



Heavy metals drive microbial community assembly process in farmland with long-term biosolids application

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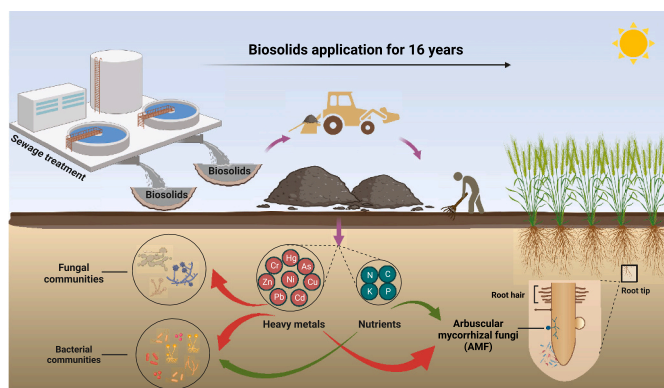
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HIGHLIGHTS

- Bacteria, fungi, and AM fungi responded differently to biosolids application.
- SOM, Cu, Pb, Cd, Zn, and Hg affected bacterial and AM fungal community.
- Fungal communities were only affected by heavy metals (Cr, Ni, and As).
- Deterministic processes shaped fungal and AM fungal community assembly.
- Stochastic processes drive bacterial community assembly.

GRAPHICAL ABSTRACT



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ABSTRACT

Biosolids are considered an alternative to chemical fertilizers due to their rich nutrients. However, long-term biosolids application can lead to heavy metals accumulation, which severely affects soil microbial community compositions. The factors influencing soil microbial community assembly were explored under a 16-year long-term experiment with biosolids applications. Our results indicated that biosolids application significantly increased fungal richness while not for bacterial and arbuscular mycorrhizal (AM) fungal richness. Besides, biosolids application significantly affected soil bacterial, fungal compositions and AM fungal community. Soil microorganisms were clustered into different modules with bacterial and AM fungal communities were affected by both organic matter and heavy metals, while fungal communities were affected by heavy metals (Cr, Ni, and As). The soil bacterial community assembly was dominated by stochastic processes while the fungal and AM fungal community assemblies were mainly driven by deterministic processes. Random forest analysis showed

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that heavy metals were identified as major drivers (Hg, Cu, Cd, and Zn for bacteria, Pb and Cr for fungi, and As and Ni for AM fungi) of the community assembly process. Overall, our study highlights the significant role of heavy metals in shaping microbial community dynamics and gives a guide for controlling biosolids application.

1. Introduction

Biosolids are mainly composed of organic matter, which can serve as a valuable nutrient source for plants, especially in terms of nitrogen (N), phosphorus (P), and potassium (K) [23,29]. Incorporating biosolids into soils could improve soil properties such as soil aeration, organic matter, and nutrient contents [2,3]. In addition, biosolids application could increase soil respiration, improve soil microbial activity, elevate microbial biomass, and alter microbial community compositions [19,55]. However, repeated or long-term application of biosolids might potentially lead to heavy metals accumulation in soils and plants, adversely affecting soil biology [30,38]. In this instance, the presence of heavy metals has been linked to a decrease in soil microbial activity, abundance, and diversity, affecting plant growth, and eventually crop yield [9,42]. However, the co-existence of the potentially positive effects of nutrients and the adverse effects of heavy metals from the application of biosolids make it complex to understand their actual impacts on soil ecosystem [31].

Soil microbial communities are susceptible to alternation following the application of biosolids, mainly as the consequence of soil properties changes [48]. However, soil microorganisms may respond differently to biosolids due to their various physiological and ecological traits [58]. Soil nutrients and organic matter contents changes may influence the equilibrium between oligotrophic microorganisms, which grow slowly but exhibit a relatively high carbon use efficiency, and saprotrophic microorganisms, which grow quickly but demonstrate a lower carbon use efficiency [1,13]. It has been reported that adding biosolids has the potential to change microbial life strategy, leading to a transition of microbial community from chemolithotrophic to heterotrophic, primarily due to the enhanced soil carbon content [53]. Furthermore, different microorganisms respond differently to heavy metal stress due to their different tolerance levels to those heavy metals [62]. For example, prolonged exposure to heavy metals could cause an increase in the population of stress-tolerant microbes, such as *Firmicutes*, and *Actinobacteria* [22]. Conversely, the presence of heavy metals often leads to a decrease in the population of sensitive or low-tolerance microbes [25,36]. Therefore, the changes in soil nutrients, carbon content, and heavy metals resulting from the application of biosolids can impact diverse microbes occupying specific niches, engaging in various biogeochemical cycles, and contributing to ecosystem functions [40]. Understanding the interplay among heavy metals, soil nutrients, and carbon content, as well as their comprehensive effects on microbial communities is crucial for improving soil health and maintaining ecosystem functions.

Soil microbial assembly processes are crucial in establishing community composition, directly impacting microbial functions within the ecosystem [32]. Deterministic processes govern the assembly of communities, relying on species-specific traits, interactions among organisms, and environmental factors like soil moisture, pH, and salinity [46]. They could result in community structures that exhibit distinct patterns of phylogenetic relatedness and turnover [47]. In contrast, stochastic processes are expected to produce phylogenetic community structures that are not significantly different from randomly assembled communities [47]. However, studies of microbial assembly processes in soils with long-term application of biosolid fertilizers are limited, and the effects of soil environmental parameters on these processes remain unclear. In this context, a previous study has revealed that the determinant roles of stochastic processes to bacterial and archaeal communities decreased with the increase of heavy metals [62]. Nevertheless, the driving process of heavy metals was reported to shift from stochastic to deterministic and finally changed to stochastic again [63]. The

variability in microbial community responses adds to the puzzle of understanding the ecological processes that govern microbial assembly in soils subjected to the long-term application of biosolids.

The objectives of our study were to determine the effects of the long-term application of biosolids on soil microbial community structure and assembly dynamics while identifying the key factors influencing soil microbial community. This study hypothesizes that (i) the biosolids applications could improve soil microbial diversity due to increased nutrient and organic matter contents, and (ii) the input of heavy metals resulting from the application of biosolids would induce a shift of soil microbial community and act as the primary drivers of microbial community assembly process.

2. Materials and methods

2.1. Site description and experiment design

The long-term experiment took place at Changping Soil Quality National Observation and Research Station in Beijing, China, situated at coordinates 40°10'N, 116°14'E. This site experiences an annual rotation of winter wheat and summer maize and is classified as flavor-aquic soil. This area experiences a sub-humid continental monsoon climate characterized by an average annual temperature (MAT) of 12.4 °C and an average yearly precipitation (MAP) of 628.9 mm.

