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Exploring the impact of fulvic acid and humic acid on heavy metal availability to alfalfa in molybdenum contaminated soil

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Humic substances, such as Fulvic acid (FA) and humic acid (HA), are widely used for the remediation of heavy metal-contaminated soils due to their ability to enhance metal mobility and facilitate plant uptake. In this study, we conducted a pot experiment with alfalfa to investigate the effects of FA and HA amendments on the mobility of molybdenum (Mo) in the soil, its uptake by alfalfa plants, and subsequent changes in the microbial community. The results demonstrated that both FA and HA influence Mo accumulation in the soil and plants. Specifically, HA treatment increased Mo concentrations in alfalfa shoots and roots by 1.08–1.19 times and 1.19–2.43 times, respectively, compared to the control. In contrast, FA enhanced Mo concentrations in alfalfa roots (1.05–1.58 times) but reduced Mo levels in the shoots (0.78–0.85 times). Furthermore, the addition of FA and HA altered the chemical speciation of Mo in the soil, promoting the conversion of reducible and oxidizable fraction to more exchangeable and residual fraction. As a result, the proportion of non-residual Mo fractions (exchangeable, reducible, and oxidizable) decreased from 87.48% to 80.30-87.35%, while residual fractions increased from 12.52% to 12.65–19.70%. Additionally, the structure of the soil bacterial community was primarily influenced by changes in soil properties such as cation exchange capacity, available phosphorus, and ammonium nitrogen levels. This finding highlight the potential of FA and HA to enhance Mo availability, uptake, and translocation in alfalfa, suggesting that their application could be an effective strategy for phytoremediation of Mo-contaminated soils, particularly when alfalfa is used as a hyperaccumulator.

Keywords Bacterial community, Contaminated soil, Fulvic acid and humic acid, Molybdenum, Phytoremediation

The levels of heavy metal pollution in soils have been significantly elevated due to human activities, such as industrial processes, mining, and agricultural practices, posing substantial risks to both human health and the environment[1,2]. This contamination is particularly concerning because of its potential to enter the food chain, affecting both ecological systems and human populations[3,4]. Consequently, there is an urgent need for cost-effective and sustainable methods to remediate heavy metal-contaminated soils. Currently, various approaches have been explored for this purpose. For example, modified biochar has shown promising results in stabilizing heavy metals such as Cd, Pb, Cu, and Zn, effectively reducing their uptake by wheat seedlings and improving soil properties[4]. Another common strategy involves the addition of EDTA to soil, which enhances the dissolution of Pb and facilitates its absorption and translocation in bamboo plants[5]. Additionally, the use of mixed chelating agents has been successful in removing Cu and Pb from contaminated agricultural soil[6]. With agents like DGPA, EDDS, and iron nanoparticles being frequently utilized[7–9]. However, many of these modifiers can lead to secondary pollution or are prohibitively expensive. Therefore, there is a pressing need to identify more affordable and environmentally benign alternatives for soil remediation. Humic substances (HS) represent a promising solution to this problem.

HS are large, stable polymers found in natural soil and aquatic systems, formed through the physical, chemical, and microbial decomposition of plant and animal residues. These complex structures contain a wide array of active functional groups that play a crucial role in the transformation, mobility, and bioavailability of heavy metals in the soil[10]. HS are typically classified into three main components based on their solubility:

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humin (HM), humic acid (HA), and fulvic acid (FA)[11]. These components differ in molecular weight, functional groups content, and elemental composition. HA, for instance, generally has a molecular weight ranging from 50 to 100 kDa, while FA has a much smaller molecular weight, typically between 0.5 and 2 kDa[12]. Numerous studies have demonstrated the beneficial effects of FA and HA in enhancing soil functions and mitigating heavy metal toxicity[13–16]. For example, research has shown that different concentrations of HA can reduce the mobilization, root uptake, and phytoaccumulation of heavy metals in cadmium-contaminated radishes[17]. The addition of FA to soils contaminated with Pb and Cd has also been found to enhance the stability of these metals[18]. Furthermore, the application of FA in wastewater irrigation of wheat has proven effective in reducing Cr toxicity, promoting plant growth, increasing biomass, and enhancing photosynthetic pigments such as chlorophyll, while also alleviating oxidative stress, lipid peroxidation, and Cr accumulation in stressed plants[19]. Additionally, FA and HA significantly increases the accumulation of Cd in plants, with concentrations reaching 2.17 and 2.78 times those of the control treatment, respectively[20]. These findings highlight the significant role of HS in influencing the behavior of heavy metals in various environmental systems.

Molybdenum (Mo), while an essential trace element for plant growth[21,22], can become an environmental hazard when its concentration in soil exceeds 5×10^{-6} in aqueous solutions, with toxicity levels falling between those of Zn(II) and Cr(III) compounds[23]. Although Mo is necessary for normal plant growth and development, excessive amounts can result in chlorosis and yellowing of leaves[24,25]. In humans, excessive Mo intake can lead to health issues, including diarrhea and anemia[26,27]. The normal concentration of Mo in agricultural soil typically ranges from 0.8 to 3.3 mg/kg[21]. However, studies have shown that in the Luoyang mining area, Mo concentrations in the soil ranged from 108.13 to 268.13 mg/kg, for exceeding the typical levels found in farmland soil[28].

