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ARBUSCULAR MYCORRHIZAL FUNGI BIOAUGMENTATION IMPROVES SOIL HEALTH IN LONG-TERM BIOSOLIDS-AMENDED FARMLAND

Tao Sun [a,b], Pierre Delaplace [b], Guihua Li [a], Anina James [a], Junting Pan [a,*], Jianfeng Zhang [a,c,**]

a State Key Laboratory of Efficient Utilization of Arable Land in China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural
Sciences, Beijing, 100081, China
b Gembloux Agro Bio Tech, University of Liège, 5030, Belgium
c Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun, 130112, China

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Soil fertility and nutrient cycling can be enhanced with application of biosolids; however, it can also pose risks associated with introduction of heavy metals. The balance between these beneficial and adverse effects in influencing soil health and multifunctionality remains unclear. Further, eco-friendly strategies to alleviate heavy metal accumulation in biosolids-applied soils are undetermined. With this objective, we sampled soil from an agricultural field that had been applied with biosolids for 16 years at rates of 0 t ha^{-1} (Control), 4.5 t ha^{-1} (SW1), 9 t ha^{-1} (SW2), 18 t ha^{-1} (SW3), and 36 t ha^{-1} (SW4). Bacterial, fungal and arbuscular mycorrhizal (AM) fungal communities were determined, and soil health index (SHI) and multifunctionality (SMF) quantified. Field experiment showed that optimal biosolids application significantly increased SHI and SMF by 3–20 % and 3–75 %, respectively, compared to the Control treatment. Bacterial and AM fungal keystone taxa abundances were positively correlated to SHI and SMF. Additionally, to corroborate these results, pot experiments were conducted to test the effect of AM fungal inoculation on soil microbial diversity and multifunctionality, and its mitigatory effect on heavy metal accumulation. AM fungal inoculation significantly increased SMF by 42–61 %, and reduced Cu and Zn contents by 7–10 % and 4–6 %, respectively. Metagenomic analyses showed that AM fungal inoculation significantly increased soil carbon, nitrogen, phosphorus and sulfur gene abundance, positively correlated to SMF. Overall, the findings underscore the benefits of judicious biosolids application combined with AM fungal bioaugmentation as a viable strategy for mitigation of heavy metal accumulation for sustainable agriculture.

Introduction

Soil health is fundamental to supporting plant and animal productivity as well as multiple ecosystem functions (Jia et al., 2025; Teague and Kreuter, 2020). Healthy soil supports high-yield crop production while maintaining optimal cycling of nutrients, including carbon storage and utilization, which is referred to as soil multifunctionality (SMF) (Adewara et al., 2024; Pandao et al., 2024). Therefore, enhancing soil health and multifunctionality is essential for maintaining sustainable agricultural systems and ensuring long-term food security. Biosolids application is increasingly recognized as a potential resource for sustainable agriculture, as it is rich in organic matter and essential nutrients that can improve soil fertility and structure, and promote high-yield crop production (Elgarahy et al., 2024; Marchuk et al., 2023; Kumar et al., 2017). However, the occurrence of heavy metals, such as cadmium and lead, and organic contaminants, such as pharmaceutical residues and microplastics in biosolids may pose long-term environmental risks (Popoola et al., 2023; Mohajerani and Karabatak, 2020). Hence, the overall impact of biosolids on soil health remains unclear. Understanding the effects of biosolids application on soil health and multifunctionality is essential for optimizing its use in sustainable agriculture while minimizing potential environmental risks.

Soil microbial communities are invaluable for soil biota and partake in several crucial functions, such as primary production, nutrient cycling and carbon storage (Jia et al., 2025). Several studies have found that soil multifunctionality, in both natural and agricultural ecosystems, is critically linked to microbial diversity (Jiao et al., 2022; Han et al., 2021; Luo et al., 2018; Delgado-Baquerizo et al., 2016). However, the benefits of microbial diversity on soil multifunctionality are mediated by a combination of natural factors and management practices (Jia et al., 2023; Jia et al., 2024; Dong et al., 2022). While biosolids have the potential to improve soil fertility and microbial diversity, they may also introduce pollutants that undermine soil microbiota and multifunctionality (Zhang et al., 2024; Zheng et al., 2021). Soil microbes are highly sensitive to changes in their microenvironment, and heavy metals found in biosolids may alter the structure of microbial communities, inhibit the growth of some microbes, and affect their ecological functions (Sun et al., 2024; Shuaib et al., 2021; Perez-de-Mora et al., 2006). For instance, Cu and Pb could disrupt microbial growth, alter community composition, and inhibit key microbial functions necessary for nutrient cycling and soil health (Sun et al., 2024). In addition to heavy metals, pollutants such as pharmaceutical residues and microplastics found in biosolids may also have long-lasting effects on soil microbial communities, reducing microbial diversity and impacting soil biological functions (Hale et al., 2022). Furthermore, it may also promote the selection of antibiotic resistance genes (ARGs) in the microbiome, raising concerns about the spread of antimicrobial resistance (Hung et al., 2022; Law et al., 2021). Given these conflicting effects, it is crucial to investigate the impact of biosolids application on soil microbial communities and multifunctionality, particularly regarding how to balance the benefits and risks to ensure its sustainable use in agriculture.

Microorganisms enable soil multifunctionality (SMF) in a context dependent manner based on the prevailing edaphic factors (Jia et al., 2024). SMF has been shown to be positively influenced by both bacteria and fungi (Luo et al., 2018), however, their influence may be different in soils with long-term biosolids application as they are highly dependent on factors such as soil type, nutrient availability, moisture, and the presence of contaminants (Morgan et al., 2024; Mohapatra et al., 2016). Soil bacteria are often more sensitive to changes in nutrient availability, pH, and the presence of contaminants (Wu et al., 2017; Oliveira and Pampulha, 2006). In biosolids-amended soils, bacteria can contribute significantly to nutrient cycling, organic matter decomposition, and soil structure improvement (Elgarahy et al., 2024; Ploughe et al., 2021). However, some bacterial species may also be negatively affected by pollutants such as heavy metals in biosolids, leading to altered microbial communities and reduced soil function (Sun et al., 2024; Olaniran et al., 2013). Soil fungi, including arbuscular mycorrhizal fungi (AMF), are essential for nutrient cycling, and contribute to soil structure by forming mycelial networks that improve soil aggregation and water retention (Wang et al., 2022a; Hooker and Black, 1995). In the context of biosolids application, fungi might be more resilient to some pollutants than bacteria and can help buffer the effects of pollutants like heavy metals. Fungi also support the growth of beneficial microbial communities by providing nutrients to plants and other microbes in exchange for carbohydrates (Devi et al., 2020). AMF, in particular, have been shown to alleviate heavy metal toxicity in plants and soil microbes by sequestering metals in their hyphal structures (Riaz et al., 2021). The potential of AMF to sequester and neutralize heavy metals in their hyphal tissues helps mitigate the negative effects of pollutants in biosolids-amended soils (Sun et al., 2025), which might make them even more crucial for promoting long-term soil health and multifunctionality. Moreover, AMF can engage with bacteria to enhance soil functionality, facilitating nutrient cycling and promoting microbial diversity (Fall et al., 2022). Nonetheless, it is still unknown how biosolids application shifts the relationship between soil microbial diversity and SMF, and which microbial groups may play critical roles in driving SMF.