The field experiment has been running since 2006 and comprises five triplicate treatments: (1) only chemical fertilization (Control), (2) 4.5 t ha⁻¹ biosolids with chemical fertilization (SW1), (3) 9 t ha⁻¹ biosolids with chemical fertilization (SW2), (4) 18 t ha⁻¹ biosolids with chemical fertilization (SW3), and (5) 36 t ha⁻¹ biosolids with chemical fertilization (SW4). The detailed information regarding fertilization can be found in Table S1. The biosolids utilized in this study underwent an air-drying process before their application in October 2021. The application procedure was akin to that of chemical fertilizers. The biosolids for this study were sourced from municipal domestic sewage, specifically acquired from Beijing Drainage Group Co., Ltd. in Beijing, China. The basic properties of the biosolids are given in the Supplementary Materials (Table S2). The application of biosolids occurred once every year between the planting cycles of maize and wheat. Spreading fertilizers evenly over the entire farm surface and then ploughing the soil.

2.2. Soil sampling and analysis

In June 2022, soils (0–20 cm) were sampled from each treatment. After removing any residues, five soil cores were sampled from each plot and mixed into a single sample. Following collection, samples were promptly transported while chilled to the laboratory, where they underwent sieving through a 2.0-mm mesh sieve. Afterward, these samples were divided into three separate subsamples for further analysis or experimentation. One subsample was kept at 4 °C for measuring biological indicators, another was kept at –20 °C for DNA sequencing, and the remaining subsample was air-dried for soil physicochemical properties analyses.

Soil moisture, pH, soil organic matter (SOM), total nitrogen (TN), inorganic nitrogen (N_{min}), available phosphorous (AP), total phosphorous (TP), available potassium (AK), total potassium (TK), microbial biomass carbon (MBC) and nitrogen (MBN), dissolved organic carbon (DOC) and nitrogen (DON), exchangeable calcium (Ca), magnesium (Mg), sodium (Na) and permanganate oxidizable carbon (POXC), were measured as described as Jia et al. [20]. The soil's heavy metal contents underwent analysis using inductively coupled plasma (ICP) optical emission

spectrometry.

2.3. Microbial DNA extraction, PCR, and Illumina sequencing

Total DNA was extracted from 0.5 g of soil using the MagaBio Kit (Bioer Technology, China). PCR amplifications targeted different regions: the V4 region for bacteria using primer pair 515 F (5'-GTGCCAGCMGCCGCGGTAA-3') / 806 R (5'-GGACTACHVGGGTWTC-TAAT-3') [54], the ITS region for fungi using primers ITS3F (5'-GCATCGATGAAGAACGCAGC-3') / ITS4R (5'-GCATCGATGAA-GAACGCAGC-3') [33], and a specific region for AM Fungi with primers AML1F (5'-ATCAACTTCGATGGTAGGATAGA-3'), AML2R (5'-GAACCCAAACACTTGGTTTCC-3'), AMV4.5NF (5'-AAGCTCG-TAGTTGAATTCG-3'), and AMDGR (5'-CCCAACTATCCCTATTAAT-CAT-3') [52] synthesized by Invitrogen. The sequencing reads, acquired from paired-end sequencing, were initially sorted into individual samples using unique barcode. Post sorting, the reads underwent merging and were subsequently subjected to denoising and chimera filtering through DADA2. Taxonomic assignment for bacteria utilized the SILVA v138 database [18], while the UNITE v8.0 database [14] was employed for fungi and the MaarjAM database [34] was employed for AM fungi. For detailed insights into the bioinformatic analyses of the data, refer to the Supporting Materials section, which contains comprehensive information about these procedures.

2.4. Statistical analyses

R (version 4.1.0) was utilized for statistical analyses. One-way ANOVAs assessed variations in soil properties, microbial diversity, and community composition. Non-metric multidimensional scaling (NMDS) was performed to evaluate the influence of biosolids application rates on soil bacterial, fungal, and AM fungal communities. Soil microbial co-occurrence networks were constructed using the 'microeco' package [28]. Robust correlations (Spearman's $\rho > 0.60$) with FDR-corrected p-values < 0.01 were used to construct networks. And subsequent network property analysis was used by the 'igraph' package. Random networks with identical edges and nodes were generated, and the topological properties of these networks were calculated using the Erdős-Rényi model [10]. The iCAMP method was used to evaluate the stochastic and deterministic processes in contributing soil microbial community. The detailed process of iCAMP analyses of the data was in the supporting Materials section, which contains comprehensive information about these procedures. Bacterial, fungal, and AMF β NTI differences were examined using one-way ANOVA. Random forest regressions identified significant drivers of soil microbial community assembly.

3. Results

3.1. Effect of biosolids application on soil parameters

Soil physical, chemical, and biological parameters were significantly affected by the application of biosolids (Table 1). Notably, soil AP, TN, SOM, and DOC contents increased with the application of biosolids. In this context, SW4 had the highest contents and control had the lowest contents. However, AK content was highest in the SW4 treatment. Furthermore, MBC and MBN were highest in the SW3 treatment. MBC and MBN increased by 11.47%, 29.70%, 64.59%, 64.40%, and 17.74%, 31.55%, 56.14%, and 42.66% in the SW1, SW2, SW3, and SW4 treatments compared to the control, respectively.

The heavy metals contents of Cu, Zn, Cd, and Hg in the soil increased with the increase in the application doses of biosolids (Fig. 1). However, although Cr and Pb contents increased with the increased application of biosolids from SW1 to SW4, Cr and Pb content was decreased in SW1 treatment (Fig. 1A and G). There was no significant difference in Ni and As content among various treatments (Fig. 1B and E).

Table 1

The effect of biosolids applications on soil parameters.