Alfalfa, a leguminous plant known for its rapid growth, substantial biomass, and high adaptability, has been extensively studied for its potential to remediate soils contaminated with heavy metals such as Cd, Zn, and Cu. Its ability to effectively mitigate soil pollution makes it an ideal candidate for this study. Therefore, in this research, we aim to investigate the potential of natural, pollution-free humic acid in combination with alfalfa for remediating Mo-contaminated soils. Specifically, we seek to: (1) evaluate the impact of FA and HA on the bioavailability of Mo under pot culture conditions. (2) examine the bioavailability, phytoextraction, and distribution of heavy metals, and (3) analyze the responses of the soil bacterial community to the presence of FA and HA. By addressing these objectives, we aim to gain insights into the potential of combining alfalfa and HS for remediating mining-polluted soil, with a specific focus on Mo bioavailability, heavy metal uptake, distribution, and their effects on soil bacterial communities.

Materials and methods

Experimental designs

For experimental purposes, soil samples were collected from agricultural land near a mining-impacted area in Luoyang city, Henan Province, China (coordinates: E111°29.294′, N33°48.829′), containing 17.00 mg/kg of Mo. The initial soil characteristics included a cation exchange capacity (CEC) of 26.79 cmol^+/kg, available phosphorus (AP) at 146.79 mg/kg, rapidly-available potassium (AK) at 90.77 mg/kg, ammonium nitrogen (AN) at 12.62 mg/kg, and a pH of 7.42. To prepare for experimentation, the samples were air-dried, cleaned of debris, crushed and sieved using 2 mm nylon sieves for pot experiment and 100-mesh sieves for microwave clean-up to ensure consistent particle size.

The FA and HA used in this study were obtained from Shanghai Yuanye Bio-Technology Co., Ltd Shanghai, China, and applied at three levels: 0.1%, 0.5%, and 1% (g/g). The control treatment (CK) contained no FA or HA. A total of 7 treatments with 3 replicates each were established CK, FA0.1, FA0.5, FA1, HA0.1, HA0.5, and HA1. For each treatment, 3 kg of soil was mixed with the designated amount of FA or HA to ensure homogeneity. After mixing, all pots were incubated at room temperature, and soils were stabilized with FA and HA for three days prior to sowing alfalfa seeds. Uniform alfalfa seeds were than planted in each pot, allowing for plant growth and development under controlled experimental conditions.

At the end of the 60-day pot experiment, the alfalfa plants were harvested, with shoots and roots separated and washed thoroughly with tap and deionized water to remove surface contaminants. The samples were then dried at 80°C for further analysis.

Rhizosphere soil was collected from each pot and divided into two portions: One air-dried at room temperature to analyze soil properties (pH, available phosphorus (AP), ammonium nitrogen (AN), rapidly-available potassium (AK), cation exchange capacity (CEC)), and heavy metal content. And the other stored at -80°C to preserve the soil bacterial community for microbiome analysis. This dual approach facilitated a comprehensive examination of both soil physicochemical properties and microbial diversity within the rhizosphere soil.

Analytical methods

CEC was measured following the hexamminecobalt trichloride solution-spectrophotometric method, as specified in the HJ 889–2017 standard of China. For the analysis of AN, AP, and AK in the acidic soil, the universal extract-colorimetry method specified in the NY/T 1849–2010 standard of China was utilized, ensuring precise quantification of these nutrient components in the soil samples.

To assess heavy metal distribution within the soil, the modified European Community Bureau of Reference (BCR) method was employed, as described in previous studiestal forms were categorized into four fractions[29]: exchangeable fraction (F1), reducible fraction (F2), oxidizable fraction (F3), and residual fraction (F4). To determine total metals content in alfalfa shoots, roots, and residual fraction, a digestion process was conducted using a mixture of HNO_3, HCl, and HF. Soil samples (0.1 g each) were digested in a microwave oven (ETHOS UP, Milestone, Italy). And the resulting solution was diluted to a final volume of 100 ml and filtered through a 0.45 μm membrane. Additional procedural details are provided in the supplementary materials. The concentration of molybdenum (Mo) was measured using an atomic absorption spectrophotometer (TAS-990 SUPER AFG, China), allowing for accurate quantification of Mo content in the samples.

Bioconcentration factor (BCF) is calculated with Eq. (1)[30].

$$\mathrm{BCF} = \frac{\text{metal concentration in shoot/root}}{\text{metal concentration in soil}} \tag{1}$$

Translocation factor (TF) is used to evaluate the ability of heavy metals to transfer within plants[31].

$$\mathrm{TF} = \frac{\text{metal concentration in shoot}}{\text{metal concentration in root}} \tag{2}$$

The primer set used for the PCR amplification consisted of 338 F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR conditions involved an initial denaturation step at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. Subsequently, the microbial community analysis in the soil was carried out using Illumina MiSeq sequencing. This sequencing technique, performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. in Shanghai, China, allowed for the generation of high-quality sequence data for further analysis and interpretation of the microbial composition in the soil samples. Detailed instructions are in the supplementary materials.

Statistical analysis

To ensure the reliability and accuracy of the study, rigorous measures were implemented for quality assurance and quality control. Duplicate samples, standard reference samples, and control treatments were utilized to validate the results. The recoveries of the chemical fractions of Mo and the total Mo were within the range of 90–105%, indicating the precision of the analytical methods employed. To account for variability, all tests were conducted in triplicate, with a standard deviation of less than 5%, ensuring consistency and reliability. The obtained results were then averaged to provide representative values. For clear and visually appealing graphical representations, all diagrams in this article were generated using Origin 2023b software.