To explore the effects of biosolids application on the interdependence of soil multifunctionality and soil microbial diversity, we collected and analyzed soil samples from agricultural field that had 16 years of continuous biosolids application annually. Further, pot experiments were conducted to determine the effect of AMF bioaugmentation on soil supplemented long-term with biosolids. The soil microbial community structure was determined and soil health index and soil multifunctionality quantified. We hypothesize that (1) appropriate application rates of biosolids can enhance soil health index and soil multifunctionality while the overuse of biosolids can reduce both parameters, (2) bioaugmented AMF could alleviate the accumulation of heavy metals introduced to soil via biosolids, promoting soil multifunctionality. This study provides a crucial roadmap on the optimum application rates of biosolids to agricultural fields, and also lays out a strategy to combat highly prevalent yet often neglected heavy metal accumulation in biosolids-applied fields via bioaugmentation of AMF.

Materials and methods

SITE DESCRIPTION AND SAMPLING

The soil samples were obtained from a 16-year long-term field experiment. The details about the crop rotations, soil type and climatic conditions of this field are available in our previous publication Sun et al. (2024). The field experiment involved a gradient of biosolids application of 0 t (Control), 4.5 t (SW1), 9 t (SW2), 18 t (SW3), and 36 t (SW4) dry biosolids ha^{-1} annually, with three replicate plots per treatment. In the year 2022, month of June, soil samples (0–20 cm) were collected from the fields undergoing the biosolids treatment. After removing surface residues, five soil cores per plot were combined into a composite sample. Soil samples were placed in a container closed with dry ice and promptly sent to the laboratory. Upon arrival, they passed through a 2 mm mesh sieve and divided into three subsamples. The first part was stored at 4 °C to study soil biological indicators, and second part was air-dried and marked for soil physicochemical properties analyses. The rest was kept at-20 °C for DNA sequencing. Additional details regarding the long-term field experiment and soil properties are available in our previous study (Sun et al., 2024).

POT EXPERIMENT SETUP AND SAMPLING

Following the wheat harvest in the field, three treatments were selected for the pot experiment: C (Control), L (SW1: low biosolids application), and H (SW4: high biosolids application). The host plant was maize (*Zea mays* L.) cv. Zhengdan958. The AMF inoculum was *Funneliformis mosseae* (HK01, Glomeraceae), propagated on maize in a soil–sand substrate for four months, containing hyphae, colonized root fragments, and approximately 50 spores g^{-1}, and applied at 8 % (*w*/w) of the root chamber substrate. The study employed a full factorial design of 3 x 2 x 2, involving three biosolids application rates in the hyphal chamber (C, L, H) and two mycorrhizal treatments (AMF-inoculated and non-inoculated) applied to the root chamber. Additionally, the hyphal chamber under each biosolids application rate was further classified based on soil sterilization status, distinguishing between soils with native microbiota (unsterilized) and those subjected to sterilization. In total there were twelve treatments with 4 replicates, totalling 48 pots. The pot experiment was conducted in a greenhouse from April to June 2023 to evaluate the role of AMF in biosolids-amended soils, following the setup detailed in Sun et al. (2025). Briefly, a compartmentalized root-hyphae system was used, where maize was grown in the root chamber (RC), while the hyphal chamber (HC), separated by a 30 μm nylon mesh, allowed AMF hyphal extension without root penetration. After eight weeks of growth, the maize plants were harvested. The aboveground biomass was clipped for biomass measurement. The top 1 cm of soil in the hyphal chamber was removed to eliminate any surface contamination. The remaining soil was sieved through a 2 mm mesh and divided into three subsamples for subsequent analyses. The first part was stored at 4 ˚C to study soil biological indicators and second part was airdried and marked for soil physicochemical properties analyses. The rest was kept at-20 ˚C for DNA sequencing.

SOIL PROPERTIES MEASUREMENT

A penetrometer was used to measure soil physical indicators including surface hardness (SurfHard: 0–10 cm soil depth) and subsurface hardness (SubHard: 10–20 cm soil depth) in the field (Zhang et al., 2023). For soil chemical indicators, pH, EC, NH4+-N, NO3̄-N, Olsen P, available K, Fe, Mn, Zn, and Mg, organic carbon (SOC), permanganate oxidizable carbon (POXC), total phosphorus (TP) and total potassium were determined based on the protocol described in Zhang et al. (2023). Heavy metals including Cu, Zn, Cd, Pb, Cr, Ni, As, and Hg were quantified as described in Sun et al. (2024).

For biological indicators, soil proteins were measured spectropho- tometrically as described in Wright and Upadhyaya (1996). Soil respiration and N_2O emissions were measured by laboratory incubation with gas chromatographic analysis. In brief, 20 g of air-dried soil was adjusted to 60 % water-holding capacity, pre-incubated at 25 °C for 7 days, and then incubated in sealed 100 mL jars at 25 °C in the dark. After 24 h, headspace gas samples were collected using gas-tight syringes and analyzed for CO_2 and N_2O concentrations (GC 7890, Agilent Technologies, Santa Clara, CA). Each sample was measured in triplicate to ensure measurement accuracy. Emission rates were calculated based on the temporal increase in gas concentrations. Soil extracellular enzyme activities including α-1,4 Glucosidase (AG: sugar degradation), β-Cellubiosidase (BC: cellulose degradation), β-1,4 Glucosidase (BG: sugar degradation), β-1,4 Xylosidase (BX: hemicellulose degradation), β-1,4-Nacetylglucosaminidase (NAG: chitin degradation), leucine- aminopeptidas (LAP: nitrogen mineralization), acid phosphatase (ACP: phosphorus mineralization) and sulfatase (SUL: sulfate hydrolysis) were measured as described in Bell et al. (2013). The glomalin-related soil protein contents including total glomalin-related soil protein (TEG) and easily extractable glomalin (EEG) were determined following the method described by Wright et al. (1998).

SOIL DNA EXTRACTION, SEQUENCING AND BIOINFORMATIC ANALYSIS

For both field and pot experiment, the MagaBio Kit from Bioer Technology, China was used for total DNA extraction from 0.5 g of soil. The primers used for targeting the V4 region of bacteria for PCR amplification was from Walters et al. (2016). The primers used for amplifying the ITS region of fungi was from Op De Beeck et al. (2014), and the primers for a specific region of AM fungi were from Van Geel et al. (2014). The primers were synthesized by InvitrogenTM (Thermo Fisher Scientific Inc.). The sequencing reads were processed according to our previously published study (Sun et al., 2024). Taxonomic assignment was done with the SILVA v138, UNITE v8.0 and MaarjAM for bacteria, fungi and AM fungi, respectively (Green et al., 2022; Eshaghi et al., 2021; Öpik et al., 2010). The raw sequencing data were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1312049.

For the pot experiments, Shanghai Biozeron Biological Technology Co., Ltd. generated and sequenced metagenomic shotgun sequencing libraries. For each sample, the sequencing libraries were created using the TruSeq DNA Library Preparation Kit (catalogue no: FC-121-2001, Illumina, USA). An NGS platform in paired-end 150 bp (PE150) mode was used for sequencing of all the samples. The raw metagenomic sequencing data were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1312532.

SOIL HEALTH SCORING AND CALCULATING AND MULTIFUNCTIONALITY ASSESSMENT

Soil health encompasses the crucial role of soil in supporting not just food and fiber production, but also its broader contributions to ecological conservation and ecosystem services (Jia et al., 2025). In this context, soil multifunctionality (EMF) is gaining recognition as a key measure of soil's capacity to deliver a range of functions concurrently (Jia et al., 2023). For soil multifunctionality, maximum method ($f(x) = x_i/x_{max}$) was used to normalize the soil extracellular enzyme activities and soil respiration to a range of 0–1. All the normalized values were averaged to quantify soil multifunctionality index (Maestre et al., 2012).