	Control	SW1	SW2	SW3	SW4
pH	7.13 ± 0.07a	7.14 ± 0.03a	7.15 ± 0.02a	7.08 ± 0.088a	7.12 ± 0.07a
Moisture (%)	15.48 ± 4.33a	14.51 ± 0.72a	11.18 ± 2.26a	20.43 ± 8.35a	16.85 ± 5.18a
AP (mg/kg)	52.71 ± 23.45c	71.09 ± 8.45bc	97.43 ± 11.72b	191.48 ± 8.67a	217.12 ± 21.58a
TP (g/kg)	1.41 ± 0.19a	1.46 ± 0.18a	1.67 ± 0.23a	1.48 ± 0.14a	1.28 ± 0.57a
AK (mg/kg)	289.61 ± 76.49b	199.82 ± 7.63b	306.91 ± 66.28b	296.41 ± 78.96b	606.45 ± 186.40a
TK (g/kg)	19.91 ± 0.73a	19.05 ± 0.91a	21.24 ± 0.99a	21.89 ± 2.25a	17.77 ± 6.36a
TN (g/kg)	1.06 ± 0.10d	1.30 ± 0.09d	1.67 ± 0.13c	2.44 ± 0.14b	3.42 ± 0.30a
SOM (g/kg)	18.83 ± 1.94e	25.30 ± 2.85d	32.67 ± 2.55c	40.91 ± 4.27b	58.95 ± 1.53a
EC (ds/cm)	138.40 ± 20.21a	140.27 ± 5.06a	134.03 ± 6.89a	193.23 ± 64.29a	183.37 ± 39.19a
NH ₄ ⁺ (mg/kg)	21.96 ± 2.64a	22.24 ± 1.19a	22.09 ± 0.399a	19.96 ± 3.58ab	17.68 ± 0.51b
NO ₃ ⁻ (mg/kg)	11.06 ± 5.85c	14.36 ± 1.89bc	15.40 ± 1.38bc	32.66 ± 16.18ab	37.81 ± 15.61a
Nmin (mg/kg)	33.02 ± 5.52c	36.61 ± 1.31bc	37.48 ± 1.34bc	52.62 ± 13.23ab	55.49 ± 15.19a
Mg (mg/kg)	436.11 ± 19.84b	418.67 ± 7.80b	422.34 ± 10.51b	440.44 ± 56.17b	500.52 ± 28.66a
Na (mg/kg)	131.87 ± 31.95a	132.30 ± 9.72a	91.23 ± 24.05ab	96.60 ± 28.87ab	75.53 ± 6.24b
Ca (mg/kg)	3819.01 ± 284.58ab	3844.08 ± 195.99ab	± 64.07b	± 320.02b	± 34.14a
MBC (mg/kg)	309.48 ± 52.18b	344.97 ± 15.70b	401.38 ± 48.66ab	509.33 ± 100.71a	508.58 ± 55.24a
MBN (mg/kg)	57.71 ± 11.36b	67.95 ± 4.63ab	75.92 ± 9.85ab	90.11 ± 27.82a	82.33 ± 17.42ab
DOC (mg/kg)	62.68 ± 9.85c	73.64 ± 3.14c	80.04 ± 5.21c	123.48 ± 18.60b	150.14 ± 15.82a
DON (mg/kg)	32.44 ± 9.33b	37.75 ± 1.29b	38.09 ± 0.95b	76.30 ± 21.01a	85.65 ± 17.07a
POXC (mg/kg)	0.20 ± 0.05b	0.19 ± 0.04b	0.18 ± 0.05b	0.25 ± 0.01ab	0.33 ± 0.08a

3.2. Effect of biosolids application on soil microbial richness and community composition

Soil bacterial richness was not significantly different among various treatments (Fig. 2A). Fungal richness increased with the increase in biosolids application rates, which was highest in SW4 treatment compared to the control, the fungal richness increased by 4.43%, 2.88%, 6.68%, and 7.48% in the SW1, SW2, SW3, and SW4 treatments (Fig. 2B). Compared with control, AM fungal richness was significantly increased in SW4 treatment. However, there was no significant difference among other treatments. Compared to the control, the AM fungal richness increased by 0.77%, 17.39%, and 41.46% in SW1, SW3, and SW4 treatments while exhibiting a decrease of 7.92% in the SW2 treatment (Fig. 2C).

The result of heatmaps showed that there were significantly positive correlations between AP, DOC content, bacterial richness, and AM fungal richness (Fig. S1). Soil moisture and DON content exclusively showed a positive correlation with AM fungal richness (Fig. S1). Positive correlations were found among TN and SOM content with fungal and AM fungal richness ($p < 0.05$) (Fig. S1). As for heavy metals, no statistically significant correlations were observed between bacterial richness and any of the heavy metals (Fig. S1). All heavy metals showed significantly positive correlations with fungal and AM fungal richness. Concurrently, Cr, Ni, As, Cd, Pb, and Hg displayed a positive association with fungal and AM fungal Shannon diversity (Fig. S1).

Both soil bacterial, fungal, and AM fungal communities were significantly influenced by the application of biosolids, which were cleared and separated along NMDS1 (Fig. 2D and E). AM fungal

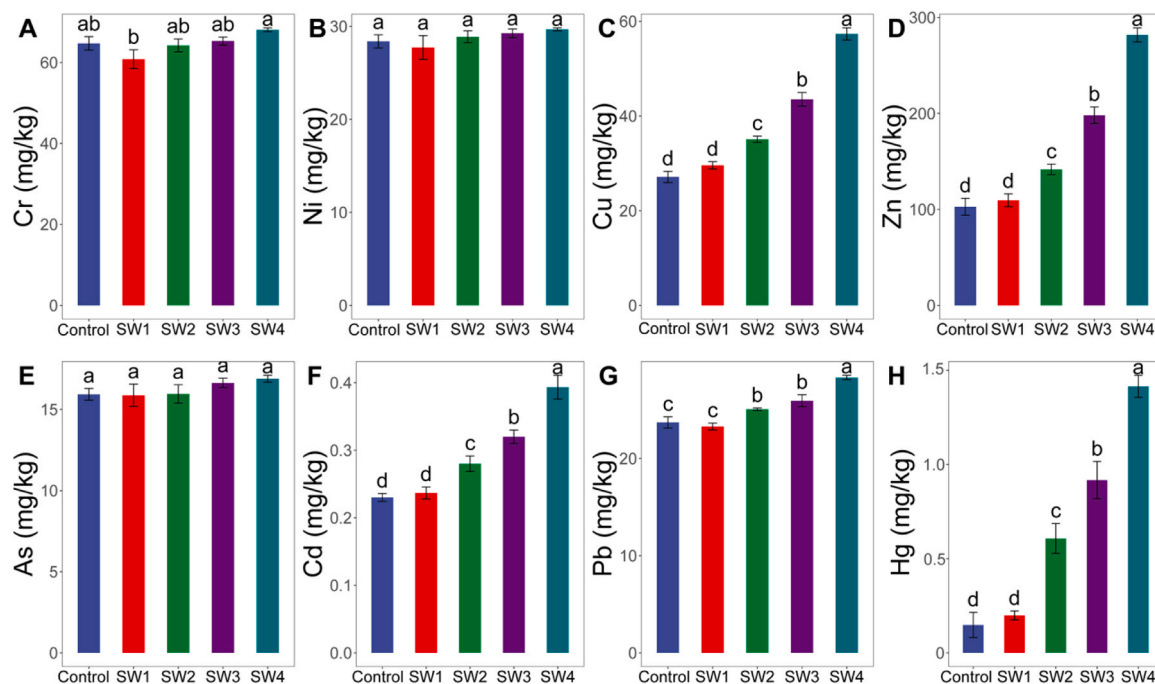


Fig. 1. The effect of biosolids application on the contents of different types of heavy metals.