Results

Effects of FA and HA on soil

Figure 1 illustrates the key soil characteristics resulting from different treatments. Compared to the control, the FA treatment (excluding FA0.1) led to an increase in CEC, with a positive correlation observed between CEC and FA concentration. In contrast, the HA treatment resulted in a decrease in CEC. All treatments exhibited higher AP content compared to the control treatment, with FA treatment generally showing an increasing trend in AP levels. The HA treatment, however, initially increased and then decreased AP content. For AN content, all treatments (except FA0.1) showed higher levels than the control, with FA0.5 and FA1 treatments resulting

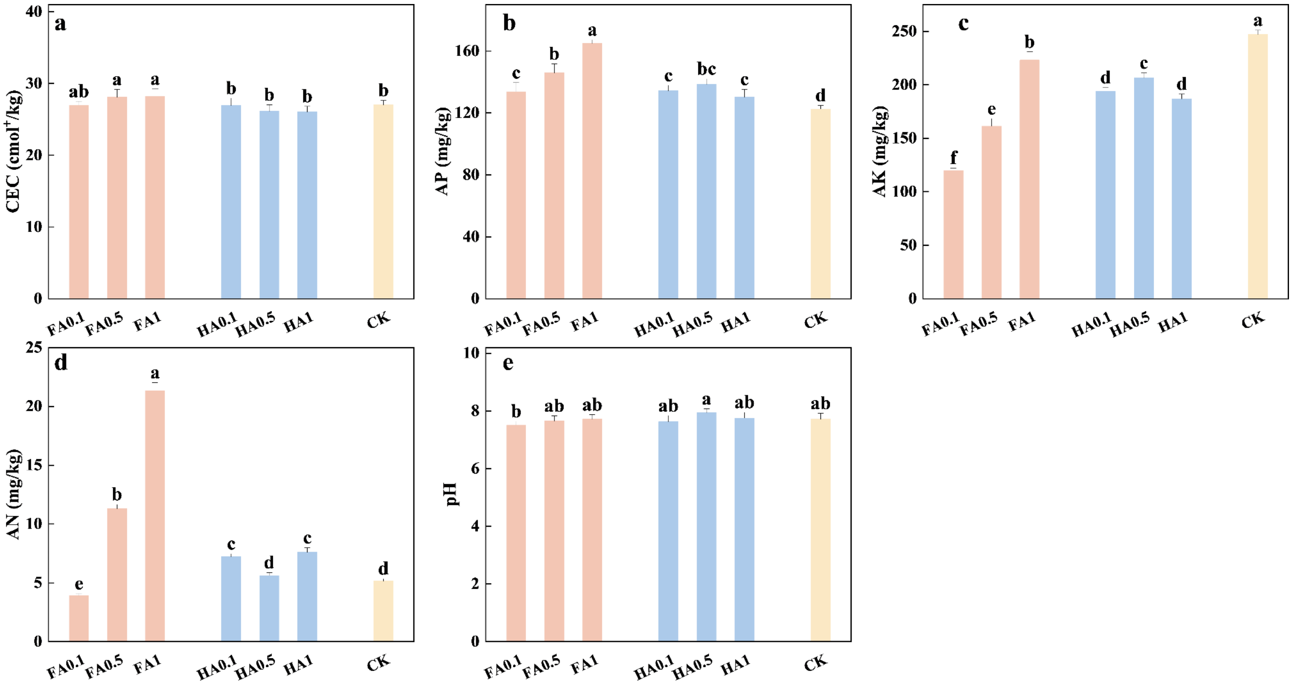

Fig. 1. Effects of on soil properties under different treatment (**a**) CEC content (**b**) AP content (**c**) AK content (**d**) AN content (**e**) the value of pH.

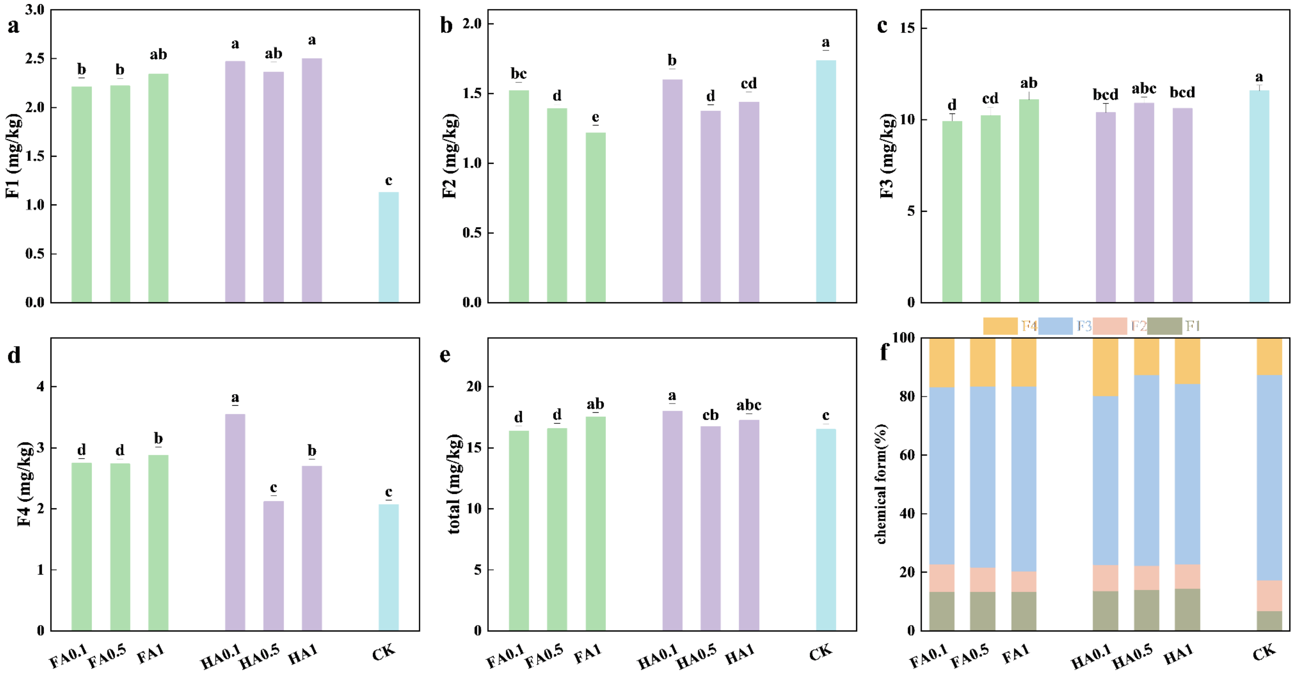

Fig. 2. Effects of different treatments on the concentrations and chemical form of Mo in soil.

