




Novel insights into the effect of arbuscular mycorrhizal fungi inoculation in soils under long-term biosolids application: Emphasis on antibiotic and metal resistance genes, and mobile genetic elements[☆]

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ABSTRACT

The application of biosolids can improve soil fertility and crop productivity but also accompanies risks of heavy metals and antibiotics introduction. In the presence of heavy metals contamination, using arbuscular mycorrhizal fungi (AMF) is a promising strategy to enhance soil microbial community stability and plant tolerance resistance to heavy metals, and to reduce the spread of antibiotic resistance genes (ARGs). The present study investigated the impacts of AMF inoculation on soil and plant heavy metal contents, and soil microbial communities by pot experiments. The results showed that AMF inoculation significantly enhanced plant biomass, and reduced soil and plant heavy metals contents. While AMF inoculation did not alter bacterial and fungal community compositions, it increased bacterial diversity at higher biosolids concentrations. Notably, AMF inoculation enhanced microbial network complexity and increased keystone taxa abundance. Furthermore, several beneficial microorganisms with high resistance to heavy metals were enriched in AMF-inoculated soils. Metagenomic analysis revealed a reduction in the mobile genetic element (MGE) gene *IS91* in AMF-inoculated soils and an increase in heavy metal resistance genes compared to soils without AMF. The possibility of reduction in MGE-mediated spread of ARGs is one of the key findings of this study. As a caution, this study also detected enrichment of few ARGs in high biosolids-amended soils with AMF inoculation. Overall, AMF inoculation could be a valuable strategy in agriculture for mitigating the environmental risks associated with biosolids, heavy metals and antibiotic resistance, thereby promoting sustainable soil management and health.

1. Introduction

The solids that are separated out during wastewater purification, and are treated physically and chemically to obtain nutrient-rich organic materials are called biosolids (Kumar et al., 2017; US EPA). The application of biosolids in agriculture has been increasingly recognized for its potential to improve soil fertility and enhance crop productivity due to their rich organic content and essential nutrients (Sharma et al., 2017; Elgarahy et al., 2024). However, the use of biosolids also carries significant risks, particularly the introduction of heavy metals and

antibiotics into the soil (Pritchard et al., 2010; Urbaniak et al., 2024). The deleterious effects of heavy metals in the environment are well-documented (Edo et al., 2024; Zhao et al., 2022), and the increasing overuse of antibiotics in domains like public health, agriculture, animal husbandry and allied sectors is transpiring as the most critical contributor to antibiotic pollution. Consequently, the spread of antibiotic resistance genes (ARGs) in the environment has become a significant concern due to its role in conferring antibiotic resistance to pathogenic bacteria overexposed to antibiotics (Nguyen et al., 2021; Ahmed et al., 2024). This issue poses serious risks to human and animal health,

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leading to increased mortality and substantial economic losses (Zainab et al., 2020). Additionally heavy metals can enhance ARGs and their trigger proliferation via a phenomenon known as co-selection (Maurya et al., 2020; Zhang et al., 2024a). The co-selection theory suggests that heavy metal contamination can lead to the simultaneous presence of metal resistance genes (MRGs) and ARGs through mechanisms such as co-resistance, cross-resistance and co-regulation, suggesting that the application of biosolids may create hotspots for both MRGs and ARGs (Naidoo, 2020; Zhang et al., 2024a). Additionally, mobile genetic elements (MGEs) such as transposons, integrons and plasmids play a crucial role in the proliferation and spread of ARGs via horizontal gene transfer among bacteria (Wu et al., 2022). In a recent study, Zhang et al. (2024b) established a link between ARGs and MGEs in rivers having discharges of aquaculture and agriculture wastewater, and industrial and urban domestic wastewater. Considering the potential risks posed by heavy metals and ARGs in biosolids, it is essential to devise effective strategies to reduce the transfer of these contaminants from biosolids to soil and crops.

There are various physical and chemical approaches to remediate heavy metal contamination in soil, such as washing of soil, soil extraction and replacement, soil solidification, incineration, chemical leaching, chemical fixing etc. (Dhaliwal et al., 2020; Liu et al., 2022). However, the laboriousness, high cost, use of chemicals and formation of harmful by-products make most of these methods unsustainable. Some environmental-friendly techniques like use of soil amendments, phytoremediation and application of specific microbial inoculants have been explored to reduce the mobility and bioavailability of heavy metals in soils (Shah and Daverey, 2020; Zheng et al., 2024). Among these, biological approaches have gained recognition for their effectiveness and minimal impact on soil and environmental health. Microorganisms use various tactics such as biosorption, bioaccumulation, biotransformation, precipitation, complexation, redox reactions, methylation and demethylation for their defense (Nivetha et al., 2023). These methods result in speciation and reduced bioavailability of heavy metals in the environment. Over the last few decades several bacteria have been identified for their potential to remediate heavy metal contaminated soils (Roy et al., 2024). However, the curb of ARGs in the environment has proven to be more challenging (Visca et al., 2024) owing to their biological nature and ability to adapt.

Recently, Dou et al. (2024) reported the in-situ remediation of Cd and acid rain co-contaminated soil using a bacterium-fungus combination. Notably, among fungi, arbuscular mycorrhizal fungi (AMF) stand out for their dual role in promoting plant growth and enhancing plant resistance to heavy metals while simultaneously reducing the spread of ARGs (Wahab et al., 2023). AMF are highly beneficial due to their ability to establish mutualistic associations with the roots of approximately 90% of terrestrial plant species, including a wide range of agricultural crops (Diagne et al., 2020). This symbiosis is particularly advantageous for plants, as AMF can significantly enhance the uptake of essential nutrients, especially phosphorus, from the soil (Khaliq et al., 2022). Extensive research has demonstrated that the symbiotic structures of mycorrhizae play a role in the sequestration and compartmentalization of heavy metals, which in turn reduces their movement from plant roots to above-ground tissues (Dhalaria et al., 2020). Moreover, AMF hyphal exudates are rich in carbohydrates, which can recruit certain microorganisms to colonize the hyphal surface, forming what is known as the hyphosphere microbiome (Zhang et al., 2022). These hyphosphere microbial communities are closely associated with AMF and mutually influence each other's growth, activity and function (Zhang et al., 2024c). AMF have also been shown to reduce the bioavailability of heavy metals like cadmium (Cd) (Boorboori and Zhang, 2022) and control the spread of ARGs (Hu et al., 2022). To the best of our knowledge, no studies have yet examined the simultaneous effects of AMF on both heavy metal toxicity and the dissemination of ARGs. Therefore, understanding the mechanisms by which AMF regulate soil heavy metal toxicity and reduces the spread of antibiotic resistance is crucial for the effective and

safe application of biosolids. Further, it remains unclear whether AMF can collaborate with other soil microorganisms to mitigate the toxicity of heavy metals and ARGs. Hence, it is crucial to also explore the synergistic effects of AMF and functional microorganisms in alleviating the pollution caused by the application of biosolids, which introduces antibiotics and heavy metals into the environment.