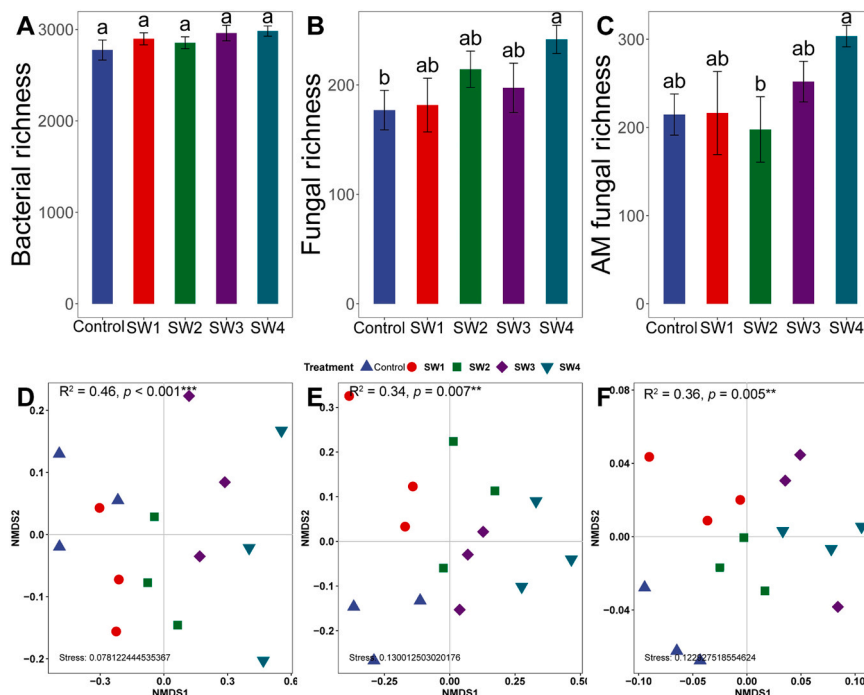


Fig. 2. The bacterial (A, D), fungal (B, E), AM fungal (C, F) richness and community compositions after biosolids application. Significance is indicated by $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

community of SW1 treatment was observed to have a higher resemblance with SW2 treatment than other treatments. A higher resemblance could also be observed between SW3 and SW4 treatments (Fig. 2F).

Proteobacteria, *Acidobacteria*, and *Ascomycota*, *Basidiomycota* were the two most abundant bacterial and fungal phyla, respectively, in all treatments (Fig. 3A and B). *Glomeraceae* and *Pycnomycaceae* were the two most abundant families in all treatments (Fig. 3). The mantel tests indicated that the soil bacterial and AM fungal community structures were markedly influenced by SOM, AP, TN, N_{min} , Cu, Pb, Cd, Zn, and Hg

contents. Furthermore, the fungal community structure was significantly affected by Cr, Ni, and As contents (Fig. 4). As for bacteria, Cu, Zn, Pb, and Hg contents exhibited positive correlations with the abundances of *Actinobacteriota* and *Chloroflexi* while showing negative associations with the abundances of *Acidobacteriota*, *Bacteroidota*, *Verrucomicrobiota*, *Armatimonadota* and *Elusimicrobiota* (Fig. S2A). Furthermore, Cr content was positively related to *Actinobacteriota* while negatively related to *Actinobacteriota* and *Verrucomicrobiota* (Fig. S2A). For fungi and AM fungi, Cu, Zn, Cd, Pb, and Hg contents were positively related to the

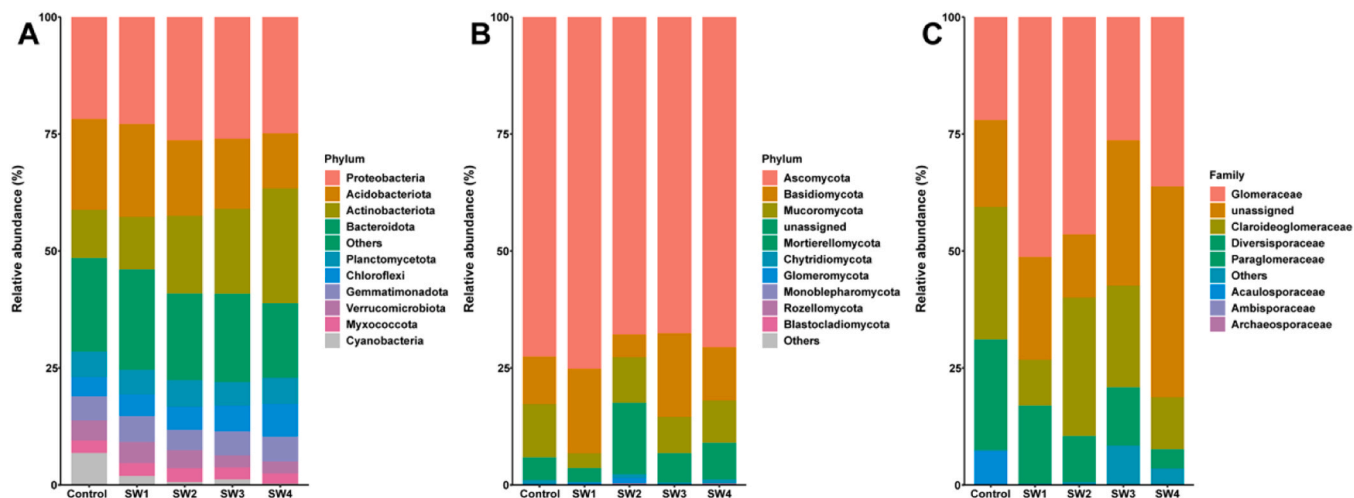


Fig. 3. The relative abundance (%) of bacterial (A), fungal (B) phyla, and AM fungal family (C) in different treatments.

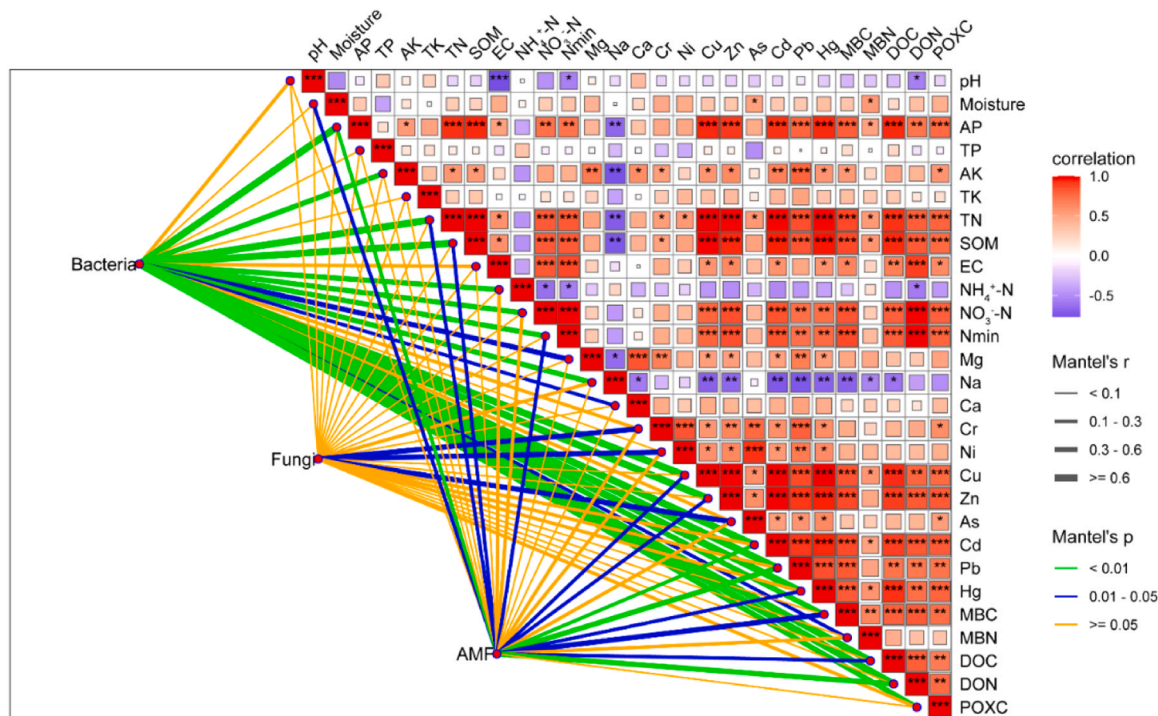


Fig. 4. Mantel test analysis showed the influence of soil parameters on bacterial, fungal, and AM fungal community composition. Significance is indicated by $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

abundances of *Rozellomycota* and *Ambisporaceae*, respectively (Fig. S2B, C). Moreover, the abundance of *Blastocladiomycota* increased with the increased Cd content (Fig. S2B). The abundance of *Archaeosporaceae* reduced with the increase of Cr, Ni, and As contents (Fig. S2C).

