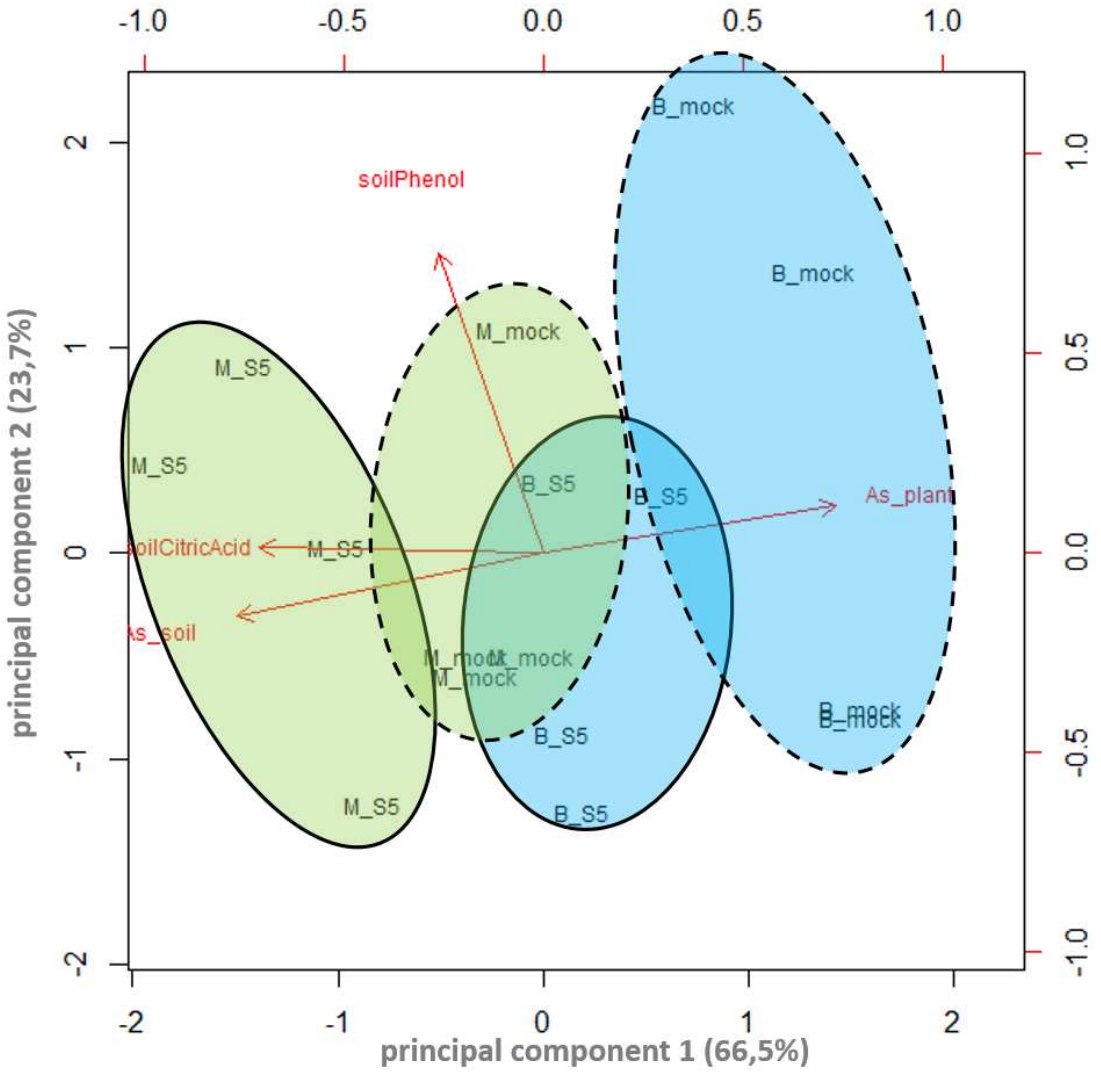

Figure 3: **The effect of inoculation with *N. lucentensis* S5 on plant biomass accumulation.**