In this context, the objectives of the current study were to investigate the effects of AMF inoculation on changes in heavy metal concentrations, microbial communities, metal resistance genes, mobile genetic elements, and antibiotic resistance genes in soil with long-term biosolids application. This study hypothesizes that (i) AMF inoculation could reduce the content of heavy metals in soil and plants; (ii) AMF inoculation, in the presence of heavy metals, could improve the stability of the microbial community by recruiting beneficial microorganisms; (iii) inoculated AMF could work with the indigenous microbial population to enhance resistance to heavy metals and reduce the presence of ARGs and MRGs.

2. Materials and methods

2.1. Pot experiment setup

2.1.1. Experiment design

The pot experiment was carried out in a glasshouse at the Chinese Academy of Agricultural Sciences between April and June 2023. The maize variety Zhengdan958 (*Zea mays* L.) was selected as the host plant. Soils from treatments with biosolids application rates of 0, 4.5, and 36 t ha⁻¹ are designated as C (control, without biosolids), L (low biosolids concentration), and H (high biosolids concentration), respectively. The biosolids used in this study were air-dried municipal sewage products obtained from Beijing Drainage Group Co., Ltd., applied without sterilization in October 2021. The experiment followed a 3 × 2 × 2 full factorial design, with three biosolids application levels in the hyphal chamber (HC) (C, L, and H), two mycorrhizal treatments in the root chamber (RC) (NM: non-AMF inoculated and AM: AMF inoculated), and each biosolids treatment in the HC further divided into sterilized and unsterilized soil conditions. The unsterilized soil contained the indigenous microbial population from the sampling site. Each of the twelve treatment combinations was replicated four times, resulting in a total of 48 pots for the experiment. Maize seeds were sterilized by soaking in a 10% (v/v) H₂O₂ solution for 30 min, then thoroughly rinsed with deionized water before being germinated in a sterile tray for 48 h in darkness. Three seedlings were initially planted per pot, and after one week, they were thinned to one plant per pot.

The dimensions of each pot were 11.5 cm long, 10 cm wide, and 15 cm high, and they were filled with 1.8 kg of soil. Microcosms (pots) were constructed with three separate chambers: a root chamber for plant growth (3.5 × 10 × 15 cm³), a buffer chamber (2 × 10 × 15 cm³), and a hyphal chamber for hyphal development (6 × 10 × 15 cm³) (Fig. S1). The system was divided into a root chamber (RC), hyphal chamber (HC), and buffer chamber (BC), all of which were separated by 30 μm nylon mesh (Li et al., 2023). The buffer chamber (BC) was positioned in the center, with the root chamber (RC) and hyphal chamber (HC) on either side. Maize was grown in the RC, allowing external hyphae to extend through the nylon mesh into both the BC and HC. The maize roots were restricted by the nylon mesh and could not pass through, and the BC acted as a barrier to limit microbial diffusion from the non-sterilized HC into the RC. Additionally, it prevented colonization of the plants by indigenous AM fungi present in the HC.

2.1.2. Plant growth substrate and AMF inoculum

The substrate consisted of a mixture of zeolite and low-phosphorus soil (2:1, w/w), which underwent γ-irradiation sterilization at 25 kGy using ⁶⁰Co to inactivate all microorganisms (Qin et al., 2020). The soil used in the root chamber (RC) and buffer chamber (BC) was sourced from an uncultivated arable field at the Shangzhuang Experimental

Station (40°08'N, 116°10'E) in Beijing, China. The basic soil properties were: pH 8.19 (in water), organic carbon (SOC) 11.5 g kg⁻¹, available phosphorus (Olsen-P) 4.18 mg kg⁻¹, inorganic nitrogen 19.9 mg kg⁻¹ and available potassium (AK) 82.4 mg kg⁻¹. Before the start of the experiment, each kilogram of soil was supplemented with 200 mg of nitrogen, 10 mg of phosphorus, and 200 mg of potassium in the forms of (NH₂)₂CO, KH₂PO₄, and K₂SO₄, respectively.

In our previous study, the relative abundance data indicated *Glomeraceae* as the most dominant family across all treatments (Sun et al., 2024). Given this predominance and its well-documented symbiotic efficiency with maize (Zhu et al., 2020), the root chamber was subsequently inoculated with the AMF *Funneliformis mosseae* (HK01), a member of the Glomeraceae family. The AMF inoculum of Glomeromycota was obtained from the Academy of Agricultural and Forestry Sciences in Beijing. The inoculum contained approximately 50 spores per gram, along with mycelium and fine root segments, propagated in a 1:1 (w/w) mixture of soil and sand, using *Zea mays* L. as the host plant grown for four months in a greenhouse. In the pot experiment, each AM treatment group received 8 % (w/w) AMF as inoculum in the root chamber.

2.2. Harvest and sample analysis

After two months' growth, the plants were harvested. In brief, maize shoots were clipped and dried to measure aboveground biomass. Maize roots were stored at -20 °C for assessment of AM colonization and hyphal length density (Table S1). Arbuscular mycorrhizal hyphae and mycorrhizal colonization were measured using the gridline-intercept method as described et al. (1984) and McGonigle et al. (1990), respectively. The heavy metal contents of both the HC soil and plant samples were determined using inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3. Microbial DNA extraction, PCR and Illumina sequencing

Total soil DNA was extracted from 0.5 g of soil. PCR amplification targeted the V4 region for bacteria with the primer pair 515F/806R and the ITS region for fungi with primers ITS3F/ITS4R, respectively (Walters et al., 2016; Op De Beeck et al., 2014). Primers were synthesized by Invitrogen™ (Thermo Fisher Scientific Inc.). Sequencing reads were demultiplexed by unique barcodes, then merged, denoised, and filtered for chimeras using the DADA2 pipeline. Bacterial taxonomy was assigned using the SILVA database (Green et al., 2022), while fungal taxonomy was determined with the UNITE database (Eshaghi et al., 2021). For a more detailed description of bioinformatics procedures, please refer to Supplementary Materials, which provide comprehensive information.

Metagenomic shotgun sequencing libraries were generated and sequenced by Shanghai Biozeron Biological Technology Co., Ltd. For each sample, the TruSeq DNA Library Preparation Kit (catalog no: FC-121-2001, Illumina, USA) was utilized to create the sequencing libraries. The concentration of the constructed libraries was determined using the High Sensitivity Double Stranded DNA Kit on a Qubit Fluorometer (Thermo Fisher Scientific Inc.). Sequencing of all samples was carried out on an NGS platform in paired-end 150 bp (PE150) mode. The detailed information on reads quality control and metagenomic de novo assembly, gene prediction, and annotation are provided in Supplementary materials.

2.4. Statistical analyses

All data analyses were performed using R software (version 4.2.0). Two-way ANOVAs were employed to evaluate the variations in microbial diversity, plant biomass, heavy metal contents. Non-metric multidimensional scaling (NMDS) was employed to assess the effects of AMF inoculation on soil microbial communities across different biosolids

application rates. A microbial co-occurrence network was constructed using the 'microeco' package by combing bacterial and fungal taxa using a threshold of 0.01%. The 'igraph' package was then utilized for analyzing network properties. Pairwise comparisons of predicted gene abundance between soils with and without AMF inoculation were performed using STAMP.