For the soil health index assessment, firstly, a total dataset including TN, pH, AP, TP, AK, TK, SOC, EC, EEG, TEG, NH_4^+, NO_3^--N, Mg, Na, Ca, POXC, CO_2, N_2O, Cr, Cd, Pb, Hg, Ni, Cu, Zn, As, SurfHard, SubHard and protein was created. Then a Minimum Dataset (MDS) was established by selecting representative parameters from the Total Datasets (Li et al., 2022). Indicator redundancy was assessed using Pearson correlation analysis, followed by principal component analysis (PCA) to reduce dimensionality (Andres-Abellan et al., 2019). Principal components (PCs) with eigenvalues exceeding 1.0 were retained for further analysis due to their significant explanatory power (Yu et al., 2018). Within each significant PC, indicators exhibiting factor loadings ≥0.5 were initially grouped (Zhang et al., 2016). In cases where indicators exhibited high loadings across multiple PCs, they were assigned to the group with the highest factor loading to avoid redundancy.

Vector norm values were computed to quantitatively evaluate each indicator's contribution to soil quality representation (Shao et al., 2020). Indicators were pre-selected when their vector norm values reached at least 90 % of the maximum within each respective group (Huang et al., 2021). If two pre-selected indicators showed a Pearson correlation coefficient above 0.5, only the indicator with the higher norm value was retained for inclusion in the MDS; otherwise, both were included.

Then, selected indicators were standardized into dimensionless values ranging from 0 to 1, linear, non-linear, and CASH scoring approaches were applied (Mahajan et al., 2020). Linear scoring included three models: "more is better," "less is better" (applicable to sand and silt), and "optimal range" (applied specifically to soil pH, optimal between 5.5 and 6.5) (Huang et al., 2021). Eqs. (1) and (2) were used based on the indicator characteristic, with Eq. (2) employed for metrics favoring higher values or below optimal ranges, and Eq. (3) for metrics favoring lower values or above optimal ranges (Li et al., 2022).

$$S_L = \frac{x}{x_{max}} \qquad (1)$$

$$S_L = \frac{x_{min}}{x} \qquad (2)$$

$$s_{NL} = \frac{a}{1 + (x/x_\mu)^b} \qquad (3)$$

The non-linear method was implemented according to Eq. (3): where is the non-linear score, is the maximum score (set at 1), $x\mu$ represents the indicator value, is the mean value, and is the slope, defined as -2.5 for "more is better" and 2.5 for "less is better" scenarios (Li et al., 2022).

The calculated indicator scores were combined to produce a comprehensive soil health index (SHI) using both additive (Eq. 4) and weighted additive (Eq. 5) approaches:

$$SHI_A = \sum_{i=1}^{n} S_i/n \qquad (4)$$

$$SHI_w = \sum\nolimits_{i=1}^{n} (w_i \times s_i) \qquad (5)$$

Where SHI_A and SHI_W denote the additive and weighted additive soil health indexes, respectively; S_i represents the transformed indicator scores derived from linear or nonlinear methods; n is the total number of indicators within the minimal data set (MDS); w_i and indicates the normalized weight of each indicator, calculated based on the explained variance of corresponding principal components (PCs).

This study evaluated and compared four distinct SHIs, generated by integrating different scoring and aggregation methods: linear scoring with additive aggregation (SHI-LA), linear scoring with weighted additive aggregation (SHI-LWA), nonlinear scoring with additive aggregation (SHI-NLA), and nonlinear scoring with weighted additive aggregation (SHI-NLWA). Higher values of SHI indicated improved soil functionality and lower contamination or degradation levels. The strong correlation between the SHI values derived from the Total Dataset (TDS) and the Minimum Dataset (MDS) (Fig. S1) indicates that the MDS-based SHI provides a reliable and representative substitute for the TDS-based SHI.

STATISTICAL ANALYSIS

The R (version 4.1.0) was used to analyze the data. One-way ANOVAs were employed to evaluate the difference in soil multifunctionality and soil health index among different treatments. The 'microeco' package was used to generate the soil microbial cooccurrence networks (Liu et al., 2021), applying robust correlations (Spearman's $\rho > 0.60$) and FDR-corrected *p*-values <0.01 for network construction. Subsequently, the network properties were analyzed using the 'igraph' package. Nodes represent individual OTUs, while edges denote pairwise associations among them. To identify keystone taxa, we calculated two topological metrics: within-module connectivity (Zi) and among-module connectivity (Pi). Based on their Zi and Pi values, nodes were categorized as network hubs (Zi > 2.5, Pi >0.62), module hubs (Zi > 2.5, Pi <0.62), connectors (Zi ≤ 2.5, Pi >0.62), or peripherals (Zi ≤ 2.5, Pi ≤0.62; Olesen et al., 2007). Partial least squares path modeling (PLS-PM) was used to explore how soil properties (pH and SOC) influence the links between microbial diversity and community composition (including bacteria, fungi, and AM fungi), and multifunctionality as well as soil health index by R package 'plspm'. Model performance was evaluated using the goodness-of-fit index.

Results

EFFECTS OF BIOSOLIDS AMENDMENTS ON SOIL MULTIFUNCTIONALITY AND SOIL HEALTH INDICES

Biosolids amendments significantly influenced soil multifunctionality and soil health indices (Fig. 1). Soil multifunctionality was significantly higher in treatments with higher biosolids application rates, particularly in SW3 and SW4 (Fig. 1A). The enzyme activities of ACP, SUL, AG, BG, BC, BX were highest in the SW3 treatment (Table S1), and the activities of LAP and NAG were highest in SW4 treatment (Table S1). For soil health indices, soil parameters including pH, TN, TP, Ca, Ni and N_2O emission were selected to quantify soil health index. Among the different treatments, there was no significant difference in soil pH and N_2O emissions. Soil TN and TP significantly increased after biosolid applications, particularly in SW3 and SW4. Soil Ca content significantly increased in SW4 while no significant difference among other treatments were observed. Biosolids application led to substantial improvements in different soil properties (Table S1). SHI-LA significantly increased in all biosolids-treated soils, with no significant differences among treatments (Fig. 1B). In contrast, SHI-LWA exhibited a significant increase in SW3 and SW4 (Fig. 1C). In terms of SHI-NLA, a significant improvement was observed in SW3 and SW4, with SW4 showing the highest values (Fig. 1D). SW1 and SW2 exhibited moderate increases but were not significantly different from the control. SHI- NWLA displayed the most pronounced variations, with a clear increase from SW1 to SW4 (Fig. 1E). The lowest values were found in control, while SW3 and SW4 significantly outperformed SW1 and SW2. The correlation matrix provides insights into the relationships between SMF and various soil health indices (Fig. 2). SMF exhibited significant positive correlations with all SHI (Fig. 2).

EFFECTS OF SOIL MICROBIAL DIVERSITY ON SOIL MULTIFUNCTIONALITY

There was significant positive correlation of SMF with bacterial and AM fungal richness but not to fungal richness (Fig. 3). As evident in the Mantel test, soil bacterial and AM fungal community dissimilarity were positively correlated to multifunctionality dissimilarity. Soil fungal community dissimilarity did not exhibit a significant correlation with multifunctionality dissimilarity (Fig. 3). A co-occurrence network for bacteria and fungi was made to assess the impact of biosolids amendments on their interactions (Fig. 4A). The network analysis revealed a total of 18 keystone taxa, comprising 7 bacterial, 5 fungal and 6 AM fungal OTUs (Fig. 4B). As presented in Table S2, Glomerales dominated the AM fungal OTUs, while Acidobacteriota and Proteobacteria were the major bacterial OTUs. Ascomycota and Glomeromycota dominated the fungal OTUs. Among the keystone, only AM fungal OTU64 and bacterial OTU372 and OTU40 were positively related to SMF and soil health index. The result of PLS-PM showed soil factors including pH and SOC had positive effects of AM fungal, bacterial diversity and community composition while they had negative effects on fungal diversity and community composition. Bacteria and AMF had positive effects on SMF which further contributed to soil health index. In addition, we found that AMF had, indirectly, positive effect on SMF (Fig. 5).