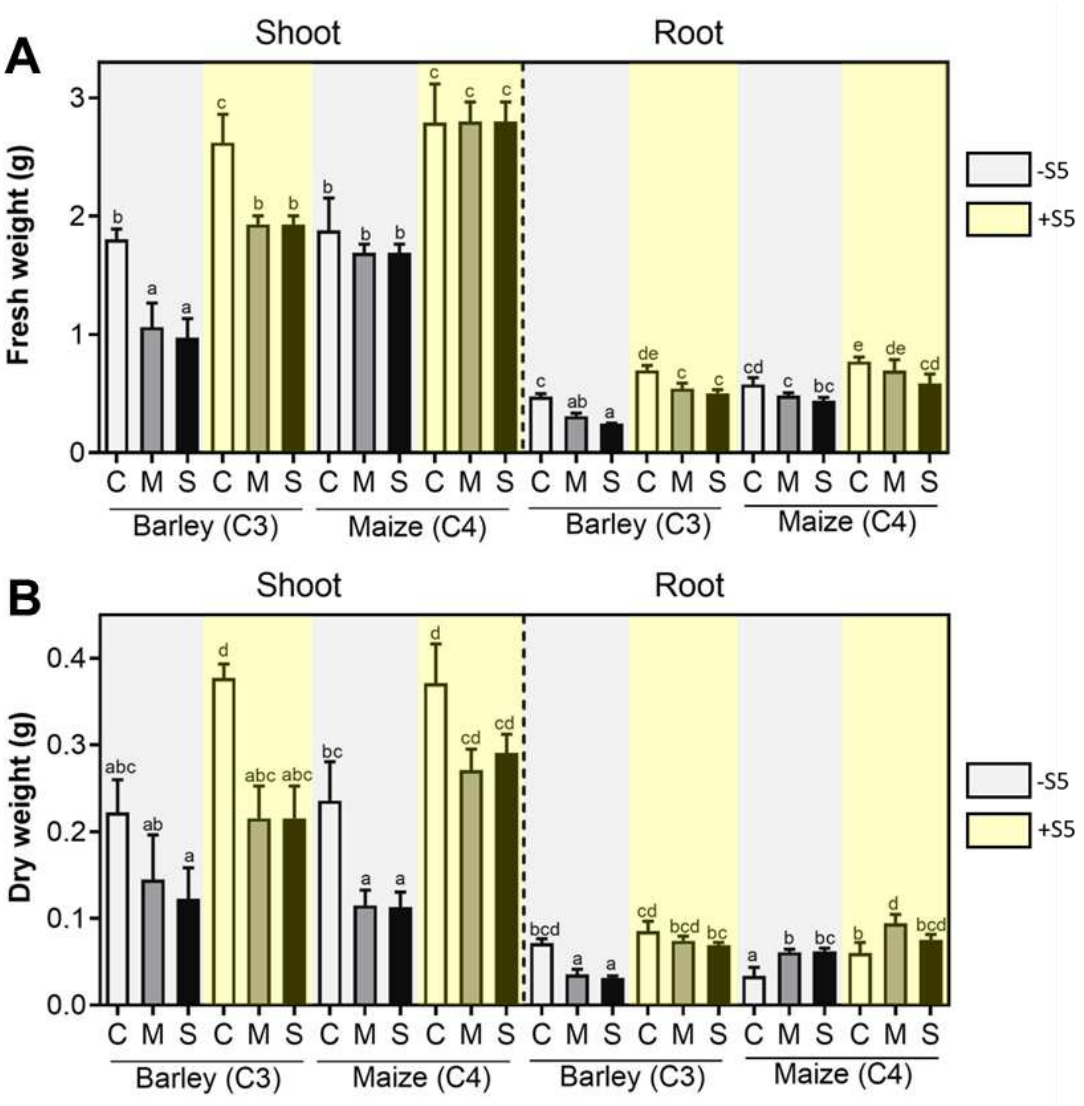

Figure 4: **The physiological/biochemical effect of As and inoculation with S5 on maize and barley shoots.**