3. Results

3.1. Effect of AMF on plant biomass, and soil and plant heavy metals contents

Biosolids application significantly increased maize biomass by 47.77% in the treatment with AMF inoculation compared to no-AMF treatment (AMF: $F = 68.33$, $p < 0.001$; Biosolids: $F = 31.81$, $p < 0.001$) (Fig. 1A; Table S2). Soil and plant heavy metals contents were also significantly affected by the AMF inoculation and biosolids application rates (Fig. 1B–F; Fig. S2; Table S2). In the soil with high application of biosolids, AMF inoculation significantly reduced Cu and Zn content by 6.96% and 5.58%, respectively. Moreover, there was a significant reduction in Zn and Hg contents by 4.69% and 31.56% in the soil with AMF inoculation, especially in unsterilized soil (Fig. 1C–F). In case of heavy metals accumulation in plant, there was a significant decrease in root Zn content by 31.51% in unsterilized soil (Fig. S2 A, B); in case of sterilized soil, there was reduction of root Cu and Zn by 4.30% and 6.10% at high biosolids application with AMF inoculation (Fig. S2 A-B; Table S2). Overall, there was a reduction in heavy metal contents in the roots with biosolids application and AMF inoculation. Notwithstanding the accumulation of heavy metals in roots, for maize leaf, a significant decrease in leaf Cu (AMF: $F = 0.66$, $p = 0.43$; Biosolids: $F = 3.43$, $p = 0.05$), Zn (AMF: $F = 14.58$, $p < 0.001$; Biosolids: $F = 6.37$, $p = 0.006$) and Cd (AMF: $F = 7.32$, $p = 0.01$; Biosolids: $F = 6.31$, $p = 0.006$) contents by 63.61%, 16.66% and 18.41% in the treatment with application of biosolids and AMF inoculation was observed (Fig. S2 D–F).

3.2. Effect of AMF on soil microbial richness and community compositions

Although there was no significant influence of AMF and biosolids application rates on the bacterial Shannon index (AMF: $F = 0.37$, $p = 0.56$; Biosolids: $F = 0.32$, $p = 0.73$), there were interactive effects of AMF and biosolids application on the bacterial Shannon index (AMF*Biosolids: $F = 3.93$, $p = 0.05$) (Fig. 2A; Table S3). A significant increase (1.97%) in bacterial Shannon index in the treatment having high application rates of biosolids with AMF inoculation was observed (Fig. 2A). However, there was no significant influence of AMF inoculation and biosolids application on the fungal Shannon index (AMF: $F = 0.05$, $p = 0.83$; Biosolids: $F = 0.64$, $p = 0.55$; AMF*Biosolids: $F = 2.52$, $p = 0.12$) (Fig. 2B; Table S3). Although there were overall no vast significant effects of AMF on soil bacterial and fungal communities, NMDS analyses revealed distinct gradual influences with the biosolids application rates' increase (Bacterial community: AMF: $F = 1.24$, $p = 0.22$; Biosolids: $F = 3.92$, $p < 0.001$; AMF*Biosolids: $F = 1.30$, $p = 0.16$) (Fungal community: AMF: $F = 0.04$, $p = 0.49$; Biosolids: $F = 3.31$, $p < 0.001$; AMF*Biosolids: $F = 0.11$, $p = 0.53$) (Fig. 2C and D).

A bacteria-fungi co-occurrence network was constructed to test the effects of AMF inoculations on microbial interactions (Fig. 3A). There were 7 keystone taxa (4 bacterial and 3 fungal amplicon sequencing variants, ASVs) found in the network (Fig. 3B). The abundance of bacterial ASV105, ASV20, ASV81 and ASV 460, and fungal ASV 31 were significantly increased with AMF inoculation (Fig. S3). In addition, a significant increase in network complexity (node number, edge number, average degree, and modularity) was also observed in the soil with AMF inoculation (Fig. 3C–F).

Metagenomics revealed that several microbial taxa were enriched and depleted in soils with AMF inoculation and without AMF

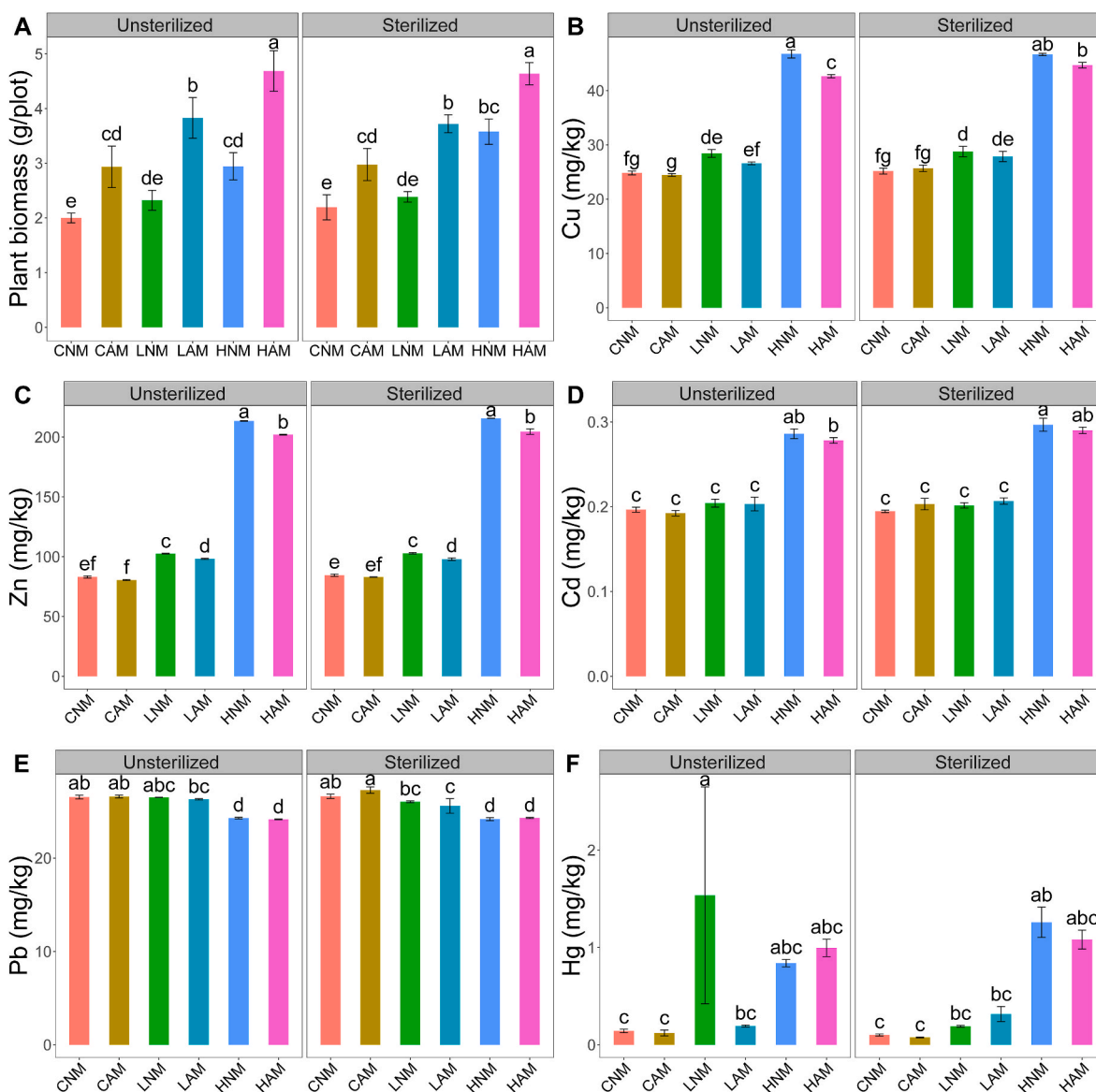


Fig. 1. The effect of AMF inoculation on plant biomass (A), and soil heavy metals contents (B, C, D, E, F) in soils with different biosolids application rates.