in particularly elevated AN concentration. In terms of AK, both FA and HA treatments exhibited lower AK levels compared to the control, indicating a reduction in AK content under treatment conditions. The initial soil solution pH in the CK was 7.71. Compared to CK, the pH in FA treatments and HA0.1 was lower, while HA0.5 and HA1 increased the pH.

Building on existing heavy metal extraction methods, BCR developed an improved three-step extraction procedure for analyzing heavy metal species[32]. The BCR method classifies heavy metals into four fractions: the exchangeable fraction (F1), the reducible fraction (F2), the oxidizable fraction (F3), and the residual fraction (F4). F1 includes water-extractable, exchangeable, and carbonate-bound metals, while F2 represents metals bound to leacheable Fe and Mn oxides and hydroxides. F3 encompasses metals associated with organic matter and sulphides, which can be separated. Finally, F4 corresponds to metals within the mineral lattice, which are not readily released into the environment.

After harvest, soil samples from the different treatments underwent BCR fractionation to analyze metal speciation. Figure 2 illustrates the distribution of Mo concentrations across the various fractions obtained from the BCR analysis. In the FA treatment, Mo was predominantly found in the F2 fraction (1.22–1.52 mg/kg), while the highest concentrations were observed in the F3 fraction (9.91–11.08 mg/kg). Similarly, in the HA treatment, Mo was mainly present in the F2 fraction (1.37–1.60 mg/kg), with the highest concentrations in the F3 fraction (10.38–10.90 mg/kg). The addition of FA and HA also led to an increased in Mo content in the F1 and F4 fractions.

Compared to the CK, the proportions of Mo in the F1 and F4 fractions increased from 6.84% to 12.52% to 13.36 ~ 13.48%, 16.44 ~ 16.76% (FA) and 13.73 ~ 14.49%, 12.65 ~ 19.70% (HA). Conversely, the proportions of Mo in F2 and F3 fractions decreased from 10.51% to 70.13% in the CK treatment to 6.95 ~ 9.27%, 60.48 ~ 63.26% (FA) and 8.20 ~ 8.89%, 57.68 ~ 65.06% (HA). These changes suggests that the addition of FA and HA facilitated the conversion of Mo from the F2 and F3 fractions to the more stable F1 and F4 fractions.

Notably, both FA and HA treatments exhibited a slight increase in Mo concentration in the F4 fraction, indicating a potential remobilization of Mo from the more labile fractions (F1, F2, and F3) to the residual fraction. This suggests that FA and HA may reduce the mobility and availability of Mo in the soil. The proportions of the residual Mo fractions were as follows: HA0.1 (19.70%) > FA0.1 (16.76%) > FA0.5(16.51%) > FA1 (16.44%) > HA1 (15.65%) > HA0.5 (12.65%) > CK (12.52%).

The content and enrichment and transport energy of Mo in plants

Throughout the experiment, robust growth was observed in Alfalfa plants across all treatments, indicating a high tolerance to Mo. The interaction between different plant species at the rhizosphere level can either promote or inhibit plant growth and metal absorption, depending on the specific crops involved[35]. The total metal concentrations in Alfalfa shoots and roots are shown in Fig. 3(a) and Fig. 3(b). The addition of FA and HA influenced the transport of Mo, particularly regarding its distribution between roots and shoots. The application of FA inhibited Mo uptake by Alfalfa shoots. As the FA concentration increased from 0.1 to 1%, Mo content in the shoots decreased from 19.56 mg/kg in the control to 16.07, 16.60, and 15.30 mg/kg, respectively. In contrast, the application of HA led to an increase in Mo content in the shoots. In the control treatment, the shoot Mo concentration was 19.56 mg/kg, which increased to 23.24, 21.08, and 21.21 mg/kg as the HA application rate