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1.1. *Soil multifunctionality influenced by AMF inoculation*

The pot experiment demonstrated that AMF inoculation, particularly under high biosolids application, significantly reduced Cu and Zn concentrations in the soil (Table 1). Moreover, soil multifunctionality and plant biomass were also significantly influenced by biosolids application rates and AMF inoculation (Fig. 6A; Fig. S2). The LAM and HAM treatments exhibited significantly higher soil multifunctionality compared to the CAM and CNM treatments, suggesting that AMF inoculation positively influenced soil health and ecosystem functions. Functional genes of carbon (Fig. 6B), nitrogen (Fig. 6C), and sulfur (Fig. 6E) cycling gene abundances did not show significant variation across treatments. However, AMF inoculation significantly increased phosphorus cycling gene abundance in no-biosolids application soil (Fig. 6D).

Fig. 1. *The effects of biosolids application on soil multifunctionality (A), and soil health index (B, C, D, F) in soils with varying rates of biosolids application. Different lowercase letters indicate significant differences (p < 0.05) among different treatments.*

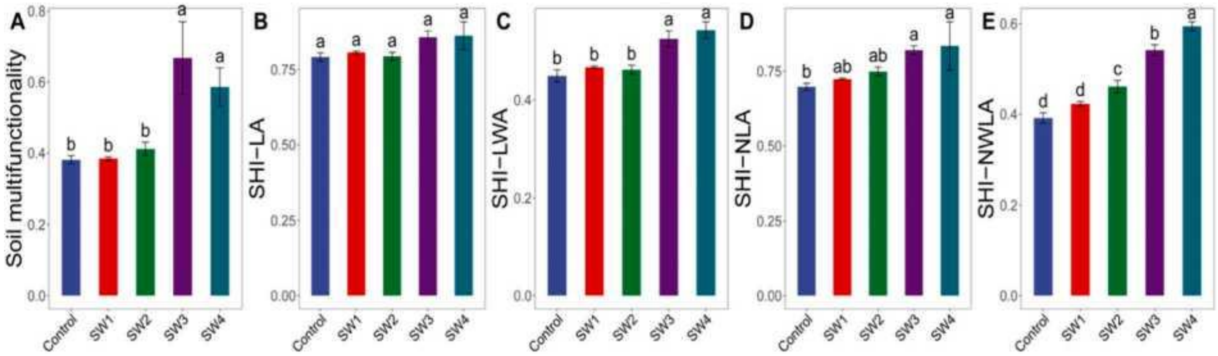

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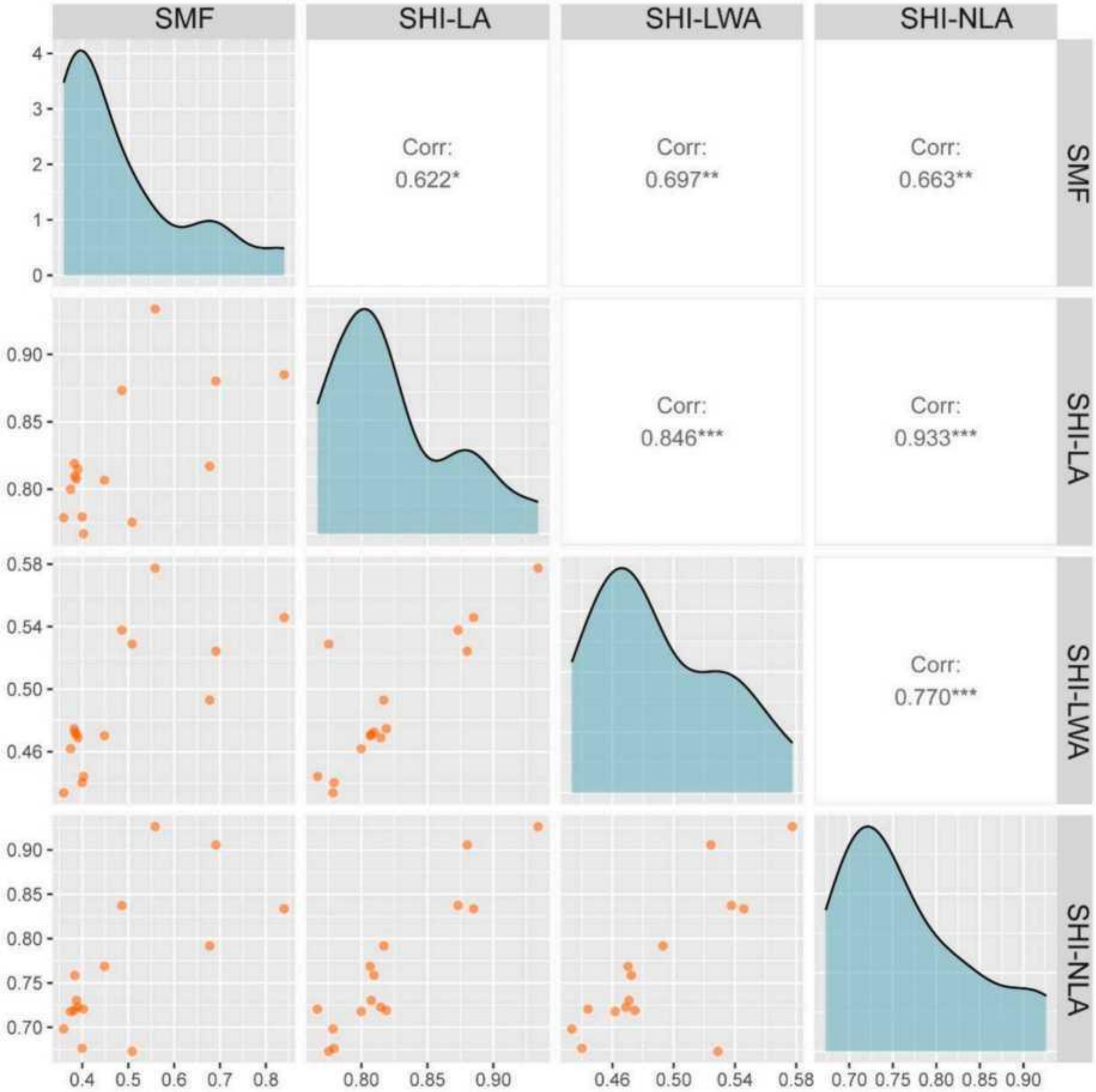
Fig. 2**. The relationship between soil multifunctionality and different soil health indices. Significance is indicated by p < 0.05*, p < 0.01** and p < 0.001*



Furthermore, correlation analyses demonstrated significant positive correlations between soil multifunctionality and maize biomass (Fig. 6F; $R = 0.49$, $p = 0.0025$), carbon cycling genes (Fig. 6G; $R = 0.59$, $p = 0.011$), nitrogen cycling genes (Fig. 6H; $R = 0.52$, $p = 0.0219$), phosphorus cycling genes (Fig. 6I; R = 0.52, $p = 0.013$), and sulfur cycling genes (Fig. 6J; $R = 0.17$, $p = 0.0022$).

Gene abundances associated with C, N, P, and S cycling exhibited distinct variation among treatments. As evident in Fig. 7, genes involved in carbon fixation and decomposition showed increased abundance in HAM and LAM treatments in comparison to the control. Key genes associated with nitrification, organic nitrogen metabolism, and denitrification were significantly enriched in AMF inoculated soils (Fig. 7). The highest expression levels were observed in the HAM and LAM treatments, indicating that biosolids application and AMF inoculation promoted nitrogen transformation processes. Notably, genes related to dissimilatory nitrate reduction (DNRA) were reduced in LNM and HNM treatments Regarding the phosphorus cycle, genes associated with inorganic phosphorus solubilization, and organic phosphorus mineralization were more abundant in the HAM and LAM treatments (Fig. 7). For the sulfur cycle, genes associated with sulfide oxidation, dissimilatory sulfate reduction, and sulfur disproportionation exhibited higher abundance in HAM and LAM treatments than in the control (Fig. 7). Overall, AMF inoculation enhanced microbial functional potential for

key biogeochemical cycling processes, including carbon degradation, nitrogen transformation, phosphorus solubilization, and sulfur reduction in biosolids application soils.