inoculation (Fig. 4, and Fig. S4, S5). In all application rates of biosolids, there were more taxa enriched in soil with AMF inoculation compared to the soil without AMF inoculation (Fig. 4, and Fig. S4, S5). The microbial taxa *Gemmatimonadetes*, *Planctomycetia*, *Oomycota* and *Binatia* were found enriched in all soils with AMF inoculation. There was also a significant increase of *Gammaproteobacteria* *MHYJ01*, *Blastocatellia*, *Verucomicrobiae*, *Methylomirabilia*, *Terriglobia*, *Bacilli*, *Polyangia*, *Clostridia*, and *Doudnabacteria* abundance observed in soil with AMF under control and low biosolids application rates. Moreover, the *UBA2214*, *Chlorophyceae*, *Mucoromycetes*, and *Dothideomycetes* were also found enriched in soil with AMF inoculation and biosolids application.

3.3. Effect of AMF inoculation on ARGs, MRGs and MGEs

In the present study, variations in antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and heavy metal resistance genes (MRGs) could be observed vis-à-vis biosolids concentrations in AMF treatments (Figs. 5–7). Regarding ARGs, *vanRO* and *mtrA* gene abundances were more enriched in soil without AMF inoculation in the control treatment (Fig. 5). These genes were not enriched in soil with AMF inoculation in the control treatment (Fig. 5). In contrast, in the soil

with biosolids application, there was a severe enrichment of ARGs in soil with AMF inoculation while not in soil without AMF inoculation. Gene *ceoB* was found to be enriched in all soils with biosolids application. Moreover, *bcrA* and *MuxB* were also enriched in soils with low biosolids application (Fig. 5).

In case of MRGs, the genes *ruvB*, *dspA*, *mntR* and *chrC*, *bfrA*, and *cueA* were found to be enriched in soil with AMF inoculation in control, low and high application of biosolids, respectively (Fig. 6). In contrast, the genes *merT-P*, *actP*, *ctpV*, *chrA*, and *arsC*, *acn*, *ctpV*, *brfA*, and *dmeF* were enriched in soil without AMF inoculations under control, low and high application of biosolids, respectively (Fig. 6). For MGEs, *IS91* was observed to be enriched in soils without AMF inoculation while *int3* was found to be enriched in soils with AMF inoculation (Fig. 7).

4. Discussion

Heavy metals have been previously reported to exert significant toxic effects on soil microecology (Wu et al., 2018; Sun et al., 2024). In the present study, it was found that AMF inoculation significantly reduced the soil heavy metals content, particularly Cu and Zn (Fig. 1), which have been found to be the two major heavy metals present in the current

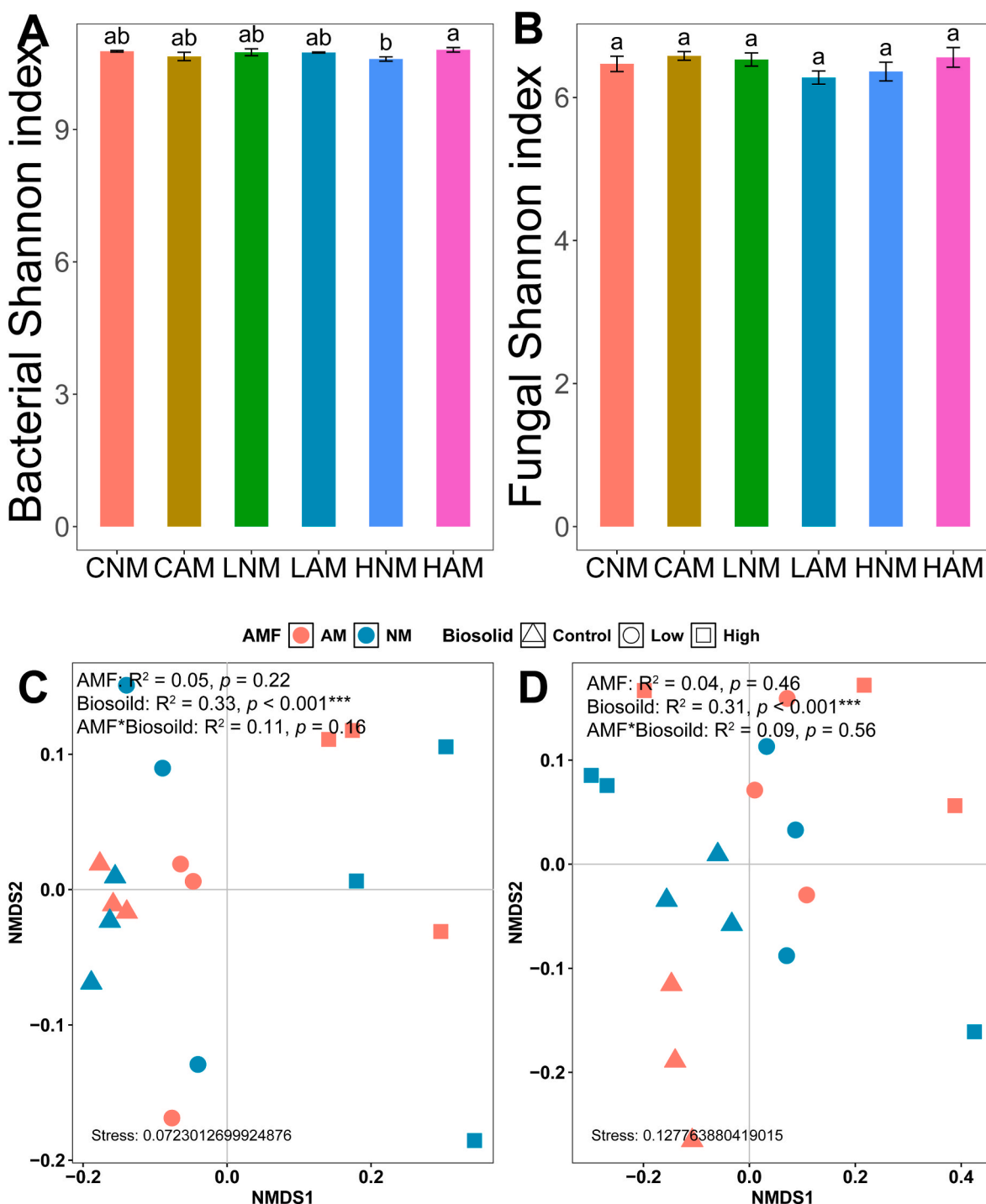


Fig. 2. Graphical representation of variation in the Shannon indexes of soil bacterial (A) and fungal (B) communities after AMF inoculation at different application concentrations of biosolids, and the effect of AMF inoculation on soil bacterial (C) and fungal (D) community compositions in soil at different concentrations of biosolids application. Significance is indicated by $p < 0.05^*$, $p < 0.01^{**}$ and $p < 0.001^{***}$.