Microbial co-occurrence patterns were examined for a more in-depth characterization of the impact of biosolids application on microbial interactions (Fig. 5). The bacterial network comprises 639 nodes and 1483 edges, the fungal network consists of 494 nodes and 1113 edges, and the AM fungal network encompasses 250 nodes and 972 edges. Other microbial network properties such as average degree and modularity were also the highest in the soil bacterial network (Table 2). Furthermore, there were 5, 8, and 8 modules in bacterial, fungal, and AM fungal networks, respectively (Fig. 5A, B, C). For the bacterial network, the relative abundance in module 1 and module 5 showed a significantly negative relationship with Cu, Zn, Cd, Pb and Hg contents (Fig. 5D).

Microbial abundance in module 2 showed positive relationships with Cr, Cu, Zn, As, Cd, Pb, and Hg contents (Fig. 5D). Moreover, microbial abundance in module 4 was positively related to Cr and As (Fig. 5D). For the fungal network, positive relationships were found between the relative abundance of module 1 and Cr, Cu, Zn, Cd, Pb, and Hg contents. And microbial abundance in module 2 was negatively related to Cu and Hg contents (Fig. 5D). In the AM fungal network, negative relationships were observed between the relative abundance of module 1 and Cr, Ni, and As contents (Fig. 5D). Furthermore, notable negative relationships were observed between microbial abundance of module 2 and Ni content (Fig. 5D). And there were incredibly positive correlations between microbial abundance in module 3 and Cr, Ni, Cu, Zn, As, Cd, Pb, and Hg content. Cu and Zn content were found to be positively related to module 7 abundance ($p < 0.05$ *) (Fig. 5D).

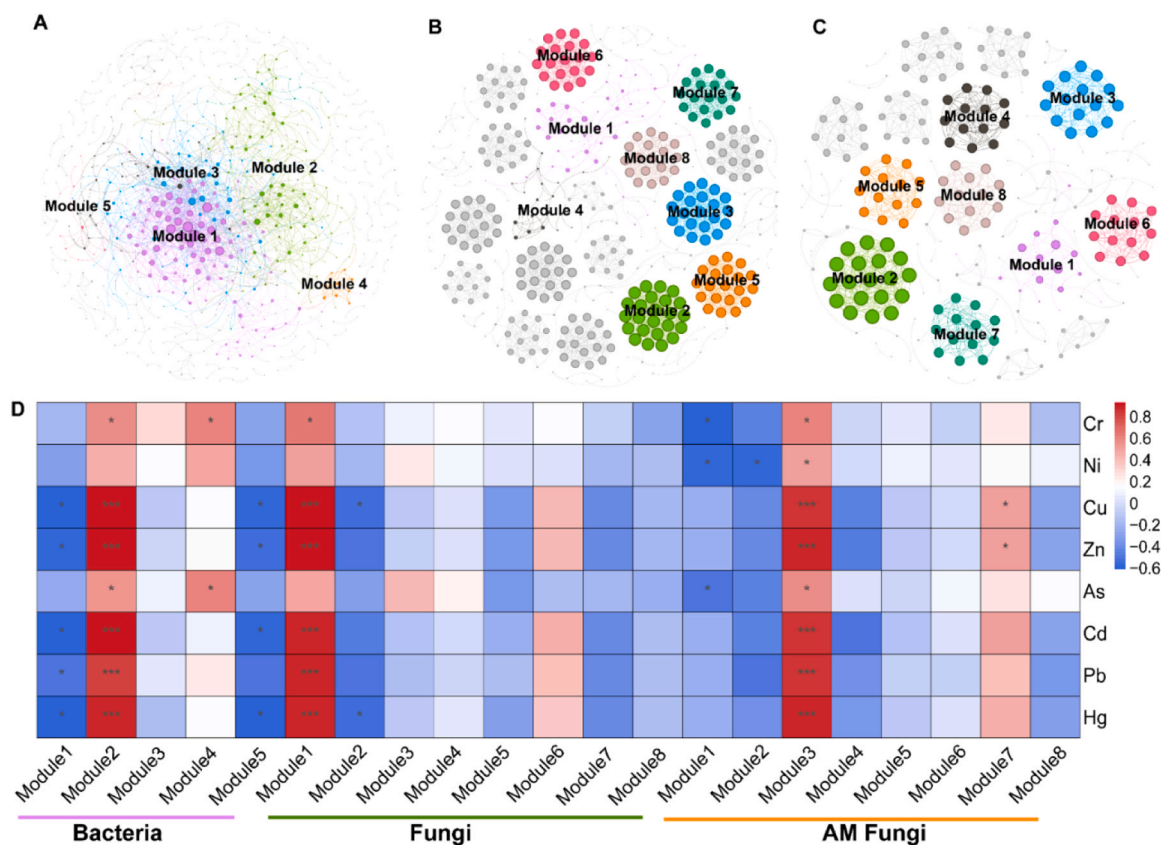


Fig. 5. Network visualization of microbial co-occurrence patterns of (A) bacterial, (B) fungal, and (C) AM fungal community. And the relationships between heavy metal contents and the microbial network abundances (D). Significance is indicated by $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

Table 2

Properties of soil bacterial, fungal, and AM fungal co-occurrence networks.

	Node	Edge	Average degree	Average path length	Network diameter	Clustering coefficient	Density	Heterogeneity	Centralization
Bacteria	639	1483	4.64	4.26	12	0.33	0.01	1.29	0.06
Fungi	94	113	2.4	1.01	2	0.98	0.03	0.78	0.05
AM Fungi	250	972	7.78	1.15	4	0.98	0.03	0.7	0.04

3.3. The effect of biosolids application on soil microbial community assembly processes

The weighted β NTI combined with RCbray was used to calculate and assess the deterministic and stochastic aspects of microbial community assembly (Fig. 6). Stochastic processes of drift predominantly governed bacterial community assembly, accounting for 80% of the observed dynamics. In comparison, deterministic methods of homogeneous selection were prevalent in shaping fungi (80%). AM fungal (80%) community assembly (Fig. 6). Bacterial β NTI decreased with the increase in biosolids application doses, except for the SW4 treatment (Fig. 6A). However, β NTI of fungal and AM fungal showed an increasing trend with the increasing application rates of biosolids (Fig. 6B and C). Deterministic factors' role in shaping the assembly of fungal communities decreased by 33.33% in the SW1, SW2, and SW3 treatments compared to the control. And the role of deterministic processes in shaping AM fungal communities exhibited an increase of 33.33% and 66.67% in both SW3 and SW4 treatments, compared to the control (Fig. 6D).