Discussion

APPROPRIATE APPLICATION RATES OF BIOSOLIDS ENHANCE SOIL HEALTH AND MULTIFUNCTIONALITY

Biosolids containing abundant organic matter and essential nutrients such as nitrogen, phosphorus, and potassium, have gained attention as a potential soil amendment with the growing emphasis on sustainable agricultural practices (Elgarahy et al., 2024). In our study, we found that long-term application of biosolids significantly improved soil health and multifunctionality. In the present study, SHI and SMF are largely complementary. They only share one common indicator—soil respiration—while other parameters represent distinct aspects: SMF mainly represents the biological and ecological functioning of soils, emphasizing processes such as carbon and nutrient cycling. In contrast, SHI is a broader composite indicator that additionally incorporates physicochemical and contamination-related parameters. Therefore, SHI and SMF are partly overlapping but largely complementary indicators that together provide a comprehensive evaluation of soil status under long-term biosolids application. We observed that biosolids application has been previously reported to improve soil organic matter, and soil structure by increasing aggregation, water retention and aeration (Elgarahy et al., 2024; Lu et al., 2012). This is particularly beneficial for soils with poor physical properties, such as sandy or compacted soils. The improved soil structure can enhance water infiltration and regulate water flow, thereby reducing erosion. Additionally, the increased organic matter content contributes to carbon sequestration, mitigating climate change (Wijesekara et al., 2021; Wijesekara et al., 2017). The nutrients in biosolids, such as nitrogen and phosphorus, provide essential elements for plant growth, reducing the need for synthetic fertilizers (Rigby et al., 2016; Lu et al., 2012). This can not only promote plant yield but also minimizes the environmental impact associated with fertilizer runoff (Rigby et al., 2016; Pritchard et al., 2010), and negate the costs incurred on fertilizer applications in agricultural fields. Furthermore, biosolids application can increase soil microbial diversity and activity, which play a crucial role in promoting nutrient cycling and organic matter decomposition (Pathma and Sakthivel, 2012). Enhanced soil microbial activity can lead to improved soil fertility and resilience, contributing to the overall health and multifunctionality of the soil ecosystem.

While the benefits of biosolids application are evident, excessive use can lead to soil degradation (Gianico et al., 2021). In our study, it was found that soil health was reduced in treatment SW4. This could be due to the heavy metals accumulation as they are often present in biosolids (Sun et al., 2024). Over time, these metals can build up in the soil, posing risks to plant health, soil organisms, and ultimately human health through the food chain. Heavy metals can disrupt soil microbial communities, reducing their diversity and activity (Abdu et al., 2017).

This can negatively impact nutrient cycling and organic matter decomposition, compromising soil health and multifunctionality (Alengebawy et al., 2021; Cardoso et al., 2013). Additionally, heavy metals can be toxic to plants, inhibiting root growth and reducing crop yields (Bharti and Sharma, 2022). In severe cases, heavy metal contamination can render soils unsuitable for agricultural use (Wu et al., 2022; Kumar et al., 2019). Therefore, it is crucial to determine the optimal application rates and monitor soil conditions to maximize the benefits of biosolids while minimizing potential risks.

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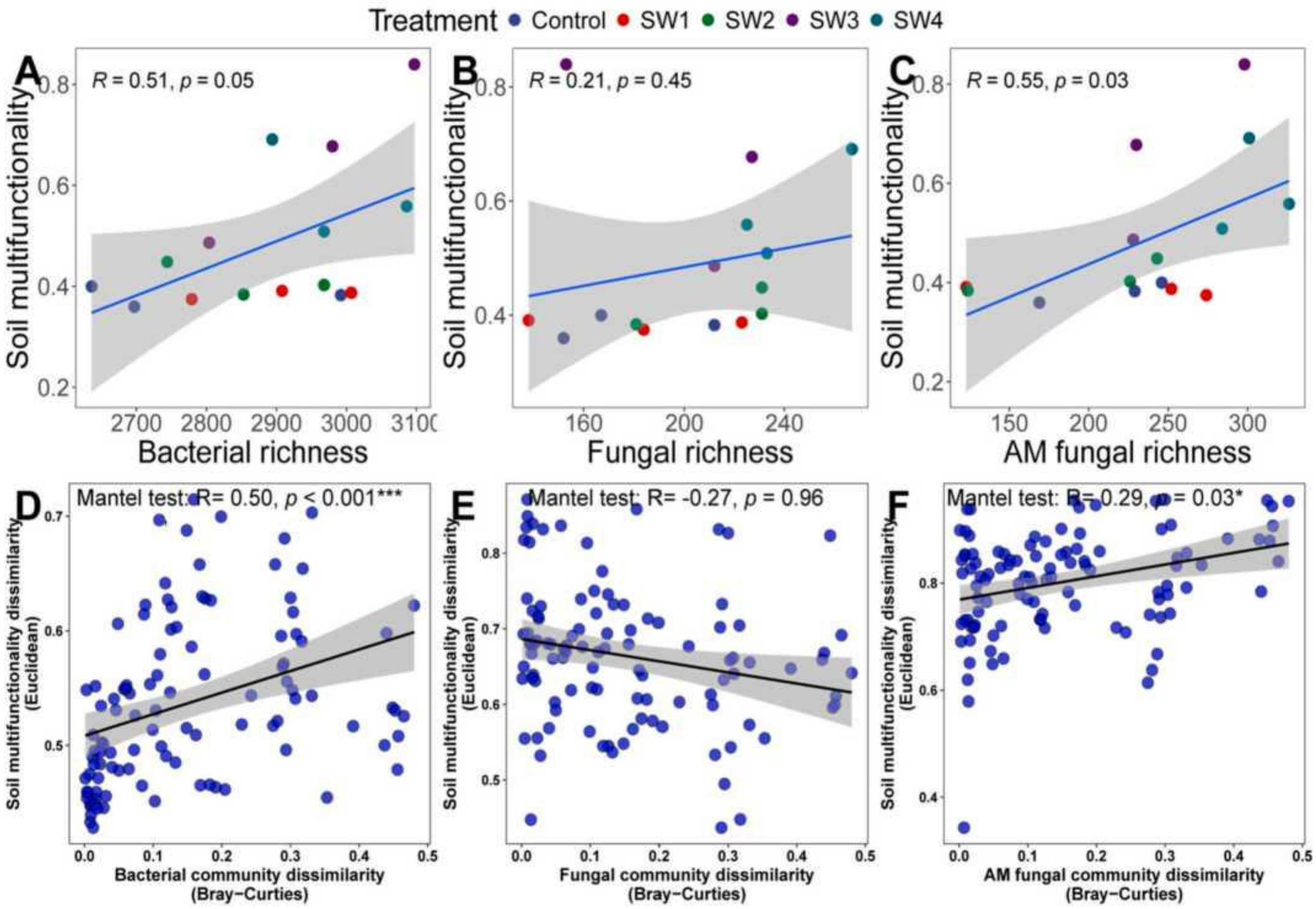
***Fig. 3**. The relationship between soil bacterial, fungal, AM fungal richness and community dissimilarity with soil multifunctionality.*



BACTERIA AND AMF AS KEY DRIVERS OF MULTIFUNCTIONALITY IN SOILS TREATED LONG-TERM WITH BIOSOLIDS

In our study, we found that bacterial and AM fungal diversity were positively related to soil multifunctionality (Fig. 3). Bacteria are among the most abundant and diverse microorganisms in soil, and their activity is essential for maintaining soil health. In soils amended with biosolids, bacteria play a key role in decomposing organic matter and releasing nutrients such as nitrogen and phosphorus (Ahmad et al., 2019; Zhu et al., 2019). This process not only enhances soil fertility but also supports plant growth. Moreover, certain bacterial species have the ability to immobilize heavy metals, reducing their bioavailability and toxicity. For example, *Pseudomonas* and *Azotobacter* have been reported to produce extracellular polymeric substances (EPS) that can bind heavy metals, intercepting them from entering the soil solution (Zeng et al., 2020; Gupta and Diwan, 2017).