study area (Sun et al., 2024). Previous studies have found that AM inoculation is a crucial and sustainable approach that boosts the ability of plants to extract and sequester pollutants from the soil (Jeffries et al., 2003; Ahmad et al., 2018; Tiwari et al., 2022). Notably, in this study it was found that the reduction of plant heavy metal content was significantly higher in soils with the presence of microbes than in sterilized soils (Fig. 1). This indicated that AMF can mitigate the toxic effects of heavy metals on soil microbial communities and may form a symbiotic association with native soil microbes to sequester heavy metals in the soil, thereby reducing their accumulation in plants. The harmful effects

of heavy metals on microbial communities occur through several mechanisms, including acting as redox catalysts in the production of reactive oxygen species (ROS), disrupting enzyme functions, affecting ion regulation, and interfering with DNA and protein synthesis, thereby decreasing bacterial survivability and community diversity (Hobman and Crossman, 2015; Hong et al., 2019). In the present study, an increase in soil bacterial richness with AMF inoculation in high biosolids input conditions was observed (Fig. 2), indicating that AMF could mitigate the deleterious effects of heavy metals on soil bacterial diversity. AMF have the potential to sequester heavy metals in soil, and

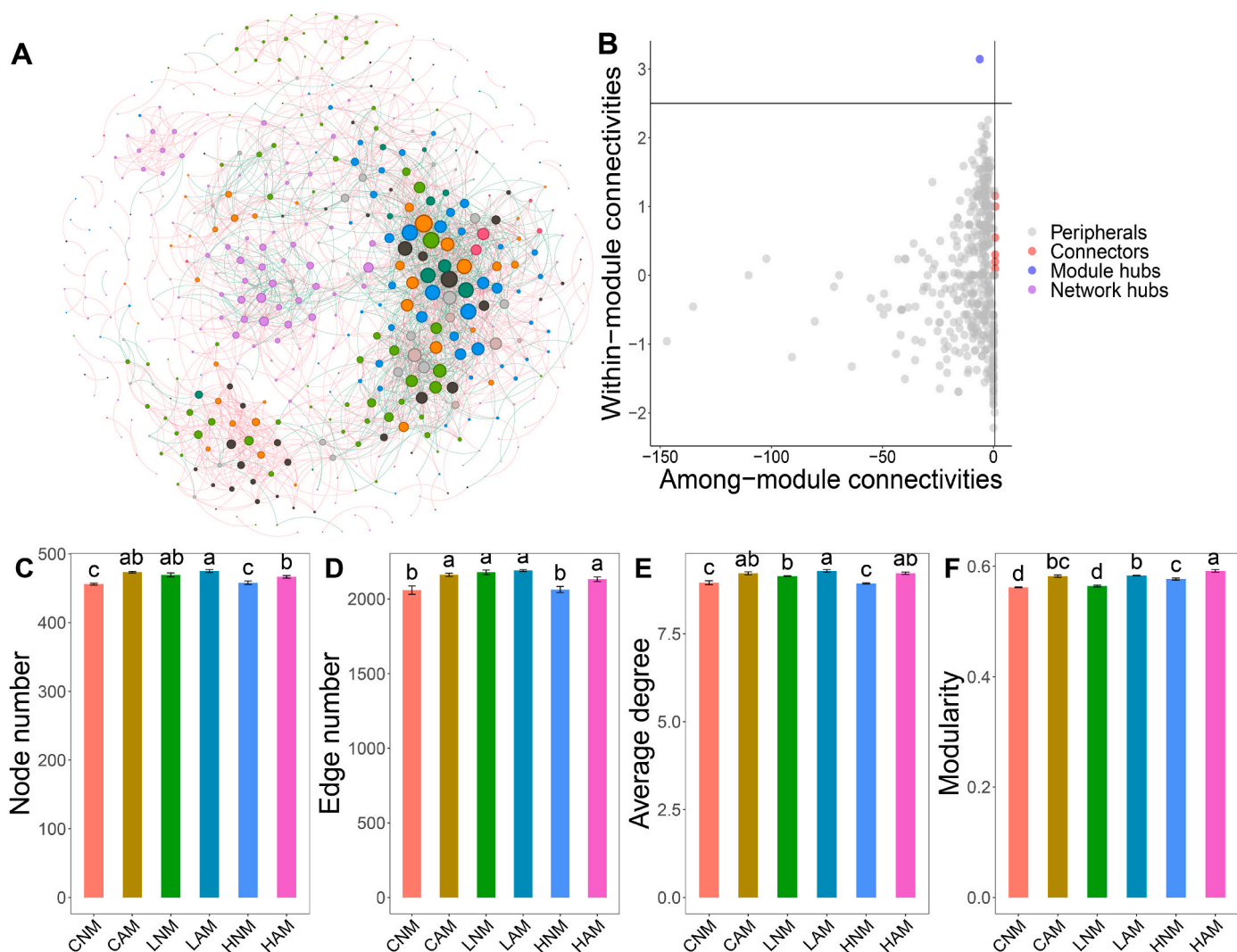


Fig. 3. Graphical representation of co-occurrence network of bacterial and fungal interactions (A), the distribution network of keystone taxa (B), and the effect of AMF inoculation on network complexity (C, D, E, F) in soils with different biosolids application rates.

they can also chelate heavy metals within their colonies (Tiwari et al., 2022; Dhalaria et al., 2020; Huang et al., 2018), reducing their bioavailability. In addition, AMF can detoxify heavy metals by directly adsorbing the metals on the fungal surface and immobilizing them in the soil through the secretion of the low-molecular weight glycoprotein, glomalin (Dhalaria et al., 2020; Tiwari et al., 2022). Furthermore, it was reported that AMF can mitigate the toxic effects of heavy metals on bacteria by enhancing soil enzyme activity as well (Riaz et al., 2021).

Edges, nodes, and average degree are widely recognized indices used to describe network properties and understand interactions between microbial groups. Microbial network complexity is frequently utilized to model these networks and evaluate their responses to stimulation (Zhai et al., 2024). This is established on MacArthur's concept that the complexity of ecosystems begets their stability (MacArthur, 1995). In the present study, AMF inoculation significantly improved the complexity and stability of the entire microbial network in soil (Fig. 3). Besides mitigating the toxicity of heavy metals on microbes, AMF also enhanced maize biomass and ameliorated heavy metal toxicity on the plants. The increase in biomass was possibly due to the improved plant nutrient uptake. It has been previously reported that AMF not only boosted plant growth but also increased the mobility of nutrients (Mitra et al., 2023). As regards to heavy metal toxicity, AMF have been reported to reduce the translocation of heavy metals to shoots and retain them in the roots enabling metal tolerance in plants (Riaz et al., 2021). This was clearly

observed in the present study with reduced presence of heavy metals in maize shoots as compared to the roots. Further, the hyphae of AMF contribute to strengthening soil aggregates and enhancing soil structure, which increases water retention, reduces erosion, and creates a more favorable environment for diverse microbial communities that can robustly support plant growth (Fall et al., 2022; Gujre et al., 2021). Additionally, AMF secrete a variety of organic compounds such as sugars and fatty acids into the rhizosphere which can promote the growth of specific beneficial microbes that contribute to plant health and soil fertility (Basu et al., 2018). Overall, AMF can potentially create a mutualistic symbiosis between the native microbes and plants (Khaliq et al., 2022), enhancing plant vitality even in the presence of heavy metal stress. In this study, AMF incubation significantly increased the abundance of *Gemmatimonadetes*, *Blastocatellia* and *Gammaproteobacteria* (Fig. 4). *Gemmatimonadetes* and *Blastocatellia* are known for their ability to survive in oligotrophic (low-nutrient) conditions and extreme environments, which often include environments contaminated with heavy metals (Wang et al., 2019; Alekseev et al., 2020). These microbes can tackle heavy metals through mechanisms such as biosorption, bioaccumulation, and possibly also transform the metals to less toxic forms (George, and Wan, 2020). Gammaproteobacteria is a large and diverse class of bacteria that include many well-known genera such as *Pseudomonas* and *Escherichia* (Gao et al., 2009; Williams et al., 2010). These bacteria are often highly adaptable and can resist heavy metals stress