Results of mantel test demonstrated that the bacterial community assembly processes could be correlated with AP, AK, TK, TN, SOM, NH_4^+ , NO_3^- , Mg, Na, Cu, Zn, Cd, Pb, Hg, MBC, DON, and POXC (Table 3). Furthermore, fungal community assembly processes exhibited

significant associations with AP, TP, Ni, Cu, and Zn contents (Table 3). AM fungal community assembly processes could be related to soil parameters such as pH, EC, NO_3^- , N_{min} , Mg, Cu, Zn, MBC, MBN, DON, and POXC contents (Table 3).

The results of random forest analysis regressions further illuminated the most crucial drivers of the community assembly processes. Spontaneous forestry regressions explained 66.67%, 36.71%, and 41.18% of variations of bacterial, fungal, and AM fungal community assembly process, respectively (Fig. 7). AP, TN, Hg, SOM, Cu, NH_4^+ , Cd, DOC, MBC, and Zn emerged as primary predictors of bacterial community assembly processes (Fig. 7A). In the case of the fungal community assembly processes, POXC, Pb, SOM, Mg and Cr were recognized as significant predictors (Fig. 7B). Additionally, TN, As, and Ni were identified as significant predictors of AM fungal community assembly process (Fig. 7C).

4. Discussion

4.1. Distinct responses of bacterial, fungal, and AM fungal communities to biosolids application

The application of biosolids could change soil physicochemical properties, such as soil pH, some essential nutrients, and organic matter

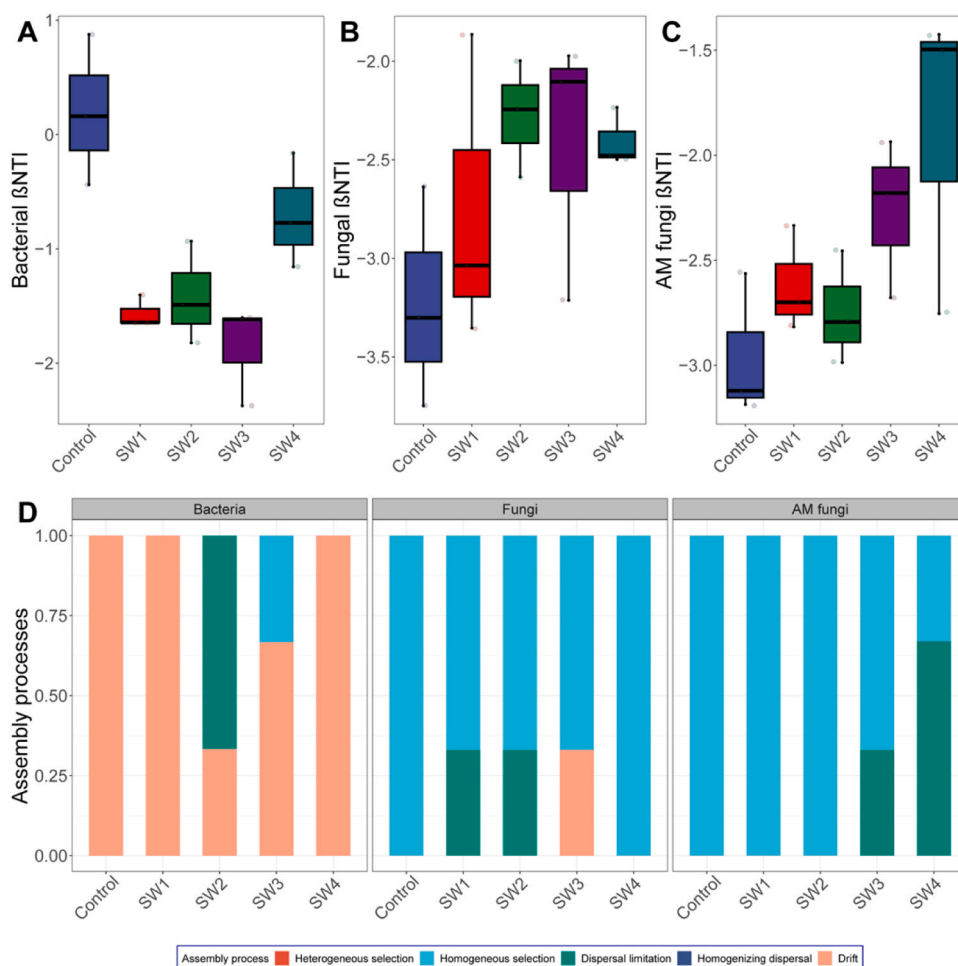


Fig. 6. Effects of biosolids application on bacterial (A), fungal (B), and AM fungal (C) β NTI and community assembly processes (D).

contents [50], which could shift the composition of soil microbial communities [19]. However, the application of biosolids had the potential to elevate the contents of heavy metals in soils. The presence of these heavy metals could potentially pose risks to both public health and the overall ecosystem functions [24].

In this study, soil microbial diversity was found to be positively correlated with SOM and nutrient contents (Fig.S1), supporting our first hypothesis that the application of biosolids increased soil microbial diversity by increasing nutrient and organic matter contents. Biosolids application significantly increased soil organic matter and nutrient contents, such as AP, and TN (Table 1). SOM functions as a vital energy and nutrient source for microorganisms, playing a pivotal role in shaping microbial communities due to its availability [21]. Soil organic matter improves microbial diversity by supplying soil substrates and stimulating indigenous organisms' growth [6]. Furthermore, soil AP and DOC content were observed to play a crucial role in describing the bacterial community patterns compared to fungi and AM fungi. This finding was similar to a previous study, which found bacteria were generally considered to be fast-growing, whose population fluctuates with soil nutrient concentrations [57]. Fungal and AM fungal richness are more sensitive to heavy metal contents, in particular to Cr and Ni than those SOM and nutrient contents. In contrast, bacterial diversity was only correlated with soil nutrient contents (Fig. S1). The different responses of bacterial and fungal richness may be due to their phylogenetic and evolution characteristics [4]. In addition, fungi, especially certain species, are known to respond more sensitively to heavy metals than bacteria. Heavy metals like Cr and Ni can have toxic effects on fungal metabolism and growth, influencing fungal richness [61]. AM

fungi create symbiotic relationships with plant roots and play a crucial role in nutrient uptake, including metals. Their sensitivity to heavy metal concentrations might be linked to their role in plant-metal interactions and the potential for metal accumulation in their structures [12]. Their sensitivity to metal concentrations suggests a dual role: facilitating metal uptake by plants, potentially influencing metal bioavailability, and acting as reservoirs for heavy metals within their structures, such as arbuscules and hyphae [37]. This sensitivity is likely associated with their known ability to modulate plant responses, playing a crucial role in coping with various stresses, including heavy metal stress. [16].