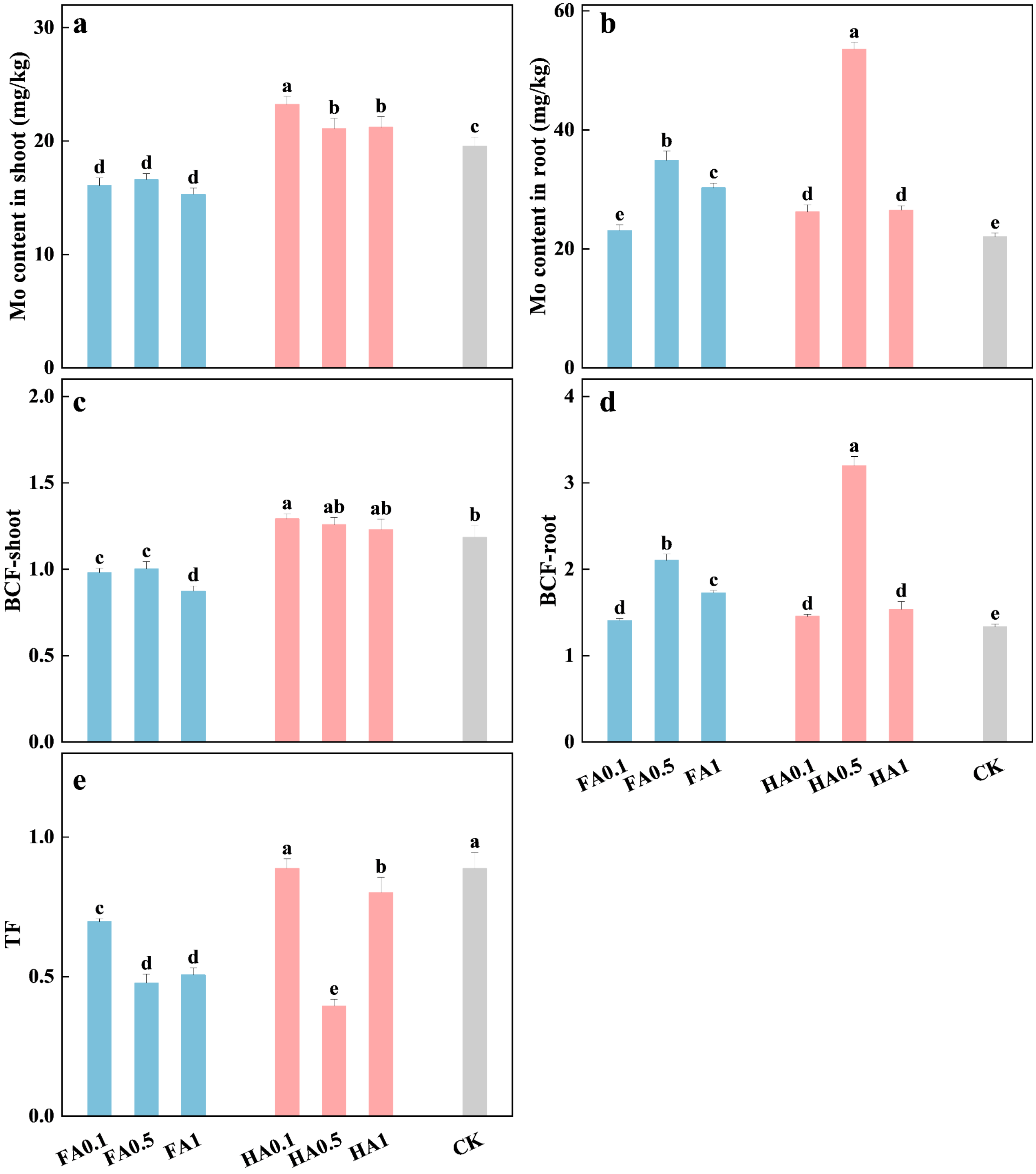

Fig. 3. (**a**) Mo content in shoots (**b**) Mo content in roots (**c**) BCF in shoots (**d**) BCF in roots (**e**) the value of TF.

increased (Fig. 3(a)). Additionally, both FA and HA treatments resulted in higher Mo concentrations in the Alfalfa roots. In the control treatment, the Mo concentration in the root was 22.06 mg/kg. The HA0.5 treatment showed the highest increase (53.58 mg/kg, 2.43 times), followed by FA0.5 (34.87 mg/kg, 1.58 times), FA1 (30.26 mg/kg, 1.37 times), HA1 (26.51 mg/kg, 1.20 times), HA0.1 (26.21 mg/kg, 1.19 times), and FA0.1 (23.06 mg/kg, 1.05 times). Mo content in the roots initially increased and then decreased with higher FA and HA application rates, suggesting that moderate application of FA and HA promotes Mo absorption by Alfalfa roots.

The BCF of plants for Mo under FA and HA treatments is shown in Fig. 3(c) and Fig. 3(d). The BCF is an indicator of a plant's ability to absorb heavy metals. The BCF for Mo in the shoot of each treatment was as follows: HA0.1 (1.29) > HA0.5 (1.26) > HA1 (1.23) > CK (1.19) > FA0.5 (1.00) > FA0.1 (0.98) > FA1 (0.87). In the

FA treatment, BCF values were generally less than 1, suggesting a limited capacity for Mo accumulation in the shoots. In contrast, HA treatments showed BCF value greater than 1, indicating a stronger enrichment capacity of Alfalfa shoots for Mo following HA application.

For the BCF of Mo in the roots, the values were as follows: HA0.5 (3.20) > FA0.5 (2.10) > FA1 (1.73) > HA1 (1.54) > HA0.1 (1.46) > FA0.1 (1.41) > CK (1.34). The addition of FA and HA enhanced Mo accumulation in the root of Alfalfa. The BCF of the roots initially increased and then decreased with the increasing FA and HA concentrations, suggesting that appropriate doses of these substances positively influence the plant's metal accumulation capacity.

The TF reflects the distribution of heavy metal between the roots and shoots, with values greater than 1 indicating greater accumulation in the shoots and values less than 1 suggesting a higher accumulation in the roots[31,37,38]. In this study, all TF values were less than 1, indicating that Mo was primarily concentrated in the roots. Furthermore, the addition of FA and HA significantly reduces the TF value, highlighting their role in limiting Mo translocation to the shoots.