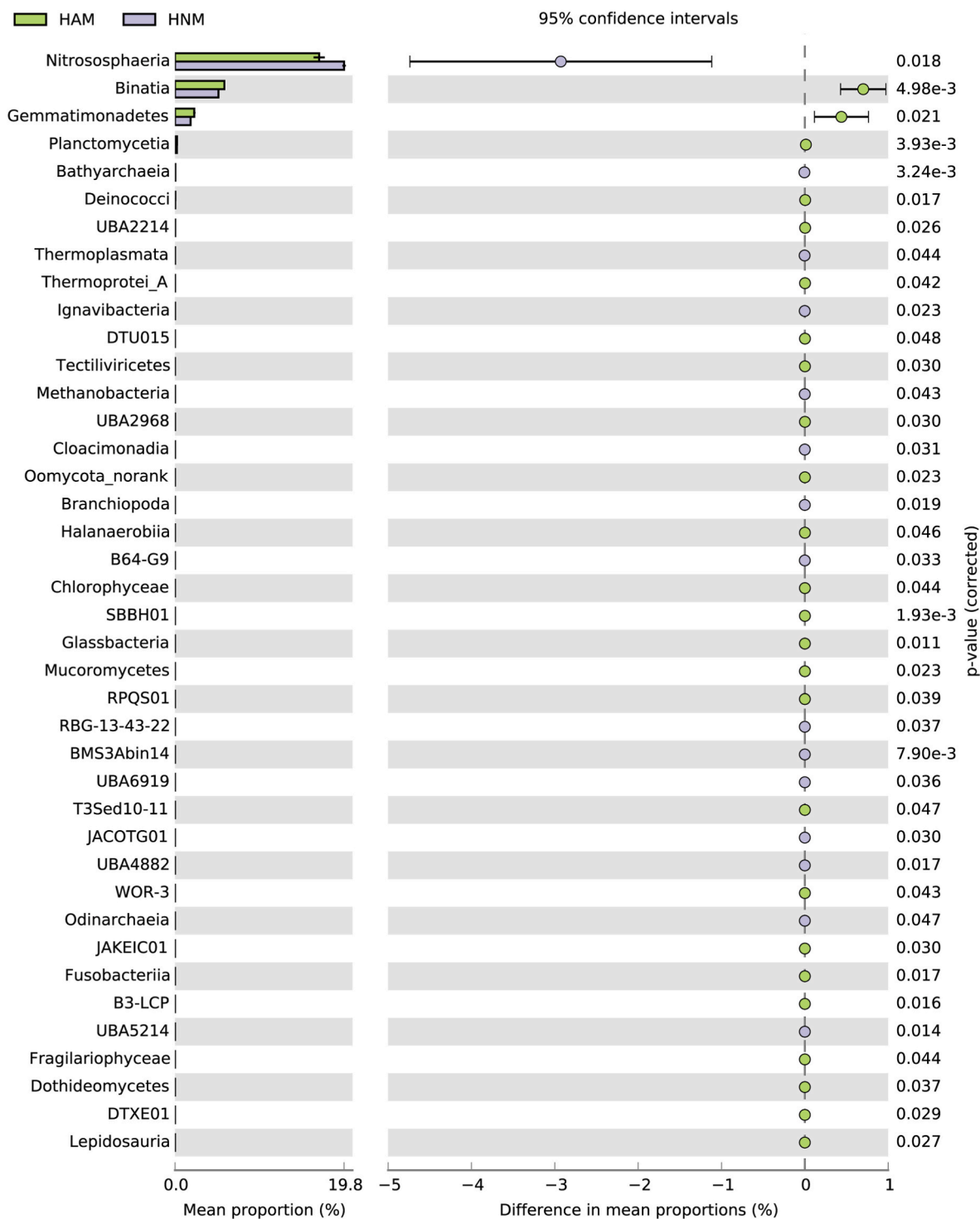


Fig. 4. Graphical representation of comparison of microbial taxa with AMF inoculation and without AMF inoculation at high biosolids application rate, determined using 95 % confidence intervals for the difference in mean proportions.

through various mechanisms, including secretion of extracellular polysaccharides and biosurfactants, and the use of efflux pumps to remove toxic metals from the cytoplasm (Chellaiyah, 2018; Mathivanan et al., 2021; Fakhar et al., 2022).

Keystone taxa are species or groups of organisms within an ecosystem that have a disproportionately large impact on their environment relative to their abundance. In the present study, seven keystone taxa (4 bacteria and 3 fungi), were enriched in soil with AMF inoculation (Fig. 3). Keystone taxa has been found to play crucial roles in

maintaining the structure and function of the ecosystem, enhancing stability and resilience of microbial communities, influencing nutrient cycling, soil structure, and improving the overall health of plants and microorganisms (Hu et al., 2024; Xiao et al., 2021). Additionally, keystone taxa possess the ability to detoxify heavy metals by either transforming them into less harmful forms or by immobilizing them, thereby reducing their bioavailability in the soil (Yu et al., 2021). Further, the several keystone genera identified in the present study (Table S5) have been previously reported to bioremediate heavy metals.