The mantel test result showed that soil bacterial and AMF community compositions were significantly influenced by SOM and heavy metals (Fig. 4). These results were consistent with previous study that organic matter could shift soil bacterial community composition by affecting heavy metals mobility and bioavailability [27]. Organic matter, such as humic and fulvic acids, has a high affinity to bind to heavy metals, thereby reducing the concentration of soil heavy metals [26].

Hence, the intricate influence of biosolids application on soil bacterial communities stems from the simultaneous introduction of nutrients and heavy metals, resulting in complex alterations in community dynamics. AM fungi act as a symbiont fungus that could help plants capture nutrients such as phosphorus and nitrogen from the soil [5,51]. Meanwhile, plants could supply carbon to AM fungi [7]. The interactions between plants and AM fungi improved plant growth by facilitating nutrient uptake from the soil. As plants thrive and gain access to more soil nutrients, they allocate more carbon compounds to AM fungi. This increased carbon supply to AM fungi is crucial in shaping the AM fungal

Table 3

The correlation between soil parameters and bacteria, fungi, and AM fungi community assembly processes by mantel test analysis.

	Bacteria		Fungi		AM fungi	
	R	<i>p</i>	R	<i>p</i>	R	<i>p</i>
pH	0.03	0.62	0.02	0.43	0.25	0.03 *
Moisture	0.08	0.21	-0.01	0.55	0.21	0.06
AP	0.37	0.01 **	0.21	0.05 *	0.02	0.42
TP	0.18	0.1	0.19	0.05 *	0.13	0.2
AK	0.31	0.01 *	-0.04	0.63	0.18	0.1
TK	0.23	0.03 *	-0.03	0.59	0.17	0.13
TN	0.55	< 0.001 ***	0.14	0.1	0.19	0.06
SOM	0.53	< 0.001 ***	0.17	0.06	0.09	0.21
EC	0.18	0.1	0.03	0.42	0.26	0.03 *
NH ₄ ⁺	0.4	< 0.01 **	-0.03	0.6	0.12	0.17
NO ₃ ⁻	0.34	< 0.01 **	-0.07	0.73	0.26	0.03 *
N _{min}	0.26	0.03 *	-0.03	0.6	0.33	0.02 *
Mg	0.39	< 0.01 **	-0.06	0.71	0.31	0.01 **
Na	0.34	< 0.01 **	-0.17	0.95	-0.27	0.99
Ca	0.19	0.07	0.02	0.56	0.19	0.03 *
Cr	0.15	0.1	0.17	0.06	-0.04	0.64
Ni	0.05	0.26	0.24	0.03 *	-0.11	0.79
Cu	0.53	< 0.001 ***	0.23	0.04 *	0.35	< 0.01 **
Zn	0.55	< 0.001 ***	0.23	0.04 *	0.36	< 0.01 **
As	0.05	0.36	0.12	0.15	-0.08	0.75
Cd	0.55	< 0.001 ***	0.11	0.18	0.15	0.1
Pb	0.51	< 0.001 ***	0.11	0.18	0.11	0.18
Hg	0.5	< 0.001 ***	0.16	0.07	0.08	0.24
MBC	0.33	< 0.01 **	0.12	0.15	0.21	0.04 *
MBN	0.1	0.2	0.12	0.15	0.3	0.01 *
DOC	0.46	< 0.001 **	0.13	0.13	0.16	0.1
DON	0.41	< 0.01 **	0.1	0.2	0.32	0.01 *
POXC	0.28	0.02	0.24	0.03	0.28	0.02 *

Note: Significance is indicated by $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***

community [5]. In addition, previous studies found that symbiotic relationships with host plants can be facilitated by the rapid growth of hyphae, particularly those that thrive under metal toxicity and challenging environmental conditions [11,39,8]. Therefore, variations of soil nutrients and heavy metals could alter the AM fungal community.

However, the fungal community was significantly affected by heavy metals, including Cr, Ni, and As. Fungi were generally more sensitive to heavy metals than bacteria, exhibiting diverse responses to such contamination [35,60]. The shift in the fungal community may be due to the selection of heavy metals-tolerance fungi phyla [43]. This study also identified positive relationships between heavy metals and fungal phyla, notably *Rozellomycota* and *Blastocladiomycota*, known for their robust tolerance to heavy metals [27]. In addition, the fungal network further confirmed the selection of fungi. Our results revealed a positive relationship between fungal abundance in module 1 and heavy metals. Notably, *Ascomycota*, the dominant fungi in module 1, was also found to have a solid tolerance to heavy metals [43]. *Basidiomycota* is another important fungus in module 1, which is widely found in agricultural soils and effectively transfers heavy metals [27]. Our results showed soil bacteria, fungi, and AM fungi respond distinctly to long-term application due to their phylogenetic characteristics, and indicate the impact of heavy metals on the soil microbial community appears to be more pronounced compared to the influence of soil nutrients.

4.2. Different ecological processes governing bacterial, fungal, and fungal assemblies

In this study, the stochastic process dominated the bacterial community, while deterministic methods shaped the fungal and AM fungal communities. The bacterial community exhibited substantial variability driven by stochastic drift processes in most soil samples. Drift represents stochastic changes in species abundance, leading to more significant than expected turnover [45]. The bacterial community may enhance its capacity to adapt to environmental changes by employing self-resistance

mechanisms and participating in synergistic interactions with other microorganisms [62]. Furthermore, compared with fungi, bacteria often demonstrate higher rates of phylogenetic and taxonomic turnover, along with increased adaptability in resource utilization. This versatility enables them to swiftly adapt to evolving conditions, establish their presence, and effectively compete in various environments [21]. However, the contribution of the stochastic process to bacterial community dynamics decreased with biosolids application, except for SW4 treatment. This shift may be due to selective pressure imposed by environmental factors [15]. The development of random forest showed that Zn, MBC, DOC, and Cd played significant roles in shaping the bacterial community assembly. Heavy metals like Zn and Cd likely exert selective pressure leading to the reduction or elimination of bacterial species with limited heavy metal tolerance [17]. Cd and Zn have the potential to diminish bacterial biomass and diversity, as well as hinder bacterial enzyme activities within soils contaminated by heavy metals [49]. Meanwhile, DOC could indeed have a positive impact on the diversity of soil bacterial communities, as it serves as a significant energy source that promotes bacterial growth and metabolic activities [59]. The findings that showed dispersal limitation increased in SW4 treatment suggest that the beneficial impacts of DOC on bacterial communities surpass the selective pressures imposed by heavy metals.

Homogeneous selection, and deterministic processes, appeared to significantly drive fungal communities' turnover [56]. Homogeneous selection commonly becomes a predominant factor in shaping communities during stable states following disturbances and is often associated with physicochemical variables [56]. In the context of long-term biosolids application, it is possible that the fungal community reached a relatively stable state as fungi gradually adapted to elevated heavy metal levels. Homogeneous selection implies that particular fungi strains or groups within a population possess genetic traits that enhance their tolerance to heavy metal exposure. As a result, these fungi thrive and become more prevalent in environments with high heavy metal concentrations [41].