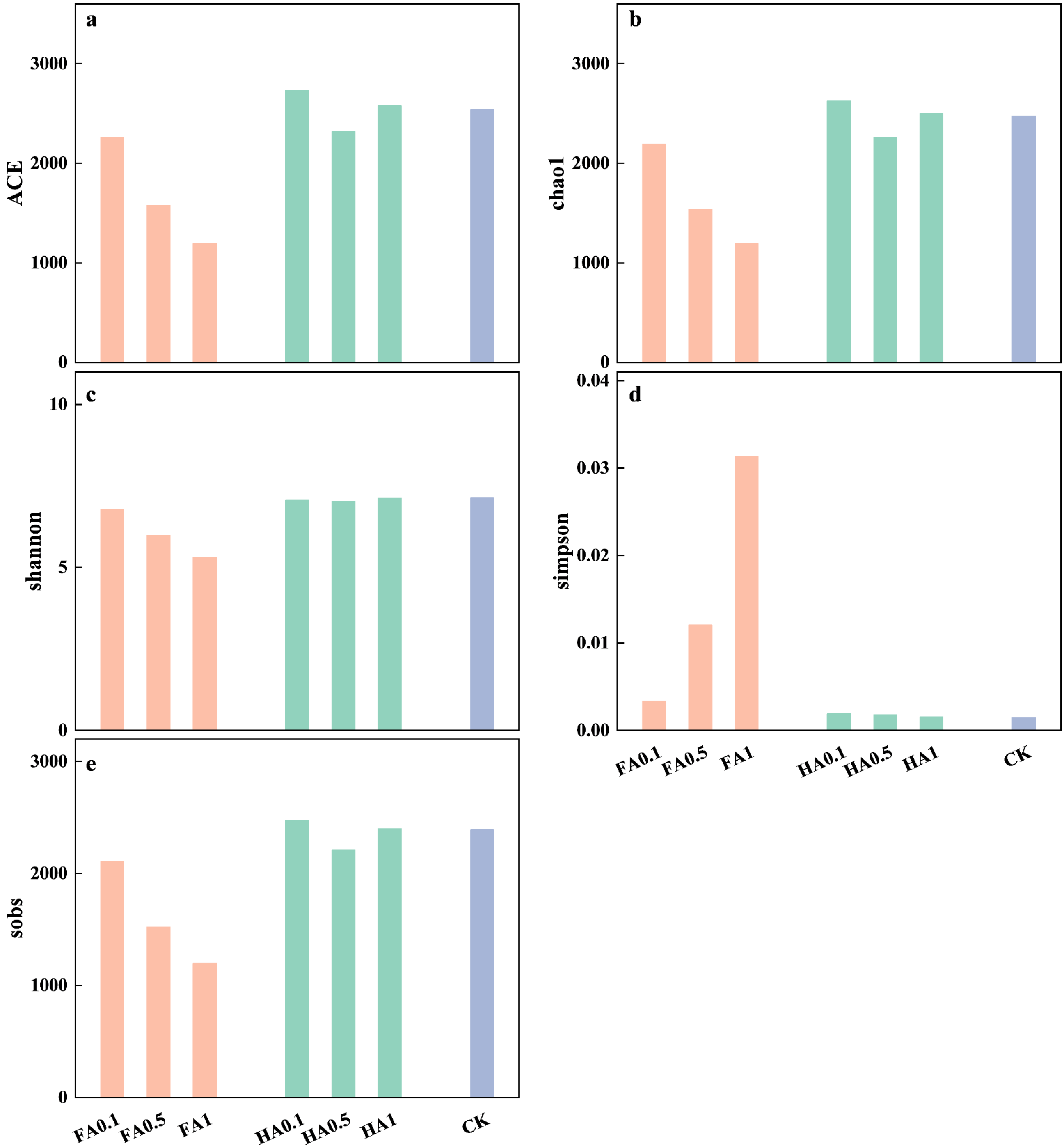

Fig. 4. (**a**) ACE index (**b**) chao 1 index (**c**) Shannon index (**d**) Simpson index (**e**) sobs index.

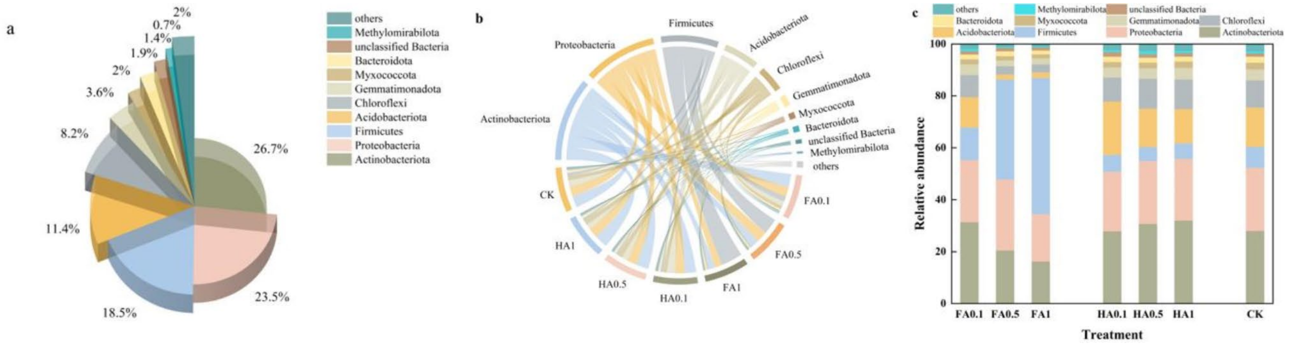

Fig. 5. (**a**) percentage of species richness on phylum level (**b**) Circos analysis on phylum level (**c**) microbial community composition on phylum level.