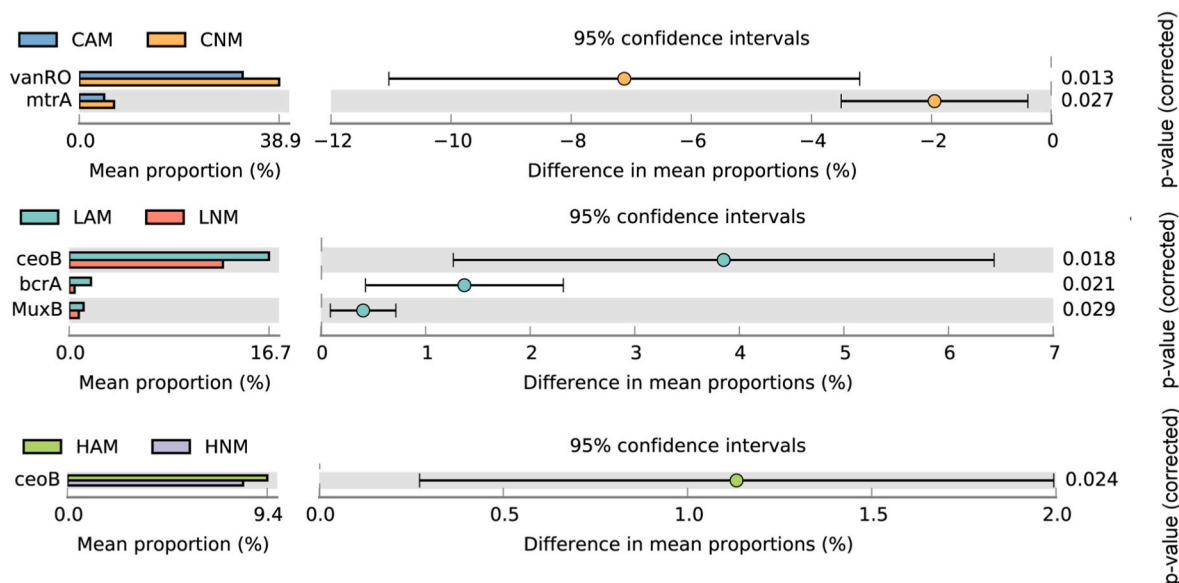


Fig. 5. The effect of AMF inoculation on ARGs in soils with different biosolids concentrations.

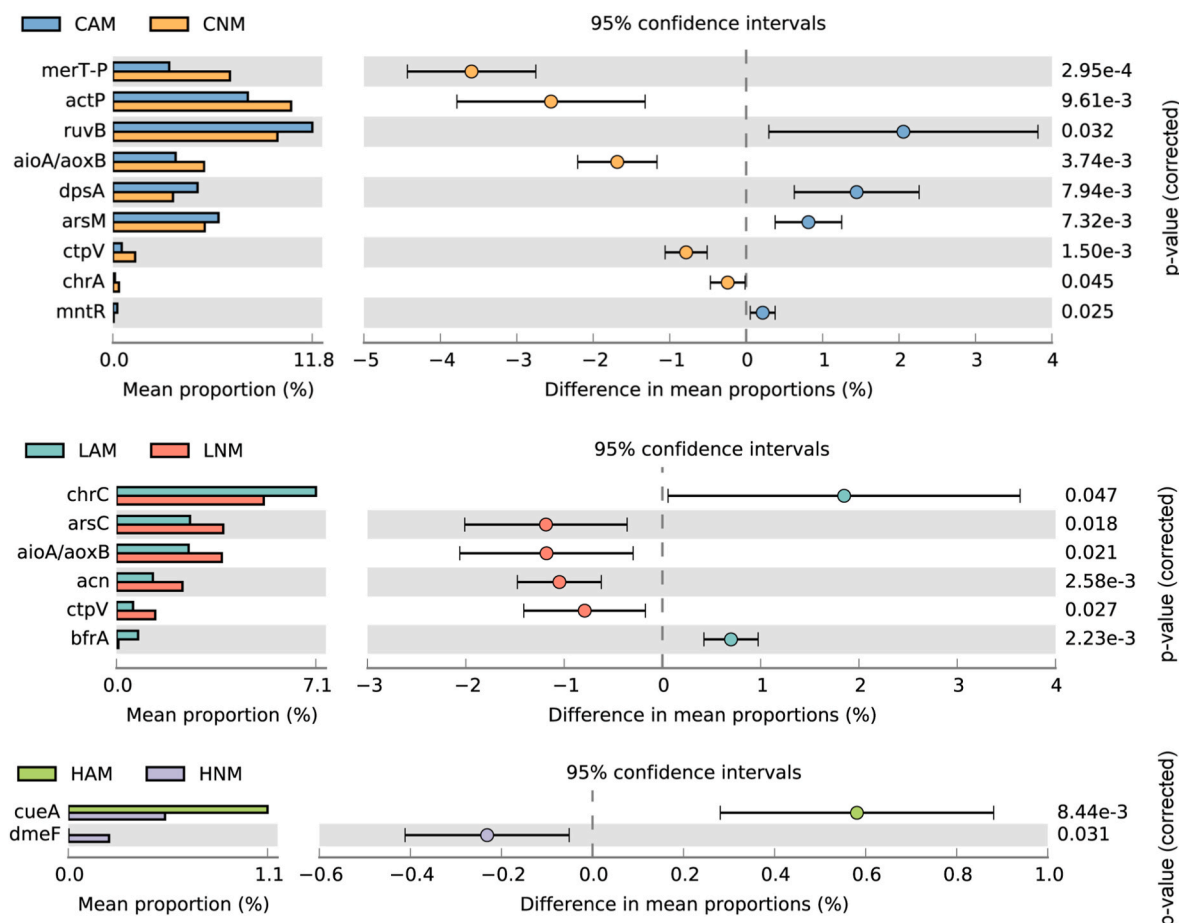


Fig. 6. The effect of AMF inoculation on MRGs in soils with different biosolids concentrations.

Sphingomonas spp. are known for their robust bioremediation capabilities of heavy metals via biosorption, secretion of extracellular polymeric substances (EPS) and bioreduction (Reddy et al., 2024). These processes help remove heavy metals from the environment or convert them into less toxic forms (Mishra et al., 2022). *Skermanella* exhibits

significant tolerance to heavy metals and may process them through mechanisms like biosorption and intracellular sequestration (George and Wan, 2020). *Cellulomonas* sp. can thrive in heavy metal-contaminated environments and can sustain heavy metals stress via biosorption and EPS production (Gupta and Diwan, 2017).

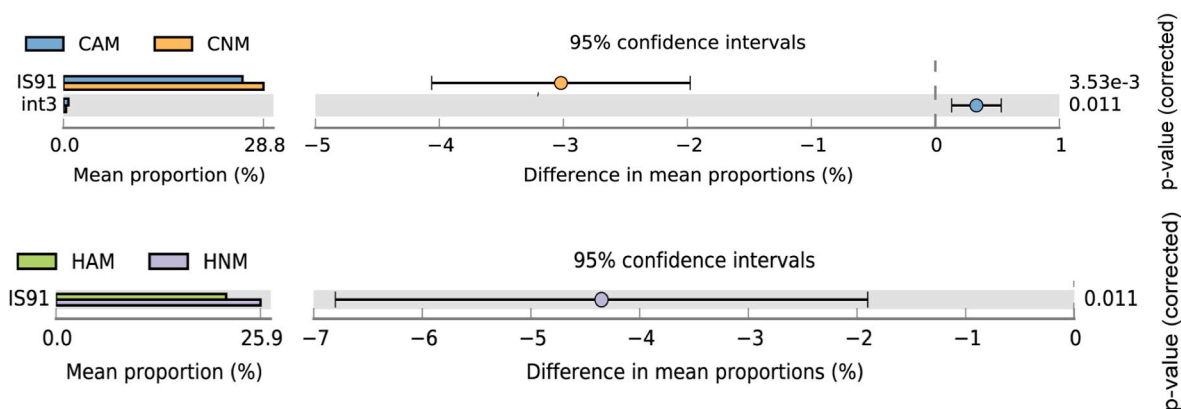


Fig. 7. The effect of AMF inoculation on MGEs in soils with different biosolids concentrations.