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Fig. 4**. Co-occurrence network of microbial interactions (A), keystone taxa distribution network (B), and relationship between keystone taxa abundance and soil multifunctionality and soil health index (C) in soils with varying rates of biosolids application. Significance is according to p < 0.05*, p < 0.01** and p < 0.001.*

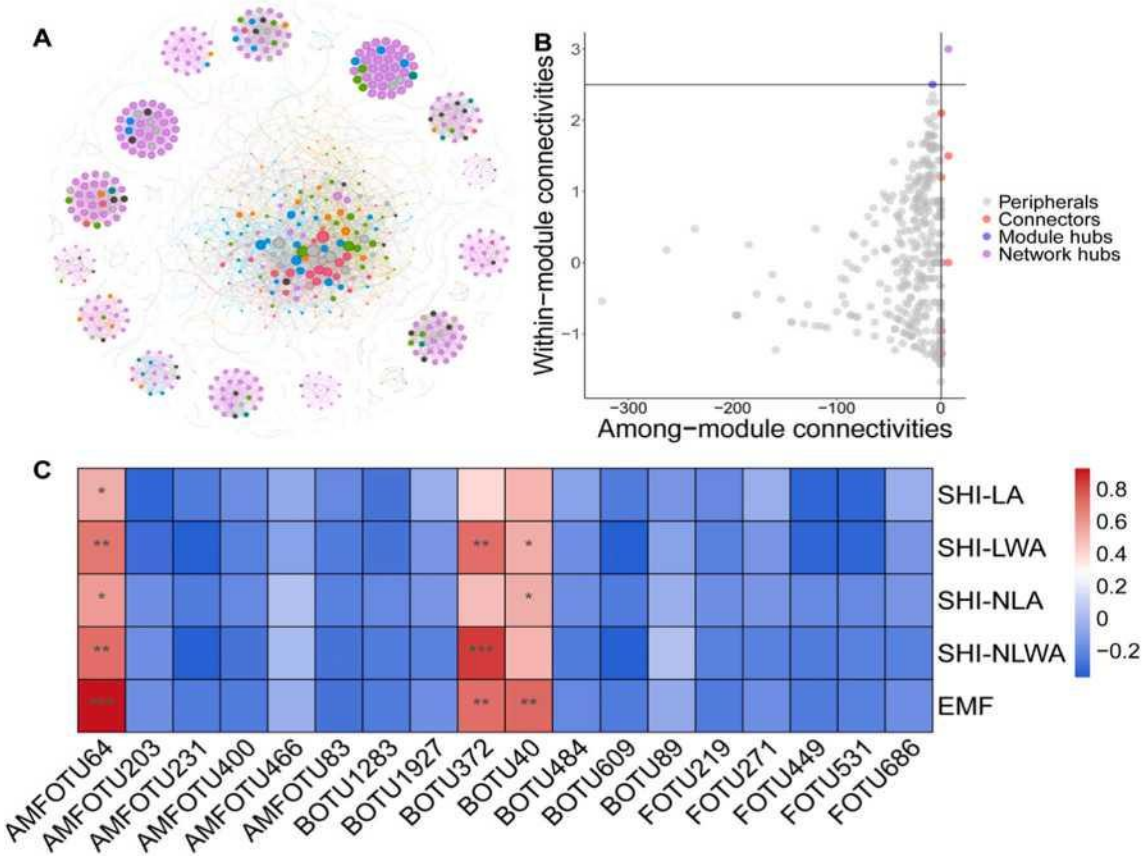
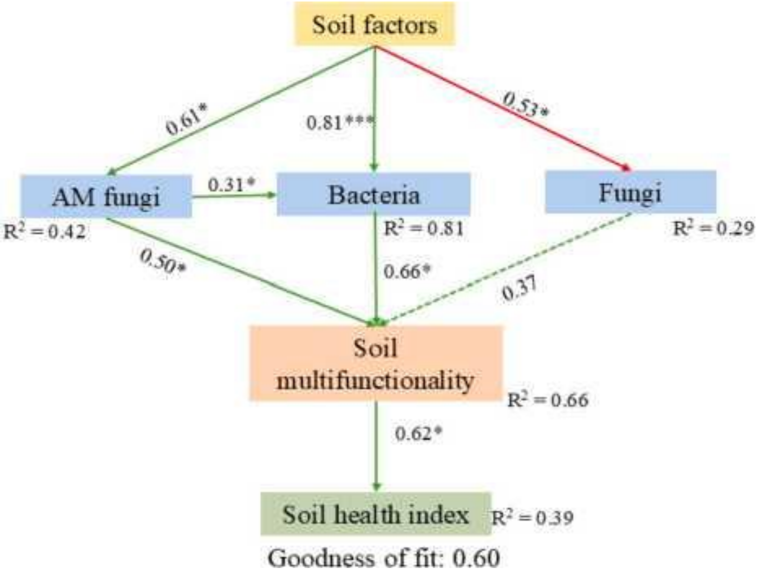


***Fig. 5**. Partial least squares path analysis for the effects soil parameters on the relationship between soil microbial diversity and soil multifunctionality as well as soil health index.*



Note: * indicates $p < 0.05$; ** indicates $p < 0.01$, *** indicates $p < 0.001$, respectively. Green and red line indicate positive and negative relationships, respectively. Continuous and dashed lines indicate significant and nonsignificant relationships, respectively. R2 denotes the proportion of variance explained.

Moreover, *Streptomyces rimosus* has Been reported to transform heavy metals into less toxic forms via processes such as oxidation, reduction, or methylation (Etesami, 2018; Das et al., 2016). These mechanisms contribute to the resilience of bacterial communities in biosolids-amended soils, allowing them to maintain their functions despite the presence of heavy metals. Further, AMF can form symbiotic relationships with plant roots, extending their hyphae into the soil to access nutrients and water for plants. In return, the fungi receive carbohydrates from the plants (Wahab et al., 2023; Wipf et al., 2019). This mutualistic relationship could be particularly beneficial in biosolids-amended soils, where AMF could enhance plant uptake of nutrients such as phosphorus, which is often present in biosolids in forms that are not readily available to plants (Table 1). AMF also plays a crucial role in mitigating the effects of heavy metals. The hyphae of AMF can bind heavy metals, reducing their translocation to plant roots (Riaz et al., 2021; Bhantana et al., 2021). Additionally, AMF have been reported to improve plant tolerance to heavy metals by enhancing antioxidant enzyme activity and reducing oxidative stress (Riaz et al., 2021; Dhalaria et al., 2020). These mechanisms suggest that AMF are an important component of soil microbial communities in biosolids- amended soils, contributing to soil multifunctionality and plant health (Table 1; Fig. S1; Fig. 6).