Effects of FA and HA on soil bacterial community

In this study, a sequencing coverage rate exceeding 98% indicated sufficient sequencing depth. Figure 4(a-e) presents the assessment of alpha diversity indicators used to evaluate the richness and diversity of the bacterial community, including Shannon, Simpson, ACE, and Chao 1. The application of FA resulted in a decrease in both the richness and evenness of the bacterial community in the soil. Specifically, the ACE and Chao indices decreased from 2540.59 to 2474.75 in the CK to 2259.73, 1575.53, and 1196.00, respectively, as the FA application rate increased from 0.1 to 1%. Similarly, the Shannon index declined from 7.13 to 5.32 with increasing FA concentration. These findings suggest that FA has detrimental effects on soil microbial ecology. In contrast, the application of HA slightly increased the ACE and Chao index, with the exception of the FA0.5 treatment. The HA0.1 treatment showed the highest bacterial richness and diversity, as evidenced by increased ACE, Chao, and Shannon index values, alongside a reduced Simpson index compared to CK.

Soil microorganisms are vital for carbon and nitrogen cycling, as well as for the decomposition of organic matter; thus, enhancing microbial communities is crucial for the restoration of contaminated soils[41]. Previous studies have shown that heavy metals can induce shifts in microbial composition, and the characteristics of rhizosphere microorganisms are closely linked to the efficacy of plant-based remediation strategies[41]. The impact of FA and HA on the composition of the rhizosphere bacterial community is illustrated in Fig. 5(a)-Fig. 5 (c). The relative abundance of soil microbial communities was analyzed at the phylum level (Fig. 5a). where five dominant bacterial phyla were identified: Actinobacteriota (26.72%), Proteobacteria (23.54%), Firmicutes (18.49%), Acidobacteriota (11.35%), and Chloroflexi (8.22%) (Relative abundance > 5%). accounting for 88.31% of the total bacterial population. Additionally, several less abundant phyla, such as Gemmatimonadota (3.63%), Myxococcota (2.05%), Bacteroidota (1.91%), and Methylomirabilota (0.74%), were also identified despite their lower relative abundances. The Circos plot (Fig. 5b) illustrates the community composition at the phylum level for each treatment and the distribution of the top 10 dominant phyla across all treatments. Regardless of the FA and HA application rates, nine dominant bacterial phyla were consistently identified in the soil samples: Actinobacteriota (16.29–32.07%), Proteobacteria (18.22–27.31%), Firmicutes (5.39–52.51%), Acidobacteriota (1.93–20.59%), Chloroflexi (3.07–11.55%), Gemmatimonadota (2.24–4.50%), Myxococcota (1.55–2.55%), Bacteroidota (1.50–2.35%), and Methylomirabilota (0.32–1.17%). The relative abundances of Proteobacteria, Myxococcota, and Bacteroidota were lower than those in the control treatment across all treatments. As the FA application rate increased from 0.1 to 1%, the relative abundances of Firmicutes increased substantially, from 8.1% in the control to 12.50%, 38.51%, and 52.51%, respectively, in the FA treatment.

Discussion

Figure 6 presents the Pearson correlation analysis, revealing significant correlations between CEC, AP, and AN with various microbial indicators. This suggests that these soil parameters are strongly associated with microbial activity.

HS are complex compounds known to form complexes with metal ions, thus influencing the mobility and bioavailability of metals[17]. HS contains diverse functional groups, including hydroxyl, aldehyde, ester, and carboxyl groups, which can participate in adsorption and complexation reactions with heavy metals, thereby altering their forms and bioavailability in soil[33]. For instance, combined applications of passivators agents (such as phosphate, humic acid, and fly ash) have been shown to convert heavy metals such as Pb and Cd from more mobility and toxicity[34]. Both FA and HA can promote stabilization of exogenous metals in soil, with the transformation effect of FA increasing with higher application rates. However, FA appears to have a relatively weaker stabilization effect and, at high doses, may even reduce metal stabilization.

Plants actively regulate the concentration of elements within their tissues under heavy metal stress[36]. In the case of Alfalfa, root tissues showed a higher Mo concentration than shoots, indicating preferential Mo accumulation in roots. Previous studies have also observed this pattern, with increased Mo concentrations in plants following HA application. For example, when the HA application rate was increased, Mo levels in shoots and roots rose from 1.74 mg/kg and 0.04 mg/kg to 2.91 mg/kg and 2.40 mg/kg, respectively[17]. Additionally, it has been reported that a 2% HA addition raised shoot concentration from 30.9 mg/kg to 39.9 mg/kg, like due to a pH reduction that facilitated Cd migration. Plants may also absorb complexes formed between heavy metals