Apiosordaria sp. can tolerate heavy metals through biosorption and EPS production, and are often used in bioremediation studies owing to their potential to grow in heavy metal-contaminated environments and reduce the toxicity of these metals (Sun et al., 2014). *Myrmecridium* sp. show tolerance to heavy metals and can process them through biosorption and sequestration mechanisms, which can help in the stabilization and detoxification of heavy metals in the environment (Lotlikar, 2019).

The presence of ARGs in municipal solid wastes is a serious concern owing to the practice of applying biosolids as fertilizers in agriculture (Sorinolu et al., 2021). MGEs and MRGs are known to aid the diffuse contamination of ARGs (Zhang et al., 2024b; Tan et al., 2024). Metagenomic analysis in the current study revealed a reduction in the *IS91* gene of the MGE gene pool in the AMF-inoculated soil (Fig. 7). *IS91* family includes insertion sequences which are essentially a type of transposable element, and it could be involved in the horizontal transfer of genes, including those responsible for antibiotic resistance or other adaptive traits (Yang et al., 2020; Pan et al., 2021). The reduction of the *IS91* gene in AMF-inoculated soil indicate that AMF could play a critical role in stabilizing the gene pool of the soil microbial community, potentially reducing the spread of MGEs that can spread undesirable traits like antibiotic resistance. This technique holds potential in mitigating MGE-mediated spread of ARGs in agricultural fields treated with biosolids and municipal solid wastes (MSWs). MRGs such as Cu-resistance-related *cueA*, multimetal(loid)-resistance-related *ruvB*, Fe-resistance-related *dpsA* and *bfrA*, As-resistance-related *arsM* and Mn-resistance-related *mntR* were enriched in soil with AMF inoculation (Fig. 6). The gene *cueA* typically encodes a copper efflux protein that helps the cell pump out excess copper ions from the cytoplasm, thereby protecting the cell from copper toxicity (Rademacher and Masepohl, 2012). *RuvB* is a gene that confers multimetal resistance, enabling it to defend against toxic, redox-active elements like Cr, Se, and Fe (Huang et al., 2023). The Fe-related *dpsA* gene encodes a protein that protects chromosomal DNA from oxidative stress, with its expression being upregulated in response to nutrient limitations, particularly nitrogen or phosphorus deficiency (Michel et al., 2003; Huang et al., 2023). Gene *bfrA* encodes a bacterioferritin, an iron-storage protein that helps in maintaining iron homeostasis by storing excess iron and releasing it when needed (Monfeli and Beeson, 2007; Abreu et al., 2019). The *arsM* gene encodes arsenite methyltransferase, which detoxifies arsenic by methylating arsenite into less toxic, more easily volatilized forms (Chen et al., 2014). The *mntR* gene encodes a regulatory protein involved in manganese homeostasis, typically controlling the expression of genes related to manganese uptake and detoxification (Pittman, 2005). These observations provide novel insights on the modulation of native soil microbial communities when in the presence of inoculated AMF. Further, these observations are in concordance with one of the previously mentioned results of the current study, where an increase in

microbial richness with AMF inoculation despite the presence of heavy metals was observed. Hence, it is possible to conjecture that AMF inoculation enriched metal-resistant microbes in the soil. Inoculation with AMF can enhance soil microbial communities' resistance to heavy metals by enriching beneficial microorganisms and keystone taxa while reducing the presence of MGEs and consequently, reducing the spread of ARGs. Overall, stable microbial population aids the maintenance of soil health and plant vitality.

Unanticipatedly, in this study, it was also found that some ARGs such as *ceoB*, *bcrA*, and *MuxB* were enriched in the soil with AMF inoculation (Fig. 5). The gene *ceoB* is part of an efflux pump system often found in bacteria, particularly within the *ceo* operon (Nair et al., 2004). This system is usually involved in conferring resistance to multiple antibiotics, particularly aminoglycosides, and other toxic compounds by actively pumping them out of the cell (Li et al., 2015). The gene *bcrA* is associated with resistance to bacitracin. It encodes a protein that is part of an ATP-binding cassette (ABC) transporter system which can expel bacitracin from bacterial cells, thereby conferring resistance to the antibiotic (Ahmad et al., 2020). *MuxB* is part of a multidrug efflux pump system in bacteria, commonly associated with resistance to a variety of antibiotics, including β -lactams and other broad-spectrum antibiotics (Sun et al., 2014). Efflux pumps like *MuxB* can contribute to multidrug resistance by actively expelling a wide range of antibiotics out of the cell. However, in the present study, some ARGs such as *vanRO* and *mtrA* showed reduced presence when the soils were treated with only AMF inoculation and without the biosolids. The gene *vanRO* confers resistance to glycopeptides and *mtrA* confers multidrug resistance (Osiris Díaz-Torres et al., 2024). These observations are timely and serves to caution the scientific community and stakeholders to further thoroughly investigate the presence and spread of ARGs in soils amended with biosolids. Additionally, more studies are warranted for the detection of the presence of other ARGs when biosolids-amended soils are inoculated with AMF. From the present study it is evident that the effect of AMF inoculation vis-à-vis the increase of ARGs is incumbent of the type of ARG.

5. Conclusions

In conclusion, AMF inoculation significantly increased plant biomass, and reduced soil and plant heavy metals contents, particularly of Cu and Zn, as well as reduced the potential spread of antibiotic resistance genes via mobile genetic elements. The influence of AMF on heavy metals was stronger in the unsterilized soil with the indigenous microbial communities compared to the sterilized soil, which indicated the development of a symbiotic relationship between the inoculated AMF and the indigenous soil microbial communities. Although there was no significant influence of AMF inoculation on the diversity of indigenous bacterial and fungal communities, there was an interactive

effect of AMF and biosolids application rates, particularly in terms of bacterial diversity in treatments with AMF inoculation in soils with high biosolids application rates. Further, AMF inoculation significantly increased microbial network complexity and keystone taxa abundance potentially aiding soil health and consequently, plant vigour. Moreover, it was also found that several beneficial microorganisms that have high resistance to heavy metals were enriched in soil with AMF inoculations. Metagenomics showed *IS91* gene was reduced in the AMF-inoculated soil, indicating that AMF could potentially reduce the spread of antibiotic resistance genes via mobile genetic elements. As far as we are aware, this is the first study to identify a reduction in the MGE *IS91* in biosolids-treated soil following AMF inoculation. This technique holds potential in mitigating MGE-mediated spread of ARGs in agricultural fields treated with biosolids or MSWs. To serve as a caution, this study revealed a higher abundance of antibiotic resistance genes in soils with AMF inoculation and biosolids amendment compared to soils without AMF inoculations and but with biosolids amendment. Overall, our findings suggest that integrating AMF inoculation into agricultural practices could be a useful strategy for managing soil health and mitigating the risks associated with heavy metals and mobile genetic elements-mediated spread of antibiotic resistance in the environment. However, further vigilance is required regarding the enrichment of antibiotic resistance genes. This study highlights the potential role of AMF in promoting sustainable soil and plant health while potentially reducing the deleterious effects of heavy metal contamination in the environment.

CRedit authorship contribution statement

Tao Sun: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Pierre Delaplace:** Writing – review & editing. **Guihua Li:** Writing – review & editing. **Anina James:** Writing – review & editing. **Junting Pan:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Jianfeng Zhang:** Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.125846>.

Data availability

Data will be made available on request.

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