Another concern associated with biosolids application is the presence of antibiotics, which can enter the soil through the application of biosolids derived from wastewater treatment plants (Elgarahy et al., 2024; Clarke and Smith, 2011). Antibiotics can disrupt soil microbial communities, reducing their diversity and activity (Cycon et al., 2019; Caracciolo et al., 2015). However, both bacteria and AMF appear resilient to antibiotics, allowing them to maintain their functions in biosolids-amended soils (Sun et al., 2025; Santaella and Plancot, 2020). For example, some bacteria possess antibiotic resistance genes, enabling them to survive in the presence of antibiotics (Jian et al., 2021). These bacteria can continue to perform essential functions such as nutrient cycling and organic matter decomposition. Similarly, AMF may tolerate certain antibiotics, maintaining their symbiotic relationships with plants and contributing to soil health (Wahab et al., 2023; Fall et al., 2022). Therefore, bacteria and AMF are key drivers of multifunctionality in soils with long-term biosolids application. Their roles in nutrient cycling, heavy metal immobilization, and plant growth promotion are essential for maintaining soil health and productivity.

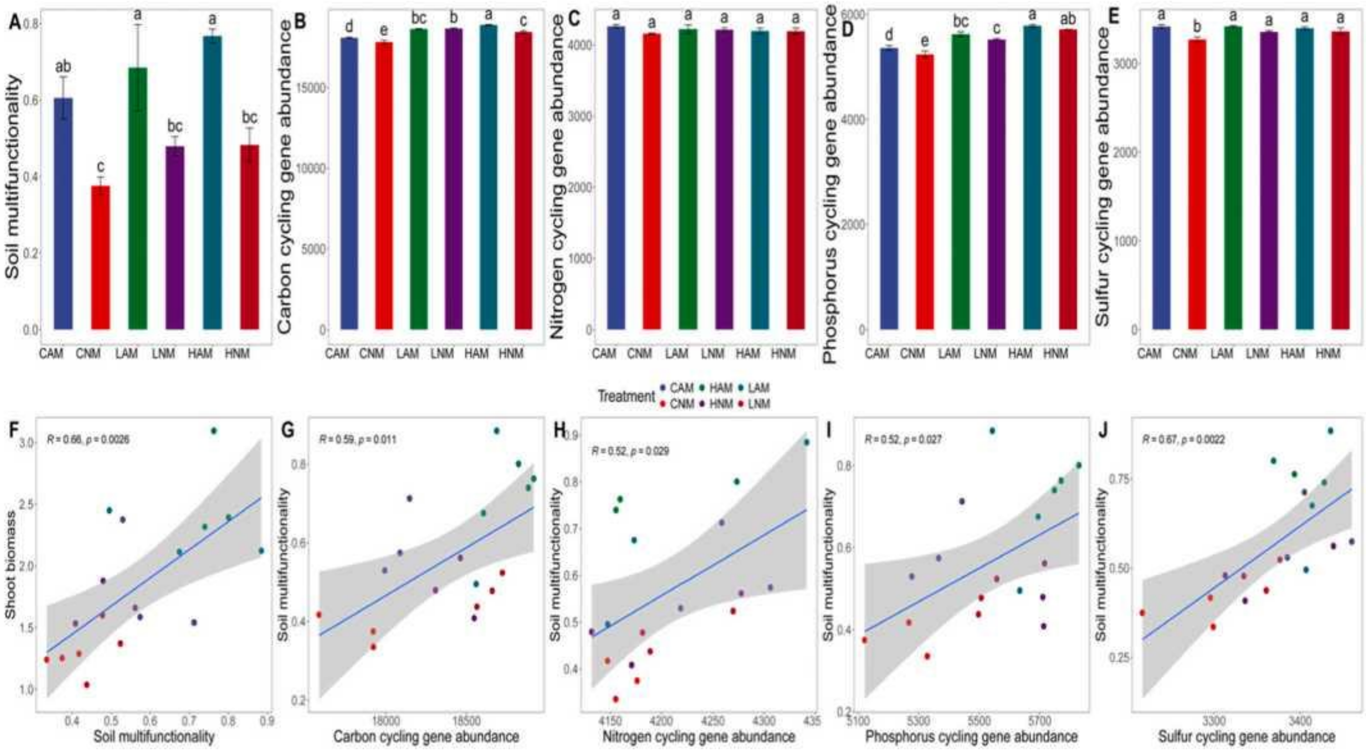
AMF MITIGATION OF HEAVY METAL STRESS AND ENHANCEMENT OF SOIL MULTIFUNCTIONALITY IN BIOSOLIDS-AMENDED SOILS

The long-term application of biosolids in agricultural soils can lead to the accumulation of heavy metals (Table 1, Table S1), posing a threat to soil health and plant growth (Gianico et al., 2021; Kumar et al., 2017). Bioaugmentation with AMF serves as a sustainable measure to increase plant resistance to heavy metal stress while suppressing the environmental spread of these contaminants (Sun et al., 2025). In this study, AMF incubation could mitigate heavy metal stress and enhance plant biomass and soil multifunctionality in biosolids-amended soils (Fig. 5; Fig. S2). AMF have been previously reported to play a crucial role in reducing the bioavailability and toxicity of heavy metals in biosolids- amended soils. Safronova et al. (2011) suggested that one of the primary mechanisms could be through the binding of heavy metals to fungal cell walls and hyphae.

Table 1*The effect of AMF inoculation on soil heavy metals contents in soils with different biosolids application rates*

Biosolids	AMF	Microbiome	Cu (mg/kg)	Zn (mg/kg)	Cd (mg/kg)	Pd (mg/kg)	Hg (mg/kg)
Control	AM	Sterilized	25.68 ± 0.6 fg	82.95 ± 0.18ef	0.2 ± 0.01c	27.29 ± 0.34a	0.08 ± 0.01c
Control	AM	Unsterilized	24.44 ± 0.25 g	80.44 ± 0.41f	0.19 ± 0c	26.59 ± 0.16ab	0.12 ± 0.03c
Control	NM	Sterilized	25.17 ± 0.53 fg	84.42 ± 0.96e	0.19 ± 0c	26.62 ± 0.23ab	0.1 ± 0.01c
Control	NM	Unsterilized	24.83 ± 0.35 fg	83 ± 0.89ef	0.2 ± 0c	26.53 ± 0.21ab	0.14 ± 0.02c
Low	AM	Sterilized	27.86 ± 0.95de	97.89 ± 1.1d	0.21 ± 0c	25.58 ± 0.78c	0.32 ± 0.08bc
Low	AM	Unsterilized	26.61 ± 0.26ef	98.25 ± 0.48d	0.2 ± 0.01c	26.29 ± 0.07bc	0.19 ± 0.01bc
Low	NM	Sterilized	28.78 ± 0.95d	102.82 ± 0.59c	0.2 ± 0c	26.02 ± 0.1bc	0.19 ± 0.01bc
Low	NM	Unsterilized	28.43 ± 0.72de	102.5 ± 0.33c	0.2 ± 0c	26.49 ± 0.01abc	1.54 ± 1.11a
High	AM	Sterilized	44.7 ± 0.53b	204.46 ± 2.31b	0.29 ± 0ab	24.3 ± 0.06d	1.08 ± 0.1abc
High	AM	Unsterilized	42.66 ± 0.29c	201.93 ± 0.32b	0.28 ± 0b	24.14 ± 0.04d	1 ± 0.09abc
High	NM	Sterilized	46.66 ± 0.22ab	215.62 ± 0.06a	0.3 ± 0.01a	24.16 ± 0.16d	1.26 ± 0.16ab
High	NM	Unsterilized	46.78 ± 0.76a	213.44 ± 0.21a	0.29 ± 0.01ab	24.28 ± 0.09d	0.84 ± 0.04abc