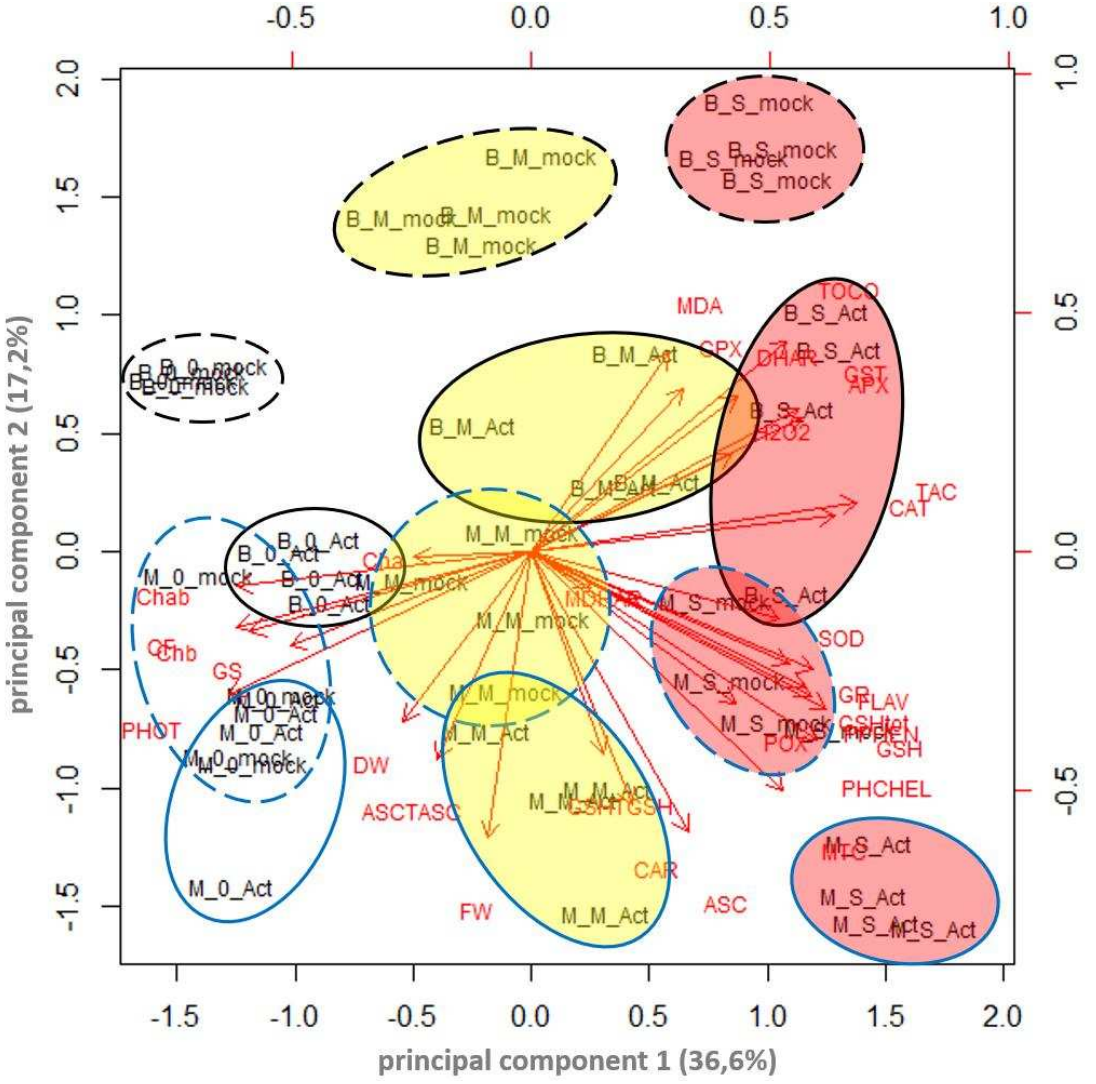

Figure 5: **The effect of As and S5 inoculation on plant detoxification.**

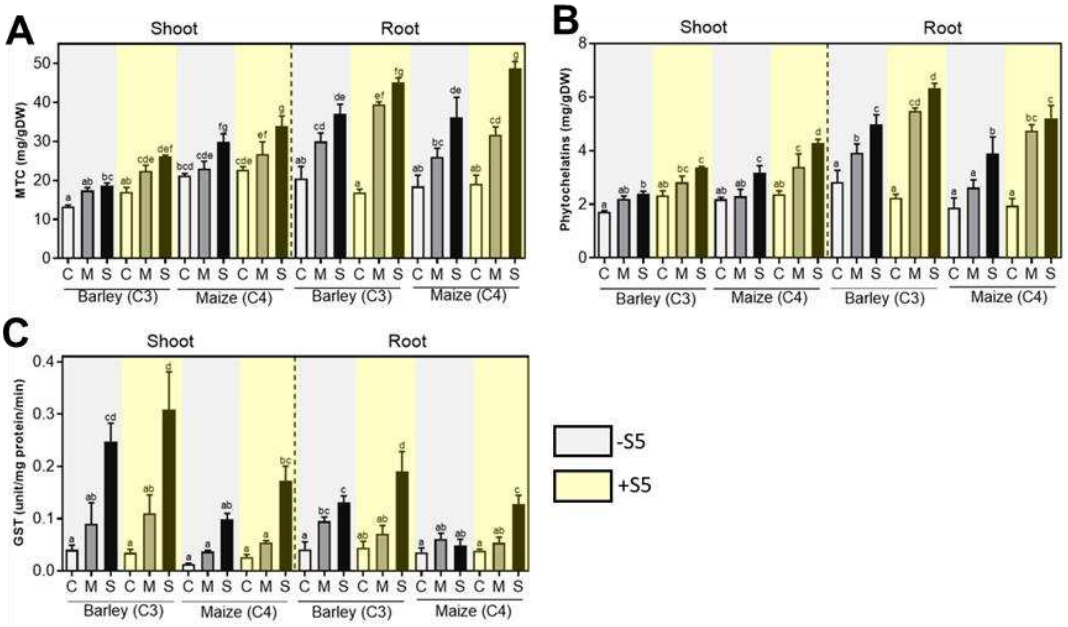

Figure 6: **The effect of As and S5 on photosynthesis and carotenoid levels.**

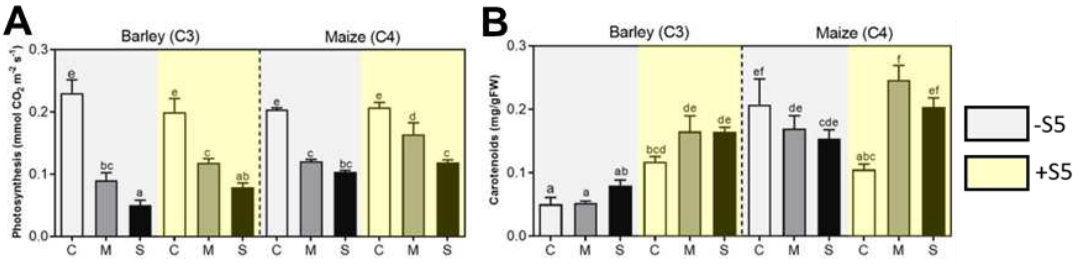

Figure 7: **The effect of As and S5 inoculation on oxidative stress/antioxidative response parameters.**