Furthermore, the dominance of homogenous selection in shifting fungal community may stem from fungi's heightened adaptability and tolerance to heavy metals and metalloids, surpassing that of bacteria [44]. Additionally, dispersal limitation contributes to shaping fungal and AM fungal community assembly. Heavy metals such as Cr and Ni recognized as the primary drivers of fungal and AM fungal community assembly, could impose more substantial dispersal limitations on the microbial community [63]. The change in dispersal limitation and homogeneous selection may stem from variations in microorganisms' dispersal/diffusion abilities across different domains [62]. Our results indicated that heavy metals drive the microbial community assembly process in long-term biosolids application farmland.

5. Conclusions

In conclusion, our study showed a significant influence of the long-term application of biosolids on various aspects including soil physicochemical parameters, heavy metal concentrations, and microbial community composition and assembly process. Notably, soil microbial communities exhibited distinctive responses to biosolids applications. While both soil organic matter (SOM) and heavy metals (Cu, Pb, Cd, Zn, and Hg) influenced bacterial and arbuscular mycorrhizal fungal communities, only the fungal community responded to heavy metals (Cr, Ni, and As). Microbial co-occurrence network analysis identified unique modules within soil microorganisms, each exhibiting differential responses to heavy metal contents. Remarkably, stochastic processes primarily governed bacterial community assembly, whereas deterministic processes had more influence on fungal and AM fungal communities. Furthermore, heavy metals played a significant role in steering microbial community assembly, with specific heavy metals acting as crucial drivers for distinct microbial groups. Zinc, Chromium, and Nickel emerged as the most influential drivers for bacterial, fungal, and AM

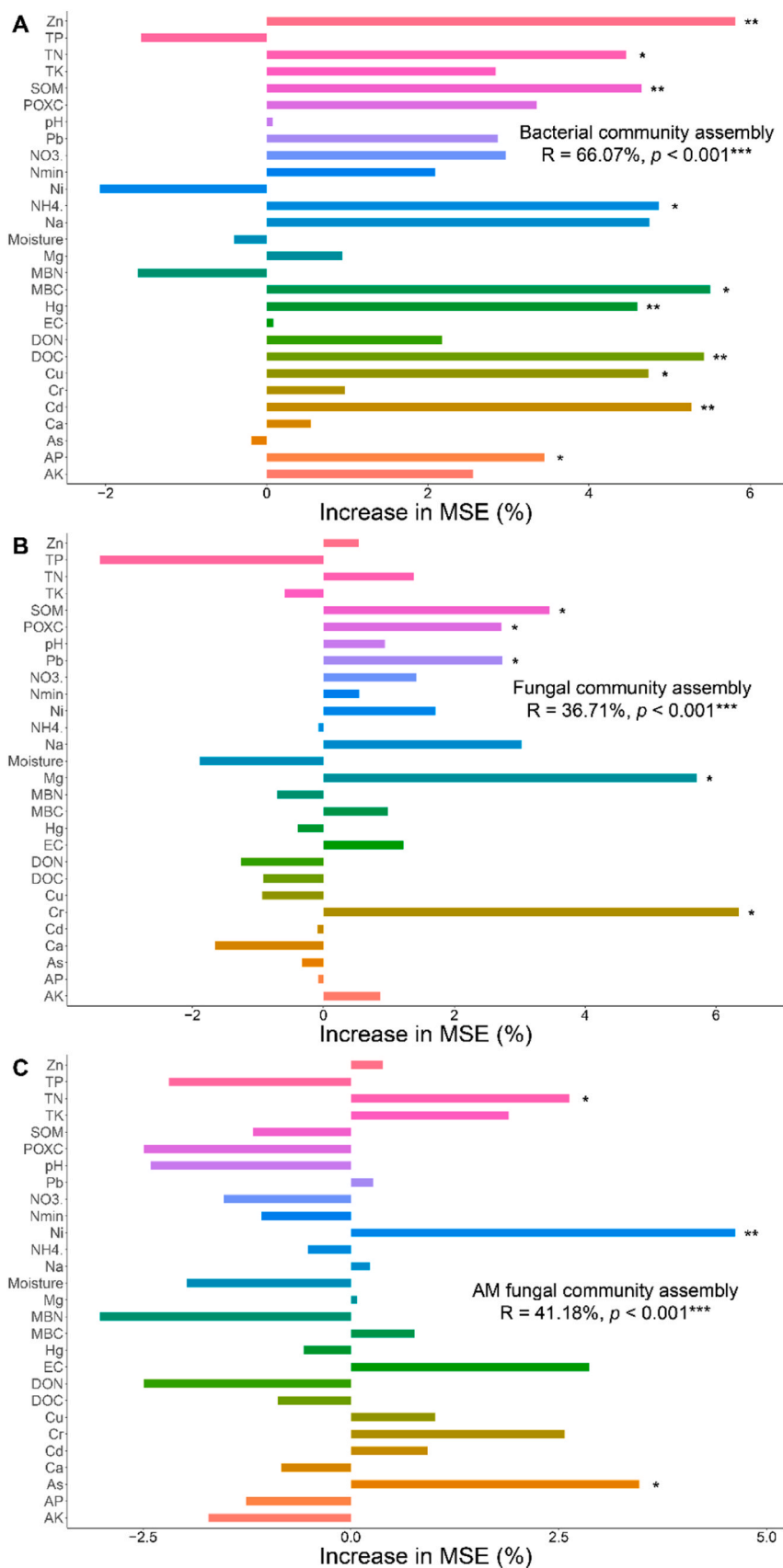


Fig. 7. Important soil parameters as predictors of microbial community processes characterized by random forest regression. Significance is indicated by $p < 0.05$ *, $p < 0.01$ **, and $p < 0.001$ ***.

fungal community assembly, respectively. These findings provide insights into the distinct responses of bacterial, fungal, and AMF communities to biosolids applications, offering valuable guidance for biosolids application practices.

Environmental implication

A complete perspective of soil microbial community structure in response to heavy metals contamination from long-term biosolids application was provided. Notably, the assembly of bacterial communities appeared to be largely governed by stochastic processes, whereas fungal and AM fungal communities were more influenced by deterministic processes. Heavy metals played an important role in driving microbial community assembly, with specific heavy metals having a significant driving effect on different microbial communities. These findings reveal complex interactions between microbial communities and their potential response to environmental changes induced by biosolids application, which is crucial for improving soil health and maintaining ecosystem functions.

CRedit authorship contribution statement

Tao Sun: Writing - original draft, Methodology, Investigation, Conceptualization. **Guihua Li:** Resources, Data curation, Conceptualization. **Mahmoud Mazarji:** Writing - review & editing. **Pierre Delaplace:** Writing - review & editing. **Xing Yang:** Writing - review & editing. **Jianfeng Zhang:** Resources, Project administration, Funding acquisition, Conceptualization. **Junting Pan:** Conceptualization, Supervision, Investigation, Writing - review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2024.133845](https://doi.org/10.1016/j.jhazmat.2024.133845).

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