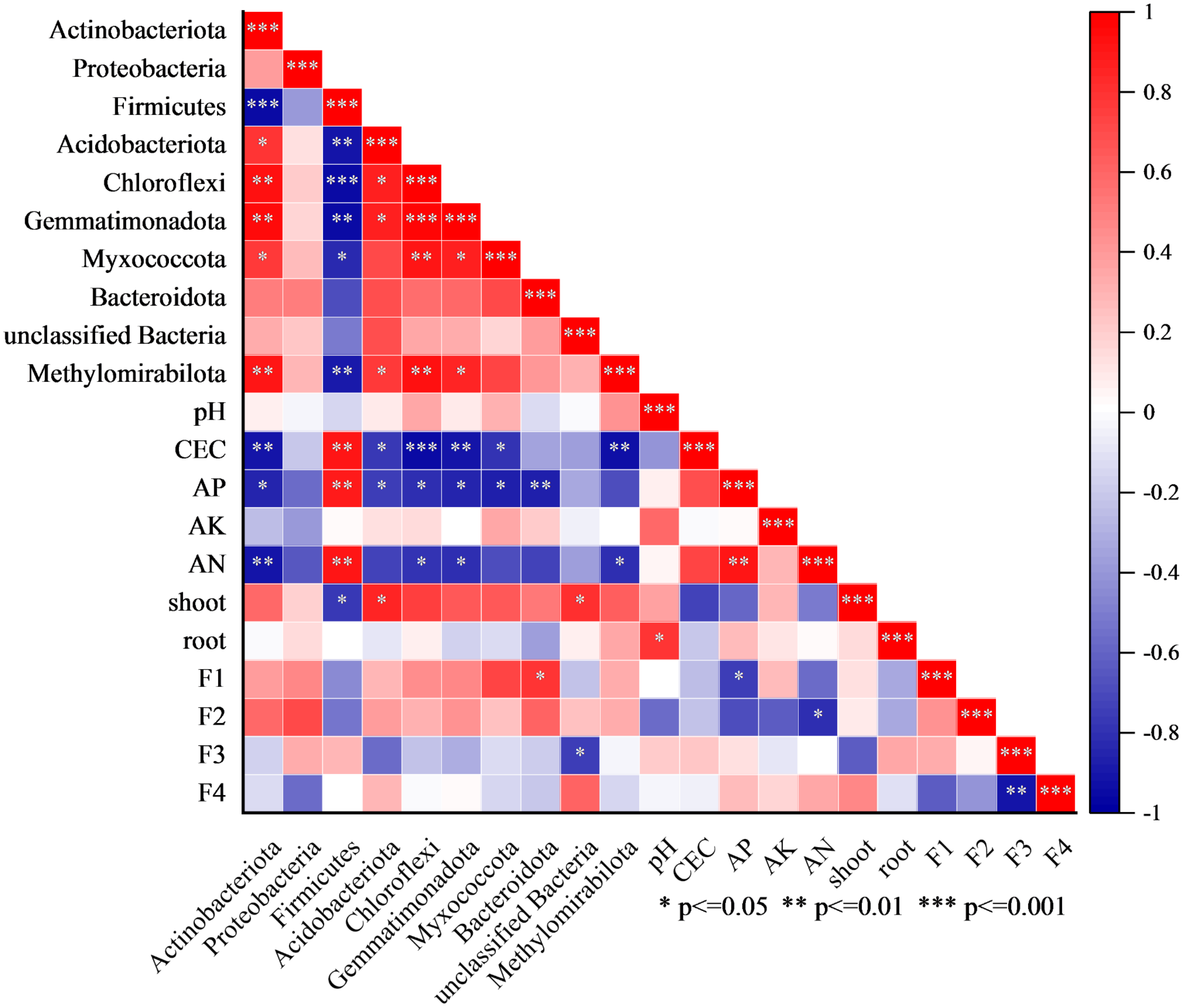

Fig. 6. Spearman correlations between soil properties and bacteria alpha diversity indices and major phyla abundance.

like Cd and humic acid fragments, which are derived from microbial decomposition or self-decomposition[15]. Humic acid's ability to form metal complexes makes it effective for bioremediation of heavy metals, while FA can inhibit meal uptake, suggesting its potential for reducing metal accumulation in acidic, contaminated soils[18].

While some studies suggest that humic substances not significantly alter the chemical form of Mo[17], contrasting findings indicate that specific inorganic metal complexes can affect Mo mobility in the rhizosphere, thereby influencing root uptake and potentially altering heavy metal accumulation in plant tissues[39,40]. These findings align with the current study's results.

Microbial diversity is a key indicator of ecosystem functionality. Soil microorganisms regulate numerous soil functions, including soil quality maintenance and plant resilience[41].

In this study, the FA and HA applications impacted alpha diversity indices, consistent with previous reports on the negative effects of Cd on bacterial diversity[42]. Biochar addition has also been shown to enhance microbial richness and diversity, thereby improving soil health across various soil types[43].

Soil microbial communities are strongly influenced by soil physicochemical properties. This study found a negative correlation between CEC and Actinobacteriota (p<0.05), Chloroflexi (p<0.05), and Methylomirabilota (p<0.05). while a positive correlation was observed with Firmicutes ($p < 0.01$) (Fig. 6). AP showed a negative correlation with Gemmatimonadota (p<0.01), Myxococcota (p<0.05), and Bacteroidota (p<0.05). A negative association was found with unclassified Bacteria (p<0.01), whereas a positive correlation was observed with Firmicutes (p<0.05). Root Mo content showed a negative correlation with Bacteroidota (p<0.05). Proteobacteria displayed a negative correlation with F4 (p<0.01) and a positive correlation with F3 (p<0.05), potentially due to their sensitivity to heavy metals. These findings suggest that CEC, AP, AN, and specific microbial indicators play a crucial role in shaping the soil bacterial communities.

Conclusions

This study utilized pot incubation experiments to evaluate the immobilization efficiency of Humic substances (FA and HA) in reducing the mobility and bioavailability of molybdenum (Mo) in agricultural soils. In Alfalfa cropping systems, FA and HA treatments effectively diminished soil Mo mobility and availability during the incubation period, influencing its transport and distribution within plant roots and shoots. Notably, FA showed a stronger impact on root Mo accumulation, whereas HA exhibited a more pronounced effect in shoots. Additionally, FA and HA applications altered soil bacterial abundance and diversity, leading to shifts in the microbial community. Specifically, FA application increased the diversity of firmicutes, while variations in Actinobacteriota, firmicutes, acidobacteriota, chloroflexi, gemmatimonadota, and myxococcota were correlated with changes in soil CEC, AP, and AN. Among the two humic substances, HA demonstrated a greater potential for remediating metal-contaminated soil. Overall, Humic substances (FA and HA) offer an eco-friendly to enhance the remediation of Mo-contaminated agricultural soils. Further studies are recommended to investigate the long-term impacts of these treatments on soil microorganisms and plants health.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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