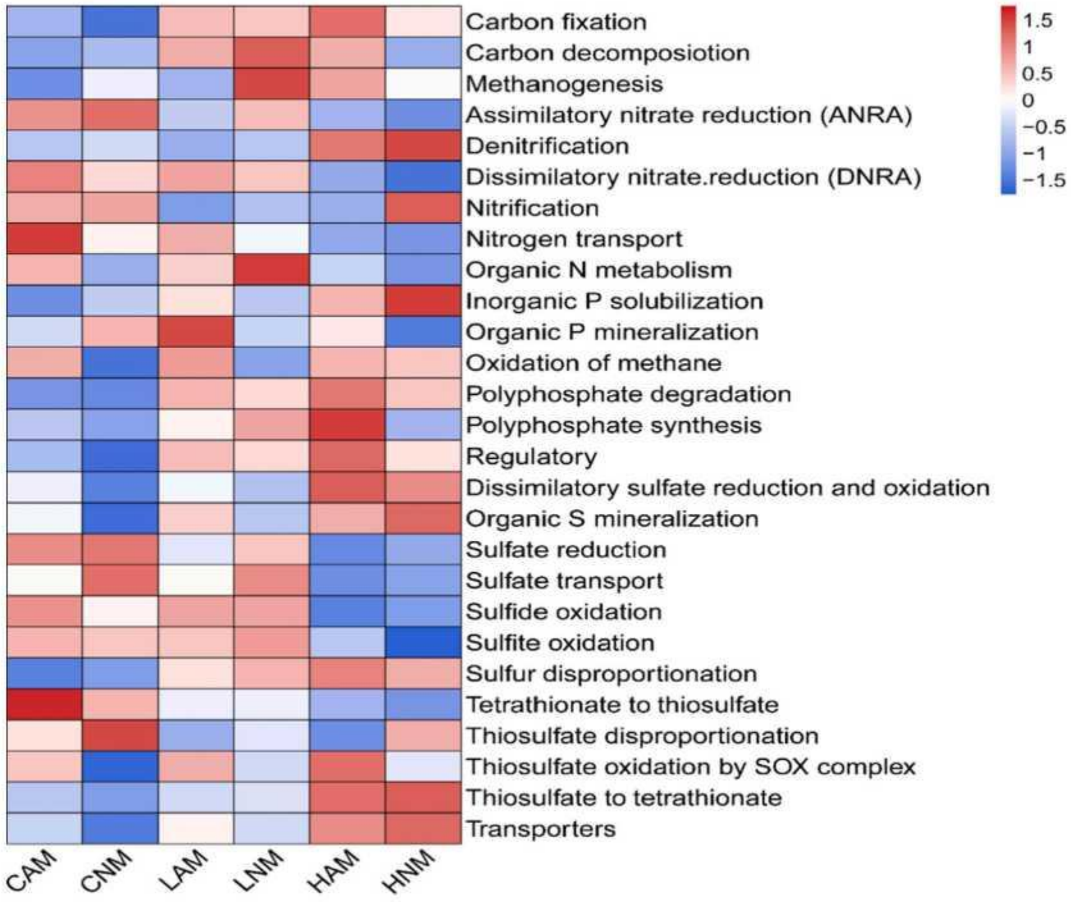
.***Fig. 6.*** *The influence of AMF inoculation on soil multifunctionality, and carbon, nitrogen, phosphorus and sulfur cycling gene abundance in soils with varying rates of biosolids application.Lowercase letters indicate significant differences (p < 0.05) between the treatments.*



The chitin and glomalin in AMF cell walls have a high affinity for heavy metals, effectively immobilizing them and preventing their uptake by plant roots (Sun and Shahrajabian, 2023; Riaz et al., 2021). Moreover, AMF modulate the chemical speciation of heavy metals in the soil, for instance, through the secretion of organic acids that reduce metal solubility and biological availability (Wang et al., 2022b). Furthermore, AMF can enhance plant tolerance to heavy metals through several mechanisms. Firstly, they improve nutrient uptake, particularly phosphorus, which is essential for plant growth and stress tolerance (Bhantana et al., 2021; Begum et al., 2019). Secondly, AMF can induce the production of antioxidant enzymes in plants, such as superoxide dismutase and catalase (Zou et al., 2021), which could neutralize reactive oxygen species (ROS) generated by heavy metal stress, reducing oxidative damage to plant cells (Rizwan et al., 2018; Liang et al., 2017).

In addition, AMF could contribute to soil multifunctionality by enhancing nutrient cycling and improving soil structure, promoting plant growth (Bhantana et al., 2021). In our study, we found that AMF inoculation significantly increased the functional gene abundance involved in carbon, phosphorus, and sulfur cycling processes (Fig. 6). The enhanced abundance of functional genes related to carbon, phosphorus, and sulfur cycling suggests an increased microbial capacity for organic matter decomposition and nutrient mineralization, which supports higher soil multifunctionality. These functional shifts align with the observed improvements in soil enzyme activities and plant performance, indicating that AMF inoculation promotes nutrient cycling through both biochemical and genetic pathways. The hyphal networks of AMF extend into the soil, increasing the surface area for nutrient absorption and facilitating the transfer of nutrients to plants (Riaz et al., 2021; Wipf et al., 2019). This process enhances soil fertility and supports sustainable agricultural production. Furthermore, previous studies have indicated that AMF could improve soil aggregation by producing glo- malin, which could enhance water infiltration, reduce erosion, and increase the resilience of soils to environmental stresses (Hartmann and Six, 2023; Keesstra et al., 2018).

Fig. 7. *The influence of AMF inoculation on specific types of soil carbon, nitrogen, phosphorus, and sulfur cycling gene abundance in soils treated with different biosolids concentrations. The colors represent Z-score standardized abundance values, with blue indicating lower and red indicating higher abundance.*



Therefore, AMF plays a vital role in mitigating heavy metal stress and enhancing soil multifunctionality in biosolids-amended soils. By leveraging the benefits of AMF, it is possible to maximize the positive impacts of biosolids application while minimizing the risks associated with heavy metal contamination. Future research should focus on optimizing AMF inoculation strategies and understanding their interactions with other soil microorganisms to further enhance soil health and productivity.

Conclusion

Overall, long-term application of biosolids at optimal rates significantly enhances soil health and multifunctionality. Soil AM fungal and bacterial diversity and communities showed positive correlations with soil multifunctionality. The abundance of AM fungal and bacterial keystone taxa was positively correlated to soil multifunctionality. The pot experiments corroborated with the results observed for the analyses of soil from the field, and showed that AM fungal inoculation significantly increased soil multifunctionality and the potential for carbon, nitrogen, phosphorus, and sulfur cycling. The carbon, nitrogen, phosphorus and sulfur cycling genes were positively related to multifunctionality. Further, AMF-bioaugmented soils showed lower accumulation of heavy metals. These findings highlight the potential of combining biosolids application with AM fungal bioaugmentation as a sustainable agricultural practice to improve soil fertility and ecosystem functioning while reducing the harmful impact of heavy metals accumulation that biosolids-applied soils frequently encounter. However, careful management with respect to application rates is essential as overuse of biosolids reduces soil health and multifunctionality while increasing the risk of heavy metal accumulation. Despite these insights, several limitations should be acknowledged. The laboratory incubation and pot experiments were conducted under controlled conditions, which may not fully capture field-scale heterogeneity and long-term environmental variability. In addition, the mechanisms linking microbial functional genes to ecosystem processes were inferred rather than directly verified through approaches such as isotopic tracing or meta- transcriptomics. Future studies should therefore emphasize field-based validation across diverse soil types and climatic regions, and integrate multi-omics analyses to link microbial functions with soil processes and ecosystem multifunctionality more precisely. This study provides a strategy for optimizing biosolids use in agriculture for maintaining soil health and multifunctionality while mitigating the risk of heavy metals accumulation for long-term sustainability.

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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.

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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.apsoil.2025.106615.

DATA AVAILABILITY

Data will be made available on